RAPPORTI ISTISAN 24|16

ISSN: 1123-3117 (cartaceo) • 2384-8936 (online)

MOOD Project
(MOnitoring Outbreak events for Disease surveillance in a data science context)

Drivers and trends of zoonotic pathogens, antimicrobial resistance and Disease X in Europe

Edited by C. Cataldo, A. Rizzoli, L. Busani

PATOLOGIE

ISTITUTO SUPERIORE DI SANITÀ

MOOD Project
(MOnitoring Outbreak events for Disease surveillance in a data science context)

Drivers and trends of zoonotic pathogens, antimicrobial resistance and Disease X in Europe

Edited by
Claudia Cataldo (a), Annapaola Rizzoli (b),
Luca Busani (a)

(a) Centro di Riferimento per la Medicina di Genere, Istituto Superiore di Sanità, Roma
(b) Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige, Trento

ISSN: 1123-3117 (cartaceo) • 2384-8936 (online)

Rapporti ISTISAN
24/16

Istituto Superiore di Sanità
MOOD Project (MOnitoring Outbreak events for Disease surveillance in a data science context). Drivers and trends of zoonotic pathogens, antimicrobial resistance and Disease X in Europe.
Edited by Claudia Cataldo, Annapaola Rizzoli, Luca Busani
2024, v, 296 p. Rapporti ISTISAN 24/16 (in Italian)

The project "MOnitoring Outbreak events for disease surveillance in a data science context" (MOOD) aims to develop innovative tools and services for the early detection, assessment, and monitoring of current and potential infectious disease threats in Europe in the context of global change. Within the MOOD project, a list of prototype infectious diseases including influenza A, tulaeremia, leptospirosis, chikungunya, dengue, Zika, West Nile, Usutu virus infection, tick-borne encephalitis, Lyme borreliosis and Crimea-Congo hemorrhagic fever was defined. Moreover, antimicrobial resistance and a so called "Disease X", an unknown but potentially pandemic infection, were included. A comprehensive literature search was performed for each disease following the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) protocol adapted for scoping reviews protocol. The selected articles, complemented with general reviews for descriptive information, were extracted to provide a complete and thorough description of the pathogen and the disease, including environmental and social covariates, epidemiological trends, diagnostics, and prevention tools available. The resulting profiles are available for epidemiologists, risk assessors, and public health officers to support them in understanding and implementing risk assessment and early warning actions.
Key words: Zoonoses; One Health; Risk assessment; Epidemiology; Ecology

Istituto Superiore di Sanità
Progetto MOOD (MOnitoring Outbreak events for Disease surveillance in a data science context). Driver e tendenze di patogeni zoonotici, resistenza antimicrobica e "Disease X" in Europa.
A cura di Claudia Cataldo, Annapaola Rizzoli, Luca Busani
2024, v, 296 p. Rapporti ISTISAN 24/16 (in inglese)

Il progetto MOOD (*MOnitoring Outbreak events for Disease surveillance in a data science context*) ha l'obiettivo di sviluppare strumenti e servizi innovativi per l'individuazione precoce, la valutazione e il monitoraggio di malattie infettive emergenti e riemergenti in Europa nel contesto del cambiamento globale. Nel progetto è definito un elenco di malattie infettive prototipo, tra cui l'influenza A, tularemia, leptospirosi, chikungunya, dengue, Zika, febbri West Nile, Usutu, l'encefalite da zecche, borrelliosi di Lyme e febbre emorragica Crimea Congo. Inoltre, sono stati inclusi i profili di resistenza agli antibiotici e "Disease X", quest'ultimo descrive una malattia sconosciuta e potenzialmente pandemica. Per ognuna è stata eseguita una ricerca della letteratura seguendo il protocollo *Preferred Reporting Items for Systematic reviews and Meta-Analyses* (PRISMA) per le *scoping review*. Gli articoli selezionati sono stati estratti per fornire una descrizione completa e approfondita dell'agente patogeno e della malattia, comprese le covariate ambientali e sociali, i trend epidemiologici, la diagnostica e gli strumenti di prevenzione disponibili. I profili risultanti sono a disposizione di epidemiologi, valutatori del rischio e responsabili della salute pubblica per aiutarli a comprendere e implementare la valutazione del rischio e le azioni di allerta precoce.
Parole chiave: Zoonosi; One Health; Valutazione del rischio; Epidemiologia; Ecologia

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 874850 and is catalogued as MOOD094. The contents of this publication are the sole responsibility of the authors and don't necessarily reflect the views of the European Commission.

Acknowledgement: E4Warning Project is joint funder for models used to develop *Aedes* distribution maps.

Per informazioni su questo documento scrivere a: luca.busani@iss.it; claudia.cataldo@iss.it

Il rapporto è accessibile online dal sito di questo Istituto: www.iss.it.

Citare questo documento come segue:
Cataldo C, Rizzoli A, Busani L (Ed.). *MOOD Project (MOnitoring Outbreak events for Disease surveillance in a data science context). Drivers and trends of zoonotic pathogens, antimicrobial resistance and Disease X in Europe.* Roma: Istituto Superiore di Sanità; 2024. (Rapporti ISTISAN 24/16).

Legale rappresentante dell'Istituto Superiore di Sanità: *Rocco Bellantone*
Registro della Stampa - Tribunale di Roma n. 114 (cartaceo) e n. 115 (online) del 16 maggio 2014

Direttore responsabile della serie: *Antonio Mistretta*
Redazione*: Sandra Salinetti*
La responsabilità dei dati scientifici e tecnici è dei singoli autori, che dichiarano di non avere conflitti di interesse.

viale Regina Elena, 299 – 00161 Roma

Working group "Disease profiles"

The following experts of the working group "Disease profiles" contributed to the document:

Daniele ARNOLDI	*Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige, Trento (Italy)*
Elena ARSEVSKA	*UMR Animals, Health, Territories, Risks, and Ecosystems (Astre), Department of Biological Systems (Bios), French Agricultural Research and International Cooperation Organization for Development (CIRAD), Campus International de Baillarguet, Montpellier (France)*
Xavier BAILLY	*Université de Lyon, INRAE, VetAgro Sup, UMR EPIA, Marcy l'Etoile; Université Clermont Auvergne, INRAE, VetAgro Sup, UMR EPIA, Saint-Genès-Champanelle (France)*
Maria BELLENGHI	*Centro di Riferimento per la Medicina di Genere, Istituto Superiore di Sanità, Rome (Italy)*
Luca BUSANI	*Centro di Riferimento per la Medicina di Genere, Istituto Superiore di Sanità, Rome (Italy)*
Rosanna CAMMARANO	*Servizio Comunicazione Scientifica, Istituto Superiore di Sanità, Rome (Italy)*[*]
Claudia CATALDO	*Centro di Riferimento per la Medicina di Genere, Istituto Superiore di Sanità, Rome (Italy)*
Alessandra CIERVO	*Dipartimento Malattie Infettive, Istituto Superiore di Sanità, Rome (Italy)*
Francesca DAGOSTIN	*Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige, Trento (Italy)*
Simon DELLICOUR	*Department of Microbiology, Immunology and Transplantation, Rega Institute, KU Leuven (Belgium); Spatial Epidemiology Lab (SpELL), Université Libre de Bruxelles (ULB), Brussels (Belgium)*
Marco DI LUCA	*Dipartimento Malattie Infettive, Istituto Superiore di Sanità, Rome (Italy)*
Mitra DRAKULOVIC	*Department for Communicable Diseases Prevention and Control, National Public Health Institute "Dr Milan Jovanovic-Batut", Belgrade (Serbia)*
Timothee DUB	*Department of Health Security, Finnish Institute for Health and Welfare, Helsinki (Finland)*
Marzia FACCHINI	*Dipartimento Malattie Infettive, Istituto Superiore di Sanità, Rome (Italy)*
Beatriz FERNANDEZ MARTINEZ	*Centro Nacional de Epidemiología, Instituto de Salud Carlos III, Consorcio de Investigación Biomédica en Red Epidemiología y Salud Pública, CIBERESP, Madrid (Spain)*
Claudia FORTUNA	*Dipartimento Malattie Infettive, Istituto Superiore di Sanità, Rome (Italy)*
Pachka HAMMAMI	*UMR Animals, Health, Territories, Risks, and Ecosystems (Astre), Department of Biological Systems (Bios), French Agricultural Research and International Cooperation Organization for Development (CIRAD), Campus International de Baillarguet, Montpellier (France)*
Soushieta JAGADESH	*International Society of Infectious Diseases, Boston (MA)(USA)*
Ferran JORI MASSANAS	*Cirad, UMR Astre, Montpellier, University of Montpellier, INRAE, Montpellier (France)*
Isabelle LEBERT	*Université de Lyon, INRAE, VetAgro Sup, UMR EPIA, Marcy l'Etoile; Université Clermont Auvergne, INRAE, VetAgro Sup, UMR EPIA, Saint-Genès-Champanelle (France)*

[*] Retired

Henna MÄKELÄ	*Department of Health Security, Finnish Institute for Health and Welfare, Helsinki (Finland)*
Giovanni MARINI	*Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige, Trento (Italy)*
Giulia MENCATTELLI	*Centro Ricerca e Innovazione, Fondazione Edmund Mach, Centro Agricultura, Alimenti, Ambiente, Università di Trento, San Michele all'Adige, Trento (Italy), Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo (Italy)*
Ewy ORTEGA	*UMR Animals, Health, Territories, Risks, and Ecosystems (Astre), Department of Biological Systems (Bios), French Agricultural Research and International Cooperation Organization for Development (CIRAD), Campus International de Baillarguet, Montpellier; Ministère de l'agriculture et de l'alimentation, Direction générale de l'alimentation (DGAL), Paris, (France)*
Sara PIACENTINI	*Dipartimento Malattie Infettive, Istituto Superiore di Sanità, Rome (Italy)*
Scilla PIZZARELLI	*Servizio Comunicazione Scientifica, Istituto Superiore di Sanità, Rome (Italy)*
Simona PUZELLI	*Dipartimento Malattie Infettive, Istituto Superiore di Sanità, Rome (Italy)*
Magalie RENE MARTELLET	*Université de Lyon, INRAE, VetAgro Sup, UMR EPIA, Marcy l'Etoile; Université Clermont Auvergne, INRAE, VetAgro Sup, UMR EPIA, Saint-Genès-Champanelle (France)*
Flavia RICCARDO	*Dipartimento Malattie Infettive, Istituto Superiore di Sanità, Rome (Italy)*
Annapaola RIZZOLI	*Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige, Trento (Italy)*
Kyla SERRES	*Spatial Epidemiology Lab (SpELL), Université Libre de Bruxelles (ULB), Brussels (Belgium)*
Valentina TAGLIAPIETRA	*Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige, Trento (Italy)*
Luciano TOMA	*Dipartimento Malattie Infettive, Istituto Superiore di Sanità, Rome (Italy)*
Wim VAN BORTEL	*Department of Biomedical Sciences, Outbreak Research Team, Institute of Tropical Medicine, Antwerp (Belgium)*
Esther VAN KLEEF	*Department of Public Health, Institute of Tropical Medicine, Antwerp (Belgium)*
Maria Fernanda VINCENTI-GONZÁLEZ	*Spatial Epidemiology Lab (SpELL), Université Libre de Bruxelles (ULB), Brussels (Belgium)*
William WINT	*Department of Biology, Environmental Research Group Oxford Ltd, Oxford (United Kingdom)*

TABLE OF CONTENTS

ACRONYMS

ADIS	Animal Disease Information System
ADNS	Animal Disease Notification System
AIVs	Avian Influenza Viruses
AMR	Antimicrobial Resistance
CAESAR	Central Asian and European Surveillance of Antimicrobial Resistance network
CCHF	Crimean Congo Haemorrhagic Fever
CDC	Centers for Disease Control and Prevention
CESME	National Reference Centre for Foreign Animal Diseases
CHIK	Chikungunya
CHIKV	Chikungunya Virus
DENV	Dengue Virus
EARS-Net	European Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EIV	Equine influenza Virus
ESVAC	European Surveillance of Veterinary Antimicrobial Consumption
EU	European Union
EU/EAA	European Union and European Economic Area
HP	Highly Pathogenic
HPAI	Highly Pathogenic Avian Influenza
IAV-S	Influenza A Viruses of Swine
IAVs	Influenza A Viruses
LP	Low Pathogenic
MS	Members States
OECD	Organisation for Economic Co-operation and Development
TBE	Tick-borne encephalitis
TESSy	The European Surveillance System of Infectious Diseases
USUV	Usutu virus
VMPs	Veterinary Medicinal Products
WAHIS	World Animal Health Information System
WNV	West Nile Virus
WOAH	World Organization for Animal Health
ZIKV	Zika Virus

INTRODUCTION

Claudia Cataldo, Luca Busani
Centro di Riferimento per la Medicina di Genere, Istituto Superiore di Sanità, Rome

The Horizon 2020 project "MOnitoring Outbreak events for Disease surveillance in a data science context" (MOOD), financed by the European Commission (Grant Agreement MOOD N° 874850), aims at the development of innovative tools and services for the early detection, assessment, and monitoring of current and potential infectious diseases threats in Europe in a context of global change.

Data processing methods and spatial or mechanistic modelling within the MOOD project integrates epidemiological and genetic data with environmental and socio-economic covariates in an inter-sectorial, interdisciplinary, One health approach. This project is attentive to any link between the risk of disease emergence and biological/sociological characteristics of both women and men.

The disease intelligence within the MOOD project is working to provide indicators, proxies and define "signals" of disease emergence. It will also provide data favouring open/accessible data sources to improve indicator, event-based surveillance, and early detection of emerging infectious threats. The project ensures that, at the European level, the results are realistic, relevant, and valid with regard to the state of disease knowledge and will create a community of experts. Disease profiles and indicators were defined for major airborne, vector-borne, foodborne, and waterborne infectious diseases, including an unknown disease "Disease X" and antimicrobial resistance.

The starting point for disease profile production has been searching for scientific and technical information for the diseases considered in the MOOD project. A multi-step approach has been followed in addressing the search:

1) description of specific objectives for each profile;
2) use of appropriate bibliographic databases;
3) definition of a common standard protocol for the bibliographic search.

A specific focus has been on social roles and behaviours potentially associated with specific disease risks, considering the gender-based differences identified from the literature.

The list of prototype significant infectious diseases that are considered in the MOOD project included influenza A for airborne pathogens, tularaemia and leptospirosis as models of neglected endemic pathogens with multiple transmission routes and reservoirs, chikungunya, dengue and Zika viruses as models of exotic pathogens transmitted by invasive mosquito species, West Nile and Usutu viruses infections as examples of exotic pathogens transmitted by endemic vectors and tick-borne encephalitis and Lyme borreliosis as models of endemic pathogens transmitted by endemic tick vectors. Moreover, antimicrobial resistance, Crimea Congo haemorrhagic fever, and a so called "Disease X", an unknown but potentially pandemic infection, were included.

Each prototype disease was profiled based on information collected from literature review and expert input. A broad approach based on the "Scoping reviews" scheme was adopted to collect and synthesize the available knowledge.

This document collects the disease profiles produced within the MOOD framework elaborated by the working group "Disease profiles", summarizing the available knowledge on several specific topics for each disease or pathogen defined.

The aim of this collection of disease profiles is to provide to risk assessors and public health officers a general One Health overview of the diseases, with historical trends and description of the drivers (related to human, animals, ecosystems, and environment) that modulate the impact of the diseases and should be considered for risk assessments.

METHODOLOGY

Claudia Cataldo (a), Scilla Pizzarelli (b), Rosanna Cammarano (b), Maria Bellenghi (a), Francesca Dagostin (c), Giovanni Marini (c), Annapaola Rizzoli (c), Valentina Tagliapietra (c), Luca Busani (a)
(a) Centro di Riferimento per la Medicina di Genere, Istituto Superiore di Sanità, Rome
(b) Servizio Comunicazione Scientifica, Istituto Superiore di Sanità, Rome
(c) Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige, Trento

The prototype infectious diseases proposed by the experts of the working group "Disease Profiles" of the project MOOD ("MOnitoring Outbreak events for disease surveillance in a data science context") are:

- influenza A for airborne pathogens with multiple host species;
- tularaemia and leptospirosis as models of neglected endemic pathogens with multiple transmission routes and reservoirs;
- chikungunya, dengue and Zika viruses as models of exotic pathogens transmitted by invasive mosquito species;
- West Nile and Usutu virus infections as examples of exotic pathogens transmitted by endemic vectors;
- tick-borne encephalitis and Lyme borreliosis as models of endemic pathogens transmitted by endemic tick vectors.

Moreover, antimicrobial resistance, Crimea Congo haemorrhagic fever, and a "Disease X", an unknown but potentially pandemic infection, were included.

The information was obtained from the literature by conducting a series of scoping reviews, one for each disease, in accordance with a standardized methodology to ensure the quality and repeatability of the process, in accordance with the guidelines provided by Tricco *et al.* (1). This was done in order to achieve a thorough understanding of the factors influencing the ecology, distribution, and trends in Europe of the prototype infectious diseases included in the MOOD project.

The key questions were harmonized, and a list of relevant keywords organized in a standardized search strategy was arranged and launched across the following databases: MEDLINE, Embase, BIOSIS, SciSearch, CABA, Scopus.

Slight modifications to the standardized protocol that simplified the research strategy and took into account disease-specific aspects were applied for scoping reviews on Crimean Congo Haemorrhagic fever, antimicrobial resistance, and a "Disease X".

Inclusion and exclusion criteria were defined considering study design, language (English or other EU languages), time frame (10 years for epidemiological and pathogen data, 30 years for environmental data), geographical location (Europe), and publication type.

Studies without data or with non-original or duplicated data (reviews, editorials, letters, modelling studies with no data), lacking denominators or reference populations, unavailable full texts, referring to data older than 2000 or gathered outside Europe, were excluded.

The final time frame covered a period from 2000 to 2022.

The results of the literature searches for each prototype pathogen were uploaded in Rayyan (2) to select and label the articles according to the main topics of interest (human, environmental, animal, vector, and reservoirs covariates). The articles of interest were selected screening titles and abstracts. Those relevant were then retrieved full text, and data were extracted using a data

extraction sheet based on the Cochrane Consumers and Communication Review Group's data extraction template. The data extraction results were analysed, providing basic statistics (numbers and frequencies) for each covariate identified.

Selected articles were summarized in the disease profiles, which were prepared by supplementing the literature search with specific bibliography, particularly for general information on pathogens. Each profile is structured as follows:

- biological, ecological and molecular features of the causative agent;
- natural history of disease in humans and vectors, including symptoms, morbidity and mortality;
- availability of preventive, therapeutic and control measures, including licensed or pipelined vaccines;
- epidemiological situation at different spatial scales: past and current trends;
- sociological and demographical dimension affecting susceptibility and exposure, including gender;
- diagnostic procedures and notification systems used at local, national and European scale;
- infrastructure capacity to identify pathogens for each Member State;
- estimated influence of environmental change on the disease future trends.

The original maps and figures were produced using official data provided by the European Centre for Disease Prevention and Control (ECDC) and World Organization for Animal Health (WOAH), and these data were those available at the time of the analysis. Some of the maps were produced in 2024. At the end of the drafting of the disease profiles, the literature search was updated to include the most recent important information on the diseases. The update included major changes in trends and distribution of the diseases, animals and vectors involved. For those diseases that were updated, references for the period 2023-2024 were included.

Specific maps on vectors distribution in Europe that integrated environmental covariates and data were produced in collaboration with E4Warning Project, a joint funder.

References

1. Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, Moher D, Peters MDJ, Horsley T, Weeks L, Hempel S, Akl EA, Chang C, McGowan J, Stewart L, Hartling L, Aldcroft A, Wilson MG, Garritty C, Lewin S, Godfrey CM, Macdonald MT, Langlois EV, Soares-Weiser K, Moriarty J, Clifford T, Tunçalp Ö, Straus SE. PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation. *Ann Intern Med.* 2018 Oct 2;169(7):467-473. doi: 10.7326/M18-0850.
2. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. *Syst Rev*. 2016 Dec 5;5(1):210. doi: 10.1186/s13643-016-0384-4.

ANTIMICROBIAL RESISTANCE

Soushieta Jagadesh (a), Claudia Cataldo (b), Annapaola Rizzoli (c), Luca Busani (b)
(a) International Society of Infectious Diseases, Boston (MA)
(b) Centro di Riferimento per la Medicina di Genere, Istituto Superiore di Sanità, Rome
(c) Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige, Trento

Priority level for EU

AntiMicrobial Resistance (AMR) in bacterial species is a significant public health challenge requiring continuous surveillance efforts globally, as well as in the European Union (EU) and the European Economic Area (EEA). AMR is responsible for over 35,000 deaths every year in the EU/EEA (1).

Twenty-nine EU/EEA countries report cases of pathogens with AMR to the European Antimicrobial Resistance Surveillance Network (EARS-Net), which has collated this data since the late 1990s, and over the previous decade coordinated by the European Centre for Disease Prevention and Control (ECDC) via the European Surveillance System (TESSy), a web-based platform for data submission and storage (1, 2).

In the 29 EU/EEA countries, a joint report from the OECD (Organisation for Economic Co-operation and Development) and the ECDC reported a shift in the overall antibiotic consumption in Europe since 2014, with the average consumption of antibiotics in humans now higher than in food-producing animals, after adjusting for biomass (3). In 2018, 4,264 tonnes of antibiotics were used in humans corresponding to a mean antibiotic consumption of 133 mg of active substance per kg estimated biomass, whereas 6,358 tonnes of antibiotics were used in food-producing animals corresponding to a mean antibiotic consumption of 105 mg per kg estimated biomass.

Overall, consumption was lower in food-producing animals than in humans in 19 of 29 EU/EEA countries. This change in paradigm highlights the efficacy of the measures to reduce the use of antibiotics in food producing animals taken at country-level. The 13th ESVAC report (4) details that, in 2022, sales of antibiotic Veterinary Medicinal Products (VMPs) used for food-producing animals represented 98.4% of total sales in tonnes (Figure 1) and ranged from 2.1 mg/PCU to 254.7 mg/PCU in the 31 participating countries (Figure 2). The total aggregated sales across all reporting countries were 73.9 mg/PCU. The report also demonstrates that in 2020, community consumption of broad-spectrum antibiotics averaged across EU/EEA countries was 3.5 times higher than consumption of narrow-spectrum antibiotics, which should generally be the first-line therapy (3).

As reported in the ECDC "Antimicrobial consumption in the EU/EEA (ESAC-Net) - Annual Epidemiological Report 2021" (later AER 2021), between 2011 and 2020, an increasing trend was observed in this ratio for the EU/EEA overall and for nine individual countries including Bulgaria, Croatia, Estonia, Hungary, Italy, Latvia, Lithuania, Slovakia, and Slovenia. While in eight of the 29 EU/EEA countries including Austria, Belgium, Denmark, Finland, France, Germany, Ireland, and Norway, narrow-spectrum antibiotics consumption was higher in comparison across the same period. In 2021, the EU/EEA population-weighted mean consumption of antibacterials for systemic use in the community was 15 DDD (Defined Daily Doses) per 1000 inhabitants per day, ranging from 7.2 in Austria to 24.3 in Romania (Figure 3) (5).

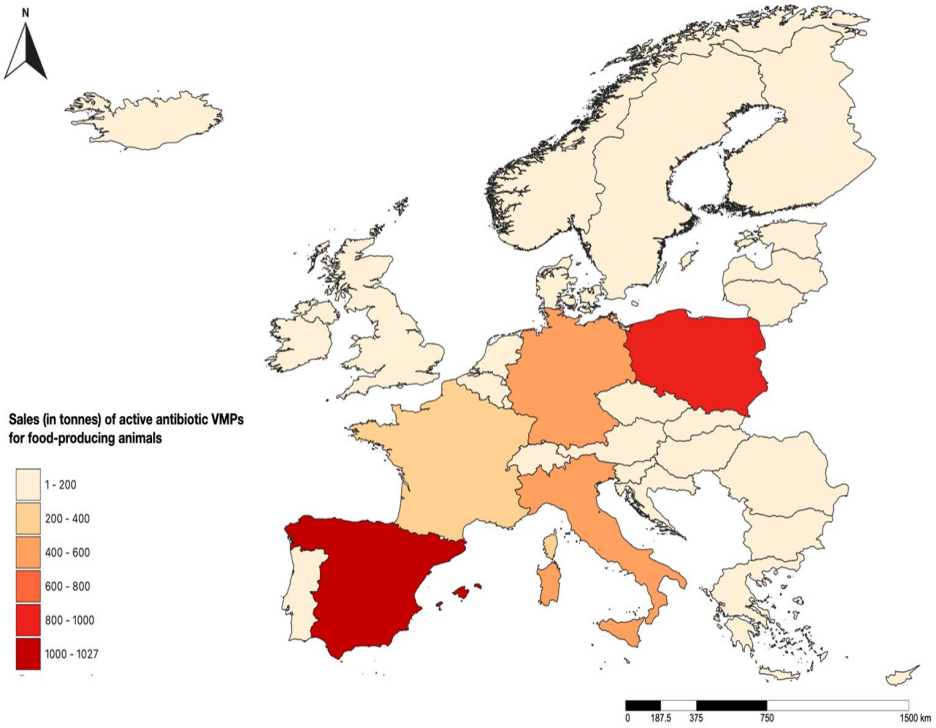

Figure 1. Overall sales, in tonnes, of antibiotic VMPs for food-producing animals in 31 European countries in 2022 – adapted from 13th ESVAC report (4)

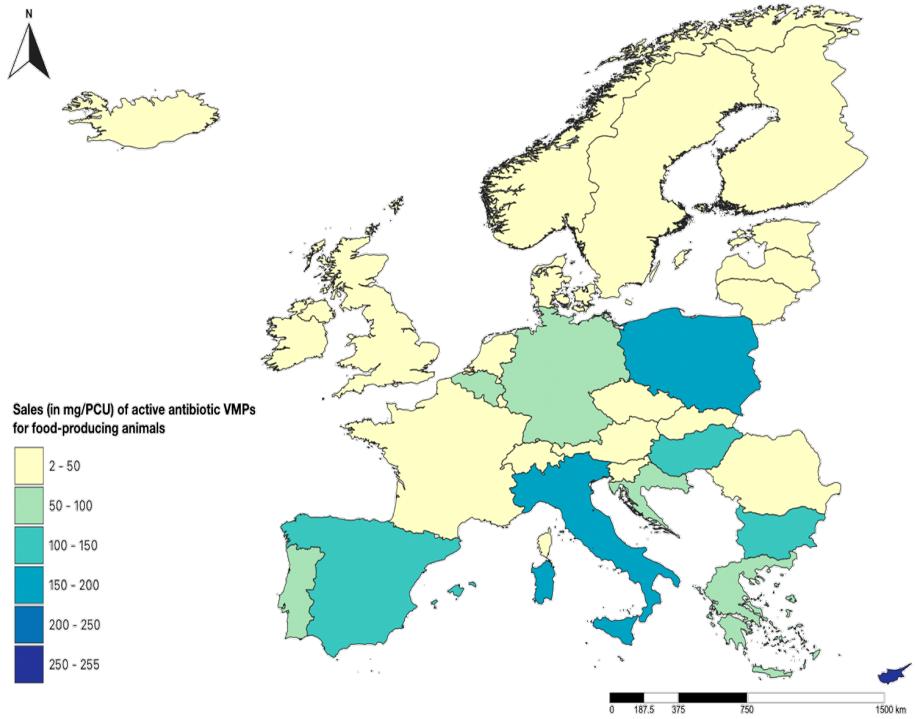

Figure 2. Overall sales, in mg/PCU, of antibiotic VMPs for food-producing animals in 31 European countries in 2022 – adapted from 13th ESVAC report (4)

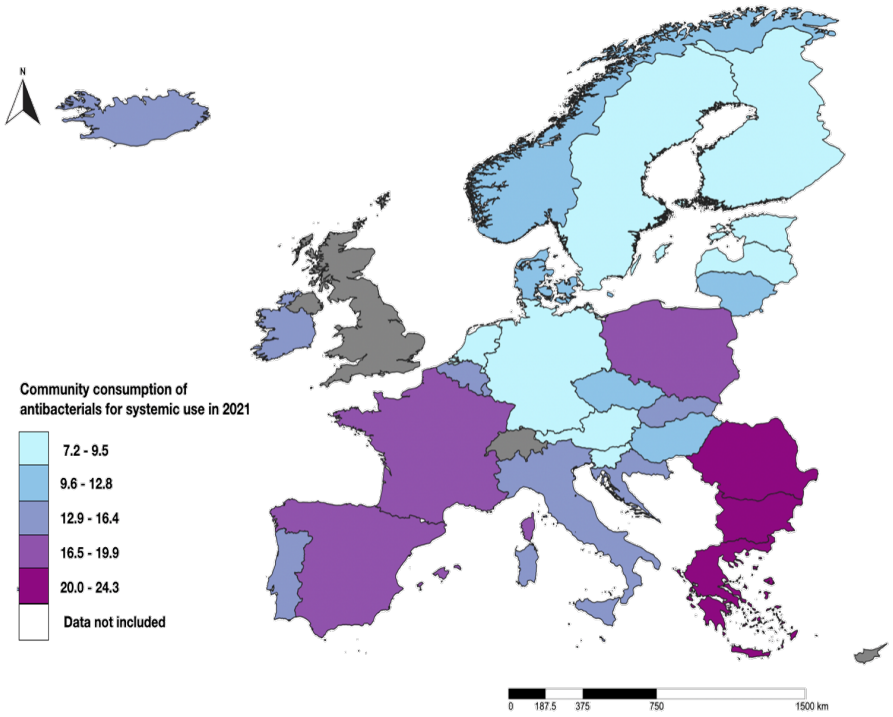

Figure 3. Community consumption (DDD per 1000 inhabitants per day) of antibacterials for systemic use among the EU/EEA countries in 2021 – adapted AER 2021 report (5)

The large variability in AMR percentages particularly in the Southern and Eastern countries of the EU/EEA remains a cause for concern (6). In 2017, 7.2% of *Klebsiella pneumoniae* isolates were identified as resistant to carbapenems, with a large variation across EU/EEA from 0% in several countries, such as Croatia, Estonia, Iceland, Luxembourg, Norway, and Slovenia, to 64.7% in Greece. The14.9% of *Escherichia coli* isolates were identified as resistant to third-generation cephalosporins, ranging from 5.9% in Norway to 41.3% in Bulgaria. Methicillin-resistant *Staphylococcus aureus* (MRSA) invasive isolates reached a proportion of 16.9%, with variations across Europe (1.0% in Norway to 44.4% in Romania). Moreover, the increase of vancomycin resistance from 9% in 2014 to 17% in 2020 in *Enterococcus faecium*, and of resistance carbapenems from 8% in 2014 to 10% in 2020 in *Klebsiella pneumoniae* was also observed (3). Recent research has also reported an increasing 2020 EU/EEA population- weighted mean resistance of 34% to third-generation cephalosporins in *K. pneumoniae*, and 18% and 38% resistance to carbapenems in Pseudomonas aeruginosa and *Acinetobacter* species, respectively (3).

Cassini *et al.* in 2019 (7) measured the health burden of five types of antibiotic-resistant infection (invasive and non-invasive) caused by eight bacteria with 16 resistance patterns in the EU/EAA. The Disability-Adjusted Life-Years (DALYs) estimated were 671,689 (95% Confidence Interval-CI: 583,148-763,966) cases of infections with antibiotic-resistant bacteria in 2015, of which 426,277 (63.5%) were associated with health care. The overall DALY rate is 170 per 100,000 population, which is similar to the combined burden of HIV, influenza, and tuberculosis in the same year in the EU and EEA. The article also identified that the burden was highest in infants (aged <1 year) and people aged 65 years or older, had increased since 2007, and was highest in Italy and Greece.

To date, the AMR percentages for the bacterial species-antimicrobial group combinations under surveillance remains high in the EU/EEA. In 2022, the ECDC estimated the burden of

infections with antibiotic-resistant bacteria under surveillance in the EU/EEA (1). The number of cases of these infections increased from 685,433 (95% Uncertainty Interval-UI: 589,451-792,873 cases) in 2016 to 865,767 (95% UI: 742,802-1,003,591 cases) in 2019, with a decrease in the estimate for 2020 to 801 517 (95% UI: 684,955-932 213 cases). These infections resulted in an estimated annual number of attributable deaths that increased from 30,730 (95% UI: 26,935-34,836 deaths) in 2016 to 38 710 (95% UI: 34,053-43,748 deaths) in 2019, decreasing slightly to 35 813 (95% UI: 31,395-40,584 deaths) in 2020.

The decrease in the community antibiotic consumption in the year 2020 in Europe is attributed to the coronavirus disease (COVID-19) pandemic (Figures 4 and 5).

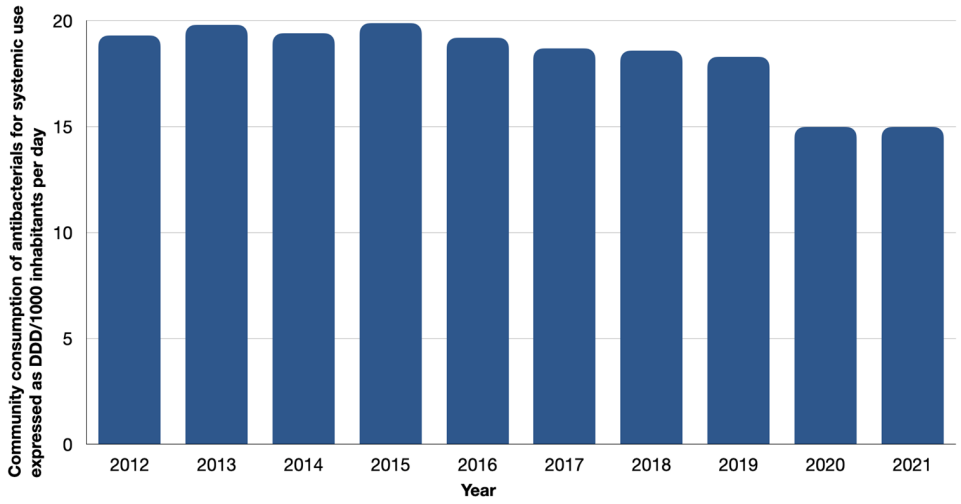

Figure 4. Trends of community consumption (DDD per 1000 inhabitants per day) of antibacterials for systemic use among the EU/EEA countries from 2012 to 2021 – adapted from AER 2021 report (5)

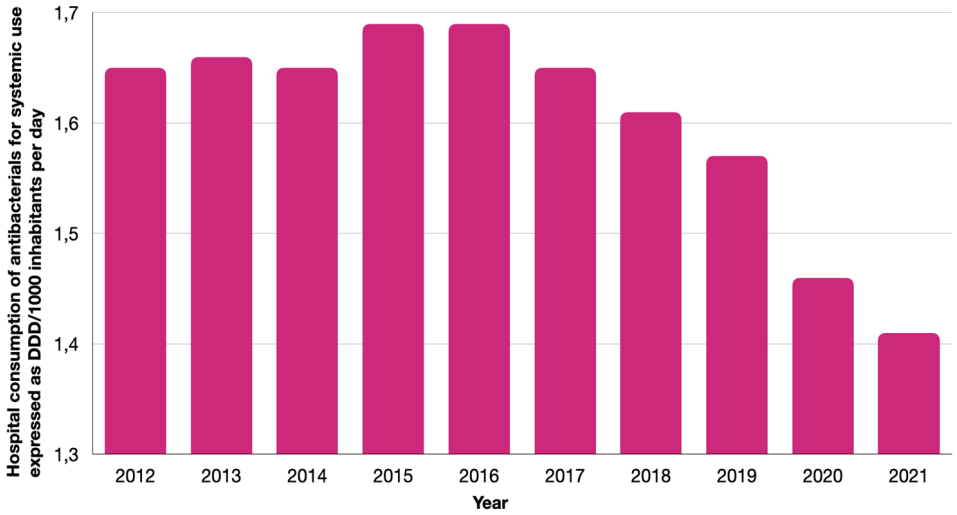

Figure 5. Trends of hospital sector consumption (DDD per 1000 inhabitants per day) of antibacterials for systemic use among the EU/EEA countries from 2012 to 2021 – adapted from the AER 2021 report (5)

The disruption of routine care in the community due to patient avoidance of health services owing to self-isolation or an inability to access healthcare reduced antibiotic access (8). Moreover, other Non-Pharmaceutical Interventions (NPIs) for COVID-19 limited physical person to person contacts and other infections (9, 10). Studies noted that the changes in antibiotic consumption during the pandemic were less consistent in the hospital sector, with increased consumption of last-line antibiotics, particularly, carbapenems (8). This was observed by the increase in the number of cases of carbapenem-resistant *Acinetobacter* spp. infections in 2020-2021, mostly in countries that had a relatively high percentage of carbapenem-resistant cases pre-pandemic. *Acinetobacter* spp., including carbapenem-resistant isolates, are known to cause outbreaks becoming difficult to eradicate once they become endemic. It is therefore predicted that carbapenem-resistant *Acinetobacter* spp. will continue to expand in the EU/EEA (8).

Distribution of pathogen

AMR surveillance for EARS-net (EU/EEA) and CAESAR (other countries in the European Region of the of the World Health Organization, WHO) networks focuses on invasive isolates of eight key bacterial species (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter species, Streptococcus pneumoniae, Staphylococcus aureus, Enterococcus faecalis* and *Enterococcus faecium*). AMR percentages are presented for a single antimicrobial agent and/or group of antimicrobial agents. Microorganisms with AMR causing notifiable diseases, such as tuberculosis, are also monitored by the WHO Regional Office. The following data is summarized from the "Antimicrobial resistance surveillance in Europe 2023" report (11). In 2021, 11 (25%) of 44 countries reporting data on *S. aureus* had Methicillin Resistant *S. aureus* (MRSA) percentages below 5%. MRSA percentages equal to or above 25% were found in 13 (30%) countries (Figure 6).

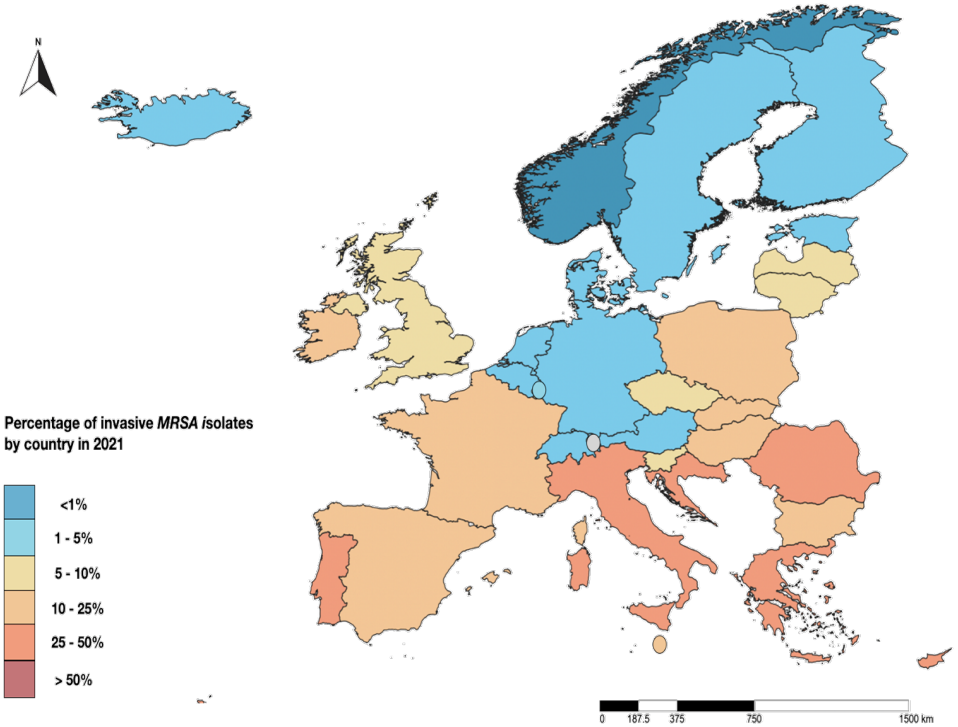

Figure 6. Invasive *Staphylococcus aureus* isolates (%) resistant to methicillin (MRSA) in 2021 – adapted from "Antimicrobial resistance surveillance in Europe 2023 report" (11)

Two (5%) of 43 countries reporting penicillin non-wild-type *S. pneumoniae* in 2021 had percentages below 5% (Estonia and Latvia), while percentages equal to or above 25% were found in five (12%) countries (Belarus, France, Romania, Serbia and Turkey).

E. coli resistance to fluoroquinolones was lowest in northern and western parts of the European Region and highest in Southern and eastern parts in 2021 (Figure 7A). An AMR percentage below 10% was observed in two (7%) of 29 EU/EEA countries (Finland and Norway). Seventeen countries (58.6%) reported a percentage of 25% or above. AMR percentages of 50% or above were observed in neighbouring countries in the European region (North Macedonia, Russia and Turkey) including Cyprus. For third-generation cephalosporin resistance in *E. coli*, 12 (27%) of the countries in Europe reported resistance percentages below 10% (Figure 7B).

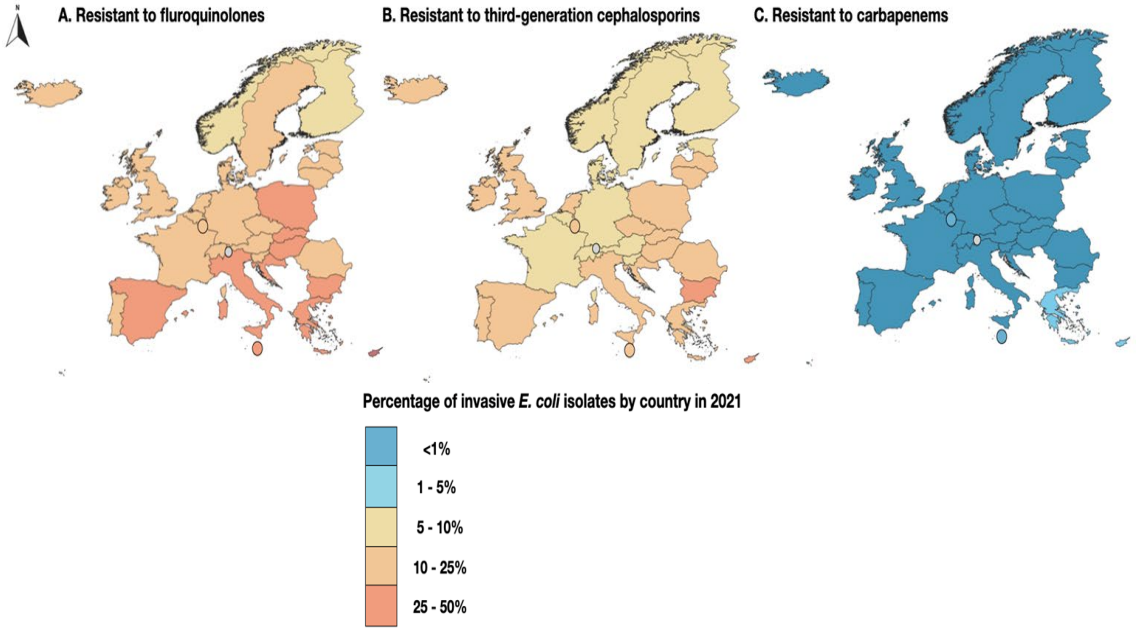

Figure 7. Invasive *Escherichia coli* isolates (%) in EU/EEA countries in 2021 resistant to: fluoroquinolones (ciprofloxacin/levofloxacin/ ofloxacin) (A), third-generation cephalosporins (cefotaxime/ ceftriaxone/ceftazidime) (B), and carbapenems (C), adapted from "Antimicrobial resistance surveillance in Europe 2023 report" (11)

In 2021, AMR percentages below 10% to third-generation cephalosporins were observed for *K. pneumoniae* in seven (16%) of 45 countries in Europe (Austria, Denmark, Finland, Iceland, Norway, Sweden and Switzerland), while 19 (42%), particularly in the Southern and Eastern parts of the Region, reported AMR percentages of 50% or above (Figure 8A). The emergence of carbapenem-resistant *E. coli* was reported in two (7%) of 29 EU/EEA countries (Greece and Cyprus) with percentages of 1% or above in 2021 (Figure 7C). Carbapenem resistance in *K. pneumoniae* was generally low in northern and western parts of Europe with 14 (31%) of 45 countries reporting AMR percentages below 1% (Figure 8B).

Fifteen (33%) countries reported percentages equal to or above 25%, eight of which (18% of 45 countries) reported AMR percentages equal to or above 50% (Belarus, Georgia, Greece, Moldova, Romania, Russia, Serbia and Ukraine).

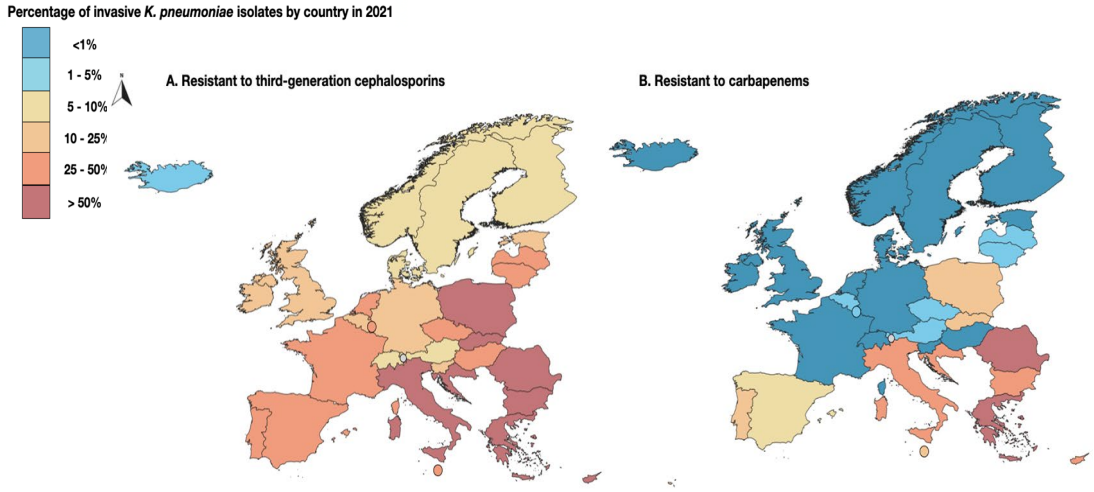

Figure 8. Invasive *Klebsiella pneumoniae* isolates (%) in EU/EEA countries in 2021 resistant to: third-generation cephalosporins (cefotaxime/ ceftriaxone/ceftazidime) (A), and to carbapenems (B), adapted from "Antimicrobial resistance surveillance in Europe 2023 report" (11)

While carbapenem resistance in *P. aeruginosa* in 2021, AMR percentages of below 5% were observed in two (5%) of 44 countries reporting data for *P. aeruginosa* (Denmark and Finland), whereas six (14%) countries reported percentages equal to or above 50% (Belarus, Georgia, Moldova, Russia, Serbia and Ukraine) (Figure 9). The percentages of carbapenem-resistant *Acinetobacter* spp. Varied widely, from below 1% in three (7%) of 45 countries reporting data (the Netherlands, Norway and Sweden) to percentages equal to or above 50% in 25 (56%) countries, mostly in Southern and eastern Europe (Figure 10).

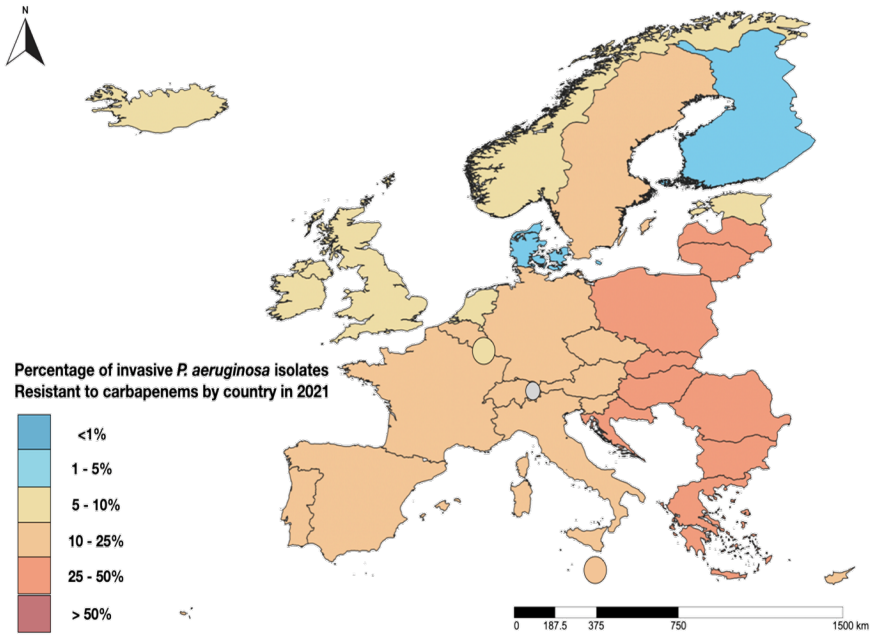

Figure 9. Invasive *Pseudomonas aeruginosa* isolates (%) in EU/EEA countries in 2021, resistant to carbapenems, adapted from "Antimicrobial resistance surveillance in Europe 2023 report" (11)

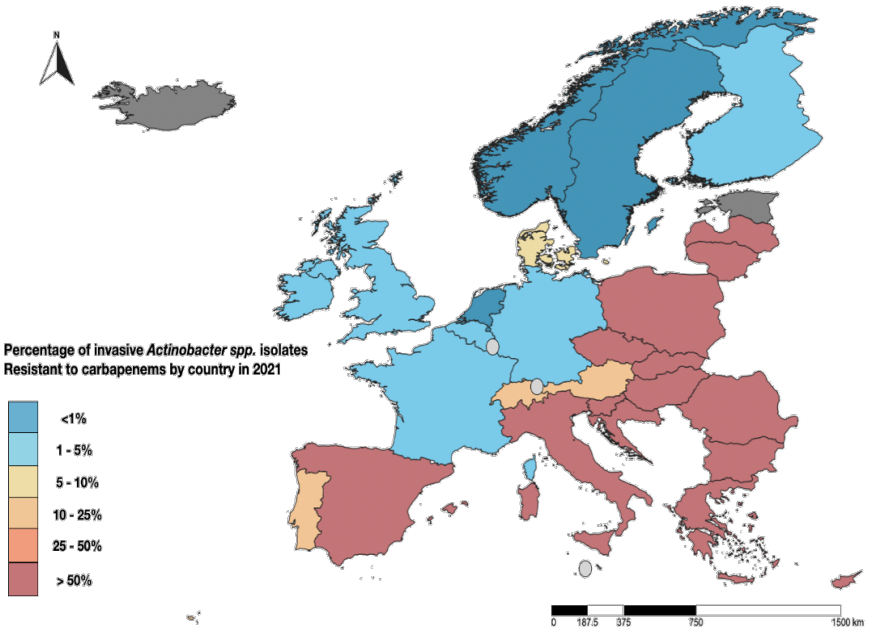

Figure 10. Invasive *Acinetobacter* species isolates (%) in EU/EEA countries in 2021, resistant to carbapenems, adapted from "Antimicrobial resistance surveillance in Europe 2023 report" (11)

Resistance to vancomycin in *E. faecium* varied substantially in Europe. In 2021, percentages of below 1% were reported by six (14%) of 44 countries providing data on this microorganism (Finland, France, Luxembourg, the Netherlands, Norway and Sweden). AMR percentages equal to or above 25% were found in 17 (39%) countries, five of which (11% of 44 countries) reported percentages equal to or above 50% (Cyprus, Lithuania, Malta, North Macedonia and Serbia) (Figure 11).

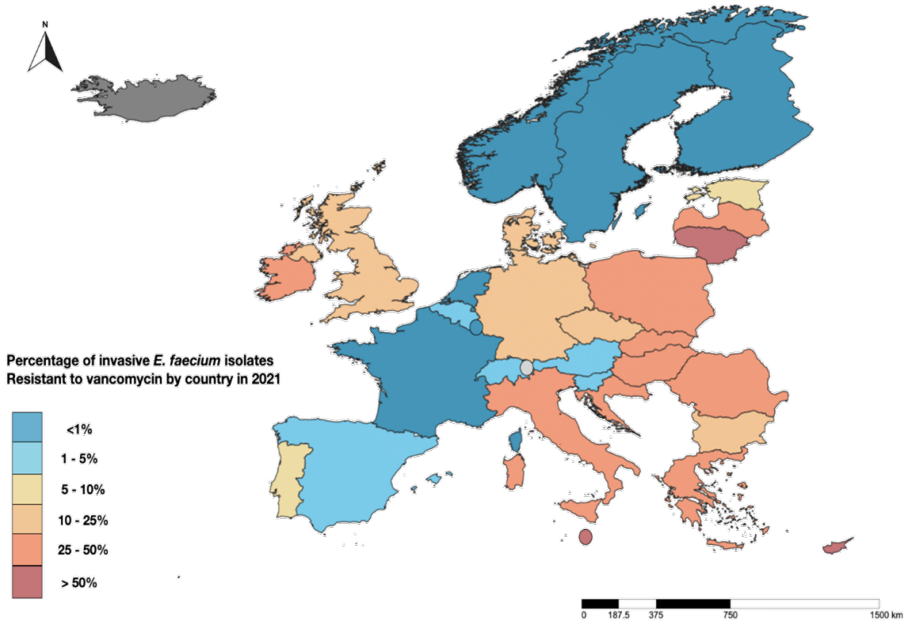

Figure 11. Invasive *Enterococcus faecium* isolates (%) in EU/EEA countries in 2021, resistant to vancomycin adapted from "Antimicrobial resistance surveillance in Europe 2023 report" (11)

Drivers of the AMR emergence and spread

The emergence and spread of AMR are driven by a multitude of factors, including human and animal movements, surface water run-off, and exchange of agricultural products (12). Although multisectoral drivers of AMR globally are poorly understood, the strong link between humans and food-producing animals is well-established (13-15). A study across 11 European countries found strong positive correlations of AMR to various antibiotics (ampicillin, aminoglycosides, third-generation cephalosporins, and fluoroquinolones) in *E. coli* isolated from food-producing animals and from humans (16). Systematic reviews have established this association on demonstrating interventions targeting drug consumption in food-producing animals influenced AMR rates in humans and animals (17, 18). Antibiotic consumption is another well-established key driver for AMR, with compelling evidence for dose dependence in humans (19) and animals (20).

Other drivers influencing AMR spread include anthropogenic factors, such as population density (21, 22) and rising incomes (23, 24). Studies show that the rise in Growth Domestic Product (GDP) and living standards in Low and Middle-Income Countries (LMICs) were positively correlated with antibiotic consumption (24, 25) as well as increase in animal protein consumption (26). Studies demonstrate that inadequate local sanitation, pollution of surrounding water bodies from agricultural run-off and release of non-metabolised antibiotics or their residues into the environment through manure/faeces also play a significant role (27-29).

An important anthropogenic driver of AMR is the misuse of antibiotics, including overprescription of antibiotics as well as overuse in livestock. In 30% to 50% of cases, incorrect prescription of antibiotics is attributed to treatment indication, choice of agent, or duration of antibiotic therapy (30, 31). Similarly, use of antibiotics in food animals has been linked to antibiotic-resistant bacteria in humans through contaminated food-derived products, and represents a threat to human health (32-34). Evidence of a proportion of human extra-intestinal extended spectrum cephalosporin-resistant *E. coli* infections originated from livestock was found in observational studies, with poultry as most probable source (35). A study reported that a targeted intervention to reduce the use of antimicrobials in animals resulted in 24% reduction in prevalence of resistant infections in humans (20). Some contradicting evidence reports that animals to human transfer of resistance is negligible (36). However, numerous studies are established that antimicrobial resistant bacteria originating in an animal can be transmitted to humans through the environment, food products, and/or by direct contact (14,33,37,38).

Climate change was found to influence AMR in Europe (39). In particular, temperature is the strongest drivers of bacterial reproduction and can also modulate aspects of horizontal gene transfer. An increasing trend of resistance in *E. coli* and increase of the minimum temperature has been observed, and European countries with 10°C warmer ambient minimum temperatures experienced more rapid resistance increases across all antibiotic classes in *E. coli* and *K. pneumoniae* (40). Another study demonstrated that higher temperatures and humidity increased colonization and infection risk for MRSA (41).

Natural history of AMR in humans and animals, including morbidity and mortality

Brief history of the AMR

The first incident of AMR was observed in 1928 with arsphenamine, also known as Salvarsan, an antibiotic introduced at the beginning of the 1910s (42). Sulphonamide resistance was detected

in certain bacteria in 1933 (43). Resistance to penicillin by bacterial β-lactamases was discovered in 1942, several years before the introduction of penicillin as a therapeutic (44). In the case of streptomycin, introduced in 1944 for the treatment of tuberculosis, mutant strains of Mycobacterium tuberculosis resistant to therapeutic concentrations of the antibiotic were found to arise during patient treatment (45). Tetracycline resistance was first observed with Shigella in 1959 (46). Methicillin resistance was identified in the bacteria *Staphylococcus aureus* in 1961 (47, 48), making methicillin resistant *S. aureus* (MRSA) one of the most difficult to treat nosocomial infections. In the mid-1950s horizontal genetic transfer of antibiotic resistance via plasmids was observed in Japan (49). Resistance to cephalosporin ceftazidime was observed in Enterobacteriaceae two years following its introduction in 1955 (50). Vancomycin resistance was identified in the bacteria *Enterococcus* and *S. aureus* in 1989 (51) and 1998 (52), respectively. Transmissible fluoroquinolone resistance was discovered in the late-1990s (27). *M. tuberculosis* strains resistant to four or more of the front-line treatments have appeared and spread rapidly in the late 2000s (53,54), with totally drug resistant strains occurring in the last decade (55). In 2015, AMR was declared a global emergency by the WHO (56).

The first report of antimicrobial usage for livestock was from Britain where Prontosil and other sulphonamides like sulphapyridine were marketed for use in animals from 1938 (57). In 1940, gramicidin was used to treat a mass outbreak of mastitis at New York's World Exhibition (58). The wartime importance of milk production was highlighted by the usage of penicillin for mastitis in both Britain and Denmark in 1943 (59, 60). In 1948, Merck's sulfaquinoxaline was the first antibiotic to be officially licensed for routine inclusion in poultry feeds (57). Antibiotic use also increased in other areas of global food production: sulphonamides were used in commercial bee hives, in aquaculture, and against mastitis in the dairy sector (57). By the 1960s, antibiotics in food production was widespread globally. In France, approximately 30 tonnes were added to animal feed in 1964 (61). In Britain, experts estimated that 41% (84/168 tonnes) of all antibiotics consumed by animals in 1967 were feed additives (62).

Sweden was the first to ban all food animal growth-promoting antibiotics in 1986 (63). Following which, the EU banned avoparcin in 1997 and bacitracin, spiramycin, tylosin and virginiamycin in 1999 (64). The growth promoter bambermycin was banned from all use in EU livestock since 2006. New regulations on veterinary medicines (Regulation (EU) 2019/6) and medicated feed (Regulation (EU) 2019/4) will enter into force within the European Union (EU) from 28 January 2022. The new veterinary Regulation (EU) 2019/6 has extended restrictions for use of some antibiotics in animals to a full ban of certain antibiotics. Around 25 EU/EEA Member States reported reduced antibiotic usage in veterinary medicine, and mainly in animal husbandry, resulting in a 47% decrease in sales of veterinary antimicrobial medicinal products between 2011 and 2021 (65).

AMR in humans

Overall, in 2021 (11), the most commonly reported bacterial species with AMR in humans was *E. coli* (39.4% of all reported cases), followed by *S. aureus* (22.1%), *K. pneumoniae* (11.9%), *E. faecalis* (8.8%), *E. faecium* (6.2%), *P. aeruginosa* (6.1%), *Acinetobacter* spp. (3.0%) and *S. pneumoniae* (2.5%). Between 2020 and 2021, the number of reported human cases increased for *Acinetobacter* spp. (+43.2%), *E. faecium* (+20.5%) and *E. faecalis* (+14.0%). Minor differences were noted for *S. aureus* (+9.4%), *P. aeruginosa* (+8.2%), *K. pneumoniae* (+8.1%), *S. pneumoniae* (+4.3%), and *E. coli* (+2.8%).

AMR in *K. pneumoniae* is of major concern in the EU/EEA. Recent outbreaks of carbapenemase (NDM-1 and OXA- 48)-producing and colistin-resistant *K. pneumoniae* were reported to have a concomitant increase in virulence (66,67), transmissibility and AMR which

pose a considerably higher risk to human health than the *K. pneumoniae* strains that previously circulated.

A recent publication demonstrated that a major part of the increase in reported *Acinetobacter* spp. in 2020-2021, during the COVID-19 pandemic years, consisted of carbapenem-resistant ventilator-associated infections in ICU patients, in the countries with carbapenem resistance percentages in this pathogen exceeding 50% in 2018-2019 with a recent estimate from 2020 attributing 3656 deaths to carbapenem-resistant *Acinetobacter* spp. (68). Multidrug resistant *Acinetobacter* spp. is notoriously difficult to eradicate from the hospital environment once established as these strains can survive on dry surfaces, readily contaminating healthcare providers' hands, and being spread by asymptomatic carriers

Despite the relative decline of MRSA and *S. pneumoniae* percentages in EU/EEA countries, they remain an important pathogen with EU/EEA health burden of MRSA for the period 2016-2020 being the second largest (1).

Vancomycin-resistant *E faecium* increased from 47124 in 2016 to 117866 in 2020, with a consequent increase in the number of attributable deaths from 1335 to 3414 (1). WHO has listed vancomycin-resistant *E. faecium* as a pathogen of high priority in its global priority list of antibiotic-resistant bacteria, emphasizing the paucity of available and effective treatment options (69).

AMR in animals

Majority (73%) of all antimicrobials are used in animals for the prevention and treatment of infections (70) and, outside the EU, are also used to improve weight gain and productivity on farms (71). In the report, AMR in animals is divided into three sections: domestic animal husbandry, companion animals, and aquaculture.

AMR in domestic animal husbandry

Antibiotic use in livestock includes treatment of infections as well as administration of subtherapeutic doses for significant weight gain among the treated animals (72-76). According to Commission Implementing Decision (EU) 2020/1729, which applies from 1 January 2021 until December 2027, monitoring of AMR is mandatory in *Salmonella* spp*., Campylobacter coli*, *Campylobacter jejuni*, and indicator *E. coli*, in the major domestically produced animal populations and their derived meat. In 2021, the population-weighted mean antimicrobial use (AMU) in food-producing animals was lower than in humans in the EU/EEA (3, 77). AMU reduction in chickens was successful in the Netherlands due to the large-scale transition from fast-growing to slow-growing chicken breeds, which required fewer antibiotic treatments (78). Studies report a substantial reduction of AMU on herd level without major impact on pig health economic performance (79, 80).

AMR in companion animals

Antimicrobial resistance of pet origin poses a major threat to human health, particularly concerning MRSA, other methicillin-resistant staphylococci, vancomycin-resistant enterococci, carbapenemase-producing *enterobacteriaceae* and Extended Spectrum Beta-Lactamase (ESBL) Gram-negative bacteria (81). A study analysing the frequency of methicillin-resistant *Staphylococcus* spp. in pets (82), dogs and cats, highlighted the prevalence of methicillin resistance traits with 40% of the resistant Staphylococcal population identified as *Staphylococcus schleiferi*, 35% *S. aureus*, and 17% *S. intermedius*. The study observed that *S. schleiferi* was more recurrent in dermatitis and ear canal infection, prevalently isolated from dogs similar to resistant

S. intermedius strain. MRSA isolation was associated with deep infections with a similar frequency in dogs and cats. Another study carried out in three Eu countries showed that 19% of the animals received at least one antimicrobial treatment six months preceding sampling and cats and dogs were treated with a standard daily dose of antimicrobials for 1.8 and 3.3 days over one year, respectively (83). The most frequently used antimicrobial was amoxicillin-clavulanate (27%). Resistance of *E. coli* to at least one antimicrobial agent was found in 27% of the isolates with 18% resistance to ampicillin and 13% multidrug resistant isolates (83).

AMR in aquaculture

Although aquaculture constitutes only 5.7% of the global antimicrobial use, it carries the highest use intensity per kilogram of biomass, 164.8 mg per kg. Some species of fish, such as catfish, are associated with antimicrobial use rates per kilogram that exceed those in terrestrial animals and humans (84). On the contrary, salmons accounted for the lowest antimicrobial use, although use coefficients in commercial salmon production varied by several orders of magnitude across countries (85-88). A recent study reported seafood as the most common food source of *E. coli* containing β-lactam resistance genes (89). Moreover, aquaculture settings utilizing antimicrobials serve as reservoirs for AMR genes, providing routes for human and animal exposure to resistant bacteria (90-94). Aquaculture and the aquatic environment have been linked to mobile genetic elements carrying resistance genes of human clinical significance (91, 92, 95). As fish and seafood supply chains are highly globalized (96), it facilitates the distribution of locally generated resistance at a global scale (97, 98). A recent study demonstrated that between 2000 and 2018, AMR in bacteria from aquaculture remained stable (33%) while the resistance from wild-caught aquatic food animals decreased sharply (52% to 22%) (99). The declining resistance observed in bacteria from wild-caught aquatic animals was attributed to reduced human and livestock faecal pollution exposure. Amongst foodborne pathogens, the study observed high rates of resistance to first-line antimicrobial classes and, for *Vibrio* and *Aeromonas* spp., moderate to high rates of resistance to antimicrobial classes of last-resort reserved for treatment of multidrug resistant pathogens in humans (99).

References

1. European Centre for Disease Prevention and Control. *Assessing the health burden of infections with antibiotic-resistant bacteria in the EU/EEA, 2016-2020*. Luxembourg: Publications Office; 2022. Available from: https://data.europa.eu/doi/10.2900/73460
2. European Centre for Disease Prevention and Control. *Antimicrobial resistance (AMR) reporting protocol 2020. European Antimicrobial Resistance Surveillance Network (EARS-Net) surveillance data for 2019*. Stockholm: ECDC; 2020.
3. OECD, ECDC, EFSA, EMA. *Antimicrobial Resistance in the EU/EEA: One Health Response*. Stockholm: European Centre for Disease Prevention and Control; 2022.
4. European Medicines Agency. *Sales of veterinary antimicrobial agents in 31 European countries in 2022. Trends from 2010 to 2022. Thirteenth ESVAC report*. Luxembourg: Publications Office of the European Union, 2023.
5. European Centre for Disease Prevention and Control. *Antimicrobial consumption in the EU/EEA (ESAC-Net) - Annual Epidemiological Report 2021*. Stockholm: ECDC; 2022.
6. European Centre for Disease Prevention and Control. *Surveillance of antimicrobial resistance in Europe – Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net) 2017*. Stockholm: ECDC; 2018.

7. Cassini A, Högberg LD, Plachouras D, Quattrocchi A, Hoxha A, Simonsen GS, *et al.* Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. *Lancet Infect Dis*. 2019 Jan;19(1):56–66.
8. Rawson T, Colles F, Terry J, Bonsall M. Mechanisms of biodiversity between Campylobacter sequence types in a flock of broiler-breeder chickens. *Ecol Evol*. 2022 Mar;12(3).
9. Zhang W, Wu Y, Wen B, Zhang Y, Wang Y, Yin W, *et al.* Non-pharmaceutical interventions for COVID-19 reduced the incidence of infectious diseases: a controlled interrupted time-series study. *Infect Dis Poverty*. 2023 Mar 9;12(1):15.
10. Müller O, Razum O, Jahn A. Effects of non-pharmaceutical interventions against COVID-19 on the incidence of other diseases. *Lancet Reg Health Eur.* 2021;6:100139. doi:10.1016/j.lanepe.2021.100139
11. European Centre for Disease Prevention and Control, World Health Organization. *Antimicrobial resistance surveillance in Europe 2023: 2021 data.* Luxembourg: Publications Office; 2023. Available from: https://data.europa.eu/doi/10.2900/63495
12. Okeke IN, Edelman R. Dissemination of antibiotic-resistant bacteria across geographic borders. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 2001 Aug 1;33(3):364–9.
13. Allel K, Day L, Hamilton A, Lin L, Furuya-Kanamori L, Moore CE, *et al.* Global antimicrobial-resistance drivers: an ecological country-level study at the human–animal interface. *Lancet Planet Health*. 2023 Apr 1;7(4):e291–303.
14. Bagcchi S. AMR in bacteria from humans, animals, and food in Europe. *Lancet Microbe*. 2023 May 1;4(5):e299.
15. Scheelbeek P, Dangour A, Jarmul S, Turner G, Sietsma A, Minx J, *et al.* The effects on public health of climate change adaptation responses: a systematic review of evidence from low- and middle-income countries. *Environ Res Lett*. 2021 Jul;16(7).
16. Vieira AR, Collignon P, Aarestrup FM, McEwen SA, Hendriksen RS, Hald T, *et al.* Association between antimicrobial resistance in Escherichia coli isolates from food animals and blood stream isolates from humans in Europe: an ecological study. *Foodborne Pathog Dis*. 2011 Dec;8(12):1295–301.
17. Torres RT, Carvalho J, Fernandes J, Palmeira JD, Cunha MV, Fonseca C. Mapping the scientific knowledge of antimicrobial resistance in food-producing animals. *One Health*. 2021 Dec 1;13:100324.
18. Tang KWK, Millar BC, Moore JE. Antimicrobial Resistance (AMR). *Br J Biomed Sci.* 2023;80:11387.
19. Browne AJ, Chipeta MG, Haines-Woodhouse G, Kumaran EPA, Hamadani BHK, Zaraa S, *et al.* Global antibiotic consumption and usage in humans, 2000–18: a spatial modelling study. *Lancet Planet Health*. 2021 Dec 1;5(12):e893–904.
20. Tang KHD. Climate change in Malaysia: Trends, contributors, impacts, mitigation and adaptations. *Sci Total Environ*. 2019 Feb 11;650:1858–71.
21. Vikesland P, Garner E, Gupta S, Kang S, Maile-Moskowitz A, Zhu N. Differential Drivers of Antimicrobial Resistance across the World. *Acc Chem Res*. 2019 Apr 16;52(4):916–24.
22. Farooqi T, Irfan M, Portela R, Zhou X, Shulin P, Ali A. Global progress in climate change and biodiversity conservation research. *Glob Ecol Conserv*. 2022 Oct;38.
23. Chaw PS, Höpner J, Mikolajczyk R. The knowledge, attitude and practice of health practitioners towards antibiotic prescribing and resistance in developing countries-A systematic review. *J Clin Pharm Ther*. 2018 Oct;43(5):606–13.
24. Klein EY, Van Boeckel TP, Martinez EM, Pant S, Gandra S, Levin SA, *et al.* Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proc Natl Acad Sci* U S A. 2018 Apr 10;115(15):E3463–70.

25. Dall C. Global antibiotic use rises, fueled by economic growth. *CIDRAP News* March 26, 2018.
26. Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, *et al.* Global trends in antimicrobial use in food animals. *Proc Natl Acad Sci* U S A. 2015 May 5;112(18):5649–54.
27. Davies J, Davies D. Origins and Evolution of Antibiotic Resistance. *Microbiol Mol Biol Rev* MMBR. 2010 Sep;74(3):417–33.
28. Tillotson GS, Zinner SH. Burden of antimicrobial resistance in an era of decreasing susceptibility. *Expert Rev Anti Infect Ther*. 2017 Jul 3;15(7):663–76.
29. Samreen, Ahmad I, Malak HA, Abulreesh HH. Environmental antimicrobial resistance and its drivers: a potential threat to public health. *J Glob Antimicrob Resist*. 2021 Dec 1;27:101–11.
30. Luyt CE, Bréchot N, Trouillet JL, Chastre J. Antibiotic stewardship in the intensive care unit. *Crit Care*. 2014;18(5):480.
31. Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, *et al.* Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. *Lancet Infect Dis*. 2014 Aug;14(8):742–50.
32. Phillips I, Casewell M, Cox T, De Groot B, Friis C, Jones R, *et al.* Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *J Antimicrob Chemother*. 2004 Jan;53(1):28–52.
33. Pokharel S, Shrestha P, Adhikari B. Antimicrobial use in food animals and human health: time to implement 'One Health' approach. *Antimicrob Resist Infect Control*. 2020 Nov 7;9(1):181.
34. Ma F, Xu S, Tang Z, Li Z, Zhang L. Use of antimicrobials in food animals and impact of transmission of antimicrobial resistance on humans. *Biosaf Health*. 2021 Feb 1;3(1):32–8.
35. Lazarus B, Paterson DL, Mollinger JL, Rogers BA. Do human extraintestinal Escherichia coli infections resistant to expanded-spectrum cephalosporins originate from food-producing animals? A systematic review. *Clin Infect Dis*. 2015 Feb 1;60(3):439–52.
36. Mather AE, Reid SWJ, Maskell DJ, Parkhill J, Fookes MC, Harris SR, *et al.* Distinguishable epidemics of multidrug-resistant Salmonella Typhimurium DT104 in different hosts. *Science*. 2013 Sep 27;341(6153):1514–7.
37. Chantziaras I, Boyen F, Callens B, Dewulf J. Correlation between veterinary antimicrobial use and antimicrobial resistance in food-producing animals: a report on seven countries. *J Antimicrob Chemother*. 2014 Mar 1;69(3):827–34.
38. Woolhouse M, Ward M, van Bunnik B, Farrar J. Antimicrobial resistance in humans, livestock and the wider environment. *Philos Trans R Soc Lond B Biol Sci*. 2015 Jun 5;370(1670):20140083.
39. Meinen A, Tomczyk S, Wiegand FN, Abu Sin M, Eckmanns T, Haller S. Antimicrobial resistance in Germany and Europe - A systematic review on the increasing threat accelerated by climate change. J Health Monit. 2023;8(Suppl 3):93-108. doi:10.25646/11404
40. McGough SF, MacFadden DR, Hattab MW, Mølbak K, Santillana M. Rates of increase of antibiotic resistance and ambient temperature in Europe: a cross-national analysis of 28 countries between 2000 and 2016. *Euro Surveill*. 2020 Nov 12;25(45):1900414.
41. Forrester JD, Cao S, Schaps D, Liou R, Patil A, Stave C, *et al.* Influence of socioeconomic and environmental determinants of health on human infection and colonization with antibiotic-resistant and antibiotic-associated pathogens: a scoping review. *Surg Infect*. 2022 Apr;23(3):209–25.
42. Stekel D. First report of antimicrobial resistance pre-dates penicillin. *Nature*. 2018 Oct 10;562(7726):192–192.
43. Shambaugh Ge Jr. History of sulfonamides. *Arch Otolaryngol*. 1966 Jan 1;83(1):1–2.
44. Abraham EP, Chain E. An enzyme from bacteria able to destroy penicillin. 1940. *Rev Infect Dis.* 1988;10(4):677–8.

45. Honoré N, Cole ST. Streptomycin resistance in mycobacteria. *Antimicrob Agents Chemother*. 1994 Feb;38(2):238–42.

46. D'agata A, Monaco R. (Crossed resistance to tetracycline of various chloramphenicol-resistant Shigella strains and of various terramycin-resistant Salmonella typhosa strains). *Boll Della Soc Ital Biol Sper*. 1954 Jun;30(6):713–5.

47. Barber M. Methicillin-resistant staphylococci. *J Clin Pathol*. 1961 Jul;14(4):385–93.

48. Jevons MP. "Celbenin" - resistant Staphylococci. *Br Med J.* 1961 Jan 14;1(5219):124–5.

49. Davies J. Vicious circles: looking back on resistance plasmids. *Genetics*. 1995 Apr;139(4):1465–8.

50. Quinn JP, DiVincenzo CA, Foster J. Emergence of resistance to ceftazidime during therapy for Enterobacter cloacae infections. *J Infect Dis*. 1987 May;155(5):942–7.

51. Murray BE. Vancomycin-Resistant Enterococcal Infections. *N Engl J Med*. 2000 Mar 9;342(10):710-21.

52. Ventola CL. The Antibiotic Resistance Crisis. *Pharm Ther*. 2015 Apr;40(4):277–83.

53. Shah NS, Wright A, Bai GH, Barrera L, Boulahbal F, Martín-Casabona N, *et al.* Worldwide Emergence of Extensively Drug-resistant Tuberculosis. *Emerg Infect Dis*. 2007 Mar;13(3):380–7.

54. Sotgiu G, Ferrara G, Matteelli A, Richardson MD, Centis R, Ruesch-Gerdes S, *et al.* Epidemiology and clinical management of XDR-TB: a systematic review by TBNET. *Eur Respir J*. 2009 Apr;33(4):871–81.

55. Velayati AA, Masjedi MR, Farnia P, Tabarsi P, Ghanavi J, ZiaZarifi AH, *et al.* Emergence of new forms of totally drug-resistant tuberculosis bacilli: super extensively drug-resistant tuberculosis or totally drug-resistant strains in iran. *Chest*. 2009 Aug;136(2):420–5.

56. World Health Organization. *Global action plan on antimicrobial resistance*. Geneva: WHO; 2015

57. Kirchhelle C. Pharming animals: a global history of antibiotics in food production (1935–2017). *Palgrave Commun*. 2018 Aug 7;4(1):1–13.

58. Budd L, Bell M, Brown T. Of plagues, planes and politics: Controlling the global spread of infectious diseases by air. *Polit Geogr*. 2009 Sep;28(7):426–35.

59. Woods A. Science, disease and dairy production in Britain, c. 1927 to 1980. *Agric Hist Rev.* 2014;62(2):294–314.

60. Cozzoli D. Penicillin and the European response to post-war American hegemony: the case of Leo-penicillin. *Hist Technol*. 2014 Apr 3;30(1–2):83–103.

61. François M. L'utilisation des antibiotiques en alimentation animale. *Bull L'Académie Natl Médicine*. 1966;150:14–23.

62. Kirchhelle C. Swann song: antibiotic regulation in British livestock production (1953–2006). *Bull Hist Med*. 2018;92(2):317–50.

63. Wierup M. The Swedish experience of the 1986 year ban of antimicrobial growth promoters, with special reference to animal health, disease prevention, productivity, and usage of antimicrobials. *Microb Drug Resist Larchmt N*. 2001;7(2):183–90.

64. Casewell M, Friis C, Marco E, McMullin P, Phillips I. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *J Antimicrob Chemother*. 2003 Aug 1;52(2):159–61.

65. European Medicines Agency. *Sales of veterinary antimicrobial agents in 31 European countries in 2021. Trends from 2010 to 2021. Twelfth ESVAC report*. Luxembourg: Publications Office of the European Union; 2022

66. Sandfort M, Hans JB, Fischer MA, Reichert F, Cremanns M, Eisfeld J, *et al.* Increase in NDM-1 and NDM-1/OXA-48-producing Klebsiella pneumoniae in Germany associated with the war in Ukraine, 2022. *Euro Surveill*. 2022 Dec 15;27(50):2200926.

67. European Centre for Disease Prevention and Control. *Outbreak of carbapenemase-producing (NDM-1 and OXA-48) and colistin-resistant Klebsiella pneumoniae ST307, north-east Germany, 2019. 28 October 2019*. ECDC: Stockholm; 2019.

68. Kinross P, Gagliotti C, Merk H, Plachouras D, Monnet DL, Högberg LD, *et al.* Large increase in bloodstream infections with carbapenem-resistant *Acinetobacter* species during the first 2 years of the COVID-19 pandemic, EU/EEA, 2020 and 2021. *Euro Surveill*. 2022 Nov 17;27(46):2200845.

69. WHO. WHO publishes list of bacteria for which new antibiotics are urgently needed. WHO News release 27 February 2017.

70. Van Boeckel TP, Glennon EE, Chen D, *et al.* Reducing antimicrobial use in food animals. *Science*. 2017;357(6358):1350-1352. doi:10.1126/science.aao1495

71. Brown K, Uwiera RRE, Kalmokoff ML, Brooks SPJ, Inglis GD. Antimicrobial growth promoter use in livestock: a requirement to understand their modes of action to develop effective alternatives. *Int J Antimicrob Agents*. 2017 Jan 1;49(1):12–24.

72. Silbergeld EK, Graham J, Price LB. Industrial food animal production, antimicrobial resistance, and human health. *Annu Rev Public Health.* 2008;29:151-69. doi:10.1146/annurev.publhealth.29.020907.090904Silbergeld

73. Economou V, Gousia P. Agriculture and food animals as a source of antimicrobial-resistant bacteria. *Infect Drug Resist*. 2015;8:49–61.

74. Hou L, Cao S, Qiu Y, Xiong Y, Xiao H, Wen X, *et al.* Effects of early sub-therapeutic antibiotic administration on body tissue deposition, gut microbiota and metabolite profiles of weaned piglets. *J Sci Food Agric*. 2022;102(13):5913–24.

75. Helm ET, Curry S, Trachsel JM, Schroyen M, Gabler NK. Evaluating nursery pig responses to in-feed sub-therapeutic antibiotics. *PLoS One*. 2019 Apr 26;14(4):e0216070.

76. Low CX, Tan LTH, Ab Mutalib NS, Pusparajah P, Goh BH, Chan KG, *et al.* Unveiling the impact of antibiotics and alternative methods for animal husbandry: a review. *Antibiot Basel Switz*. 2021 May 13;10(5):578.

77. Mulchandani R, Wang Y, Gilbert M, Van Boeckel TP. Global trends in antimicrobial use in food-producing animals: 2020 to 2030. *PLOS Glob Public Health*. 2023 Feb 1;3(2):e0001305.

78. Speksnijder DC, Mevius DJ, Bruschke CJM, Wagenaar JA. Reduction of Veterinary Antimicrobial Use in the Netherlands. The Dutch Success Model. *Zoonoses Public Health*. 2015 Apr;62(s1):79–87.

79. Raasch S, Collineau L, Postma M, Backhans A, Sjölund M, Belloc C, *et al.* Effectiveness of alternative measures to reduce antimicrobial usage in pig production in four European countries. *Porc Health Manag*. 2020 Mar 2;6:6.

80. Collineau L, Rojo-Gimeno C, Léger A, Backhans A, Loesken S, Nielsen EO, *et al.* Herd-specific interventions to reduce antimicrobial usage in pig production without jeopardising technical and economic performance. *Prev Vet Med*. 2017 Sep 1; 144:167–78.

81. Pomba C, Rantala M, Greko C, Baptiste KE, Catry B, van Duijkeren E, *et al.* Public health risk of antimicrobial resistance transfer from companion animals. *J Antimicrob Chemother*. 2017 Apr 1;72(4):957–68.

82. Morris DO, Rook KA, Shofer FS, Rankin SC. Screening of Staphylococcus aureus, Staphylococcus intermedius, and Staphylococcus schleiferi isolates obtained from small companion animals for antimicrobial resistance: a retrospective review of 749 isolates (2003-04). *Vet Dermatol*. 2006 Oct;17(5):332–7.

83. Joosten P, Ceccarelli D, Odent E, Sarrazin S, Graveland H, Van Gompel L, *et al.* Antimicrobial Usage and Resistance in Companion Animals: A Cross-Sectional Study in Three European Countries. *Antibiotics*. 2020 Feb 16;9(2):87.
84. Schar D, Klein EY, Laxminarayan R, Gilbert M, Van Boeckel TP. Global trends in antimicrobial use in aquaculture. *Sci Rep*. 2020 Dec 14;10(1):21878.
85. Burridge L, Weis JS, Cabello F, Pizarro J, Bostick K. Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. *Aquaculture*. 2010 Aug 15;306(1):7–23.
86. Lillehaug A, Børnes C, Grave K. A pharmaco-epidemiological study of antibacterial treatments and bacterial diseases in Norwegian aquaculture from 2011 to 2016. *Dis Aquat Organ*. 2018 May 7;128(2):117–25.
87. Miranda CD, Godoy FA, Lee MR. Current status of the use of antibiotics and the antimicrobial resistance in the Chilean salmon farms. *Front Microbiol*. 2018 Jun 18;9:1284.
88. Love DC, Fry JP, Cabello F, Good CM, Lunestad BT. Veterinary drug use in United States net pen Salmon aquaculture: Implications for drug use policy. *Aquaculture*. 2020 Mar 15;518:734820.
89. Pearce R, Conrady B, Guardabassi L. Prevalence and types of extended-spectrum β-Lactamase-producing bacteria in retail seafood. *Foods*. 2023 Aug 12;12(16):3033.
90. Heuer OE, Kruse H, Grave K, Collignon P, Karunasagar I, Angulo FJ. Human health consequences of use of antimicrobial agents in aquaculture. *Clin Infect Dis*. 2009 Oct 15;49(8):1248–53.
91. Cabello FC, Godfrey HP, Tomova A, Ivanova L, Dölz H, Millanao A, *et al.* Antimicrobial use in aquaculture re-examined: Its relevance to antimicrobial resistance and to animal and human health. *Environ Microbiol*. 2013;15(7):1917–42.
92. Aedo S, Ivanova L, Tomova A, Cabello FC. Plasmid-related quinolone resistance determinants in epidemic vibrio parahaemolyticus, uropathogenic Escherichia coli, and marine bacteria from an aquaculture area in Chile. *Microb Ecol*. 2014 Aug 1;68(2):324–8.
93. Larsson DGJ, Andremont A, Bengtsson-Palme J, Brandt KK, de Roda Husman AM, Fagerstedt P, *et al.* Critical knowledge gaps and research needs related to the environmental dimensions of antibiotic resistance. *Environ Int*. 2018 Aug;117:132–8.
94. Lulijwa R, Rupia EJ, Alfaro AC. Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review of the top 15 major producers. *Rev Aquac*. 2020;12(2):640–63.
95. Shen Y, Lv Z, Yang L, Liu D, Ou Y, Xu C, *et al.* Integrated aquaculture contributes to the transfer of mcr-1 between animals and humans via the aquaculture supply chain. *Environ Int*. 2019 Sep;130:104708.
96. FAO. *The State of World Fisheries and Aquaculture 2020: sustainability in action*. Rome: FAO; 2020 FAO. https://doi.org/10.4060/ca9229en 2020.
97. Cabello FC, Godfrey HP, Buschmann AH, Dölz HJ. Aquaculture as yet another environmental gateway to the development and globalisation of antimicrobial resistance. *Lancet Infect Dis*. 2016 Jul 1;16(7):e127–33.
98. Thornber K, Verner-Jeffreys D, Hinchliffe S, Rahman MM, Bass D, Tyler CR. Evaluating antimicrobial resistance in the global shrimp industry. *Rev Aquac*. 2020;12(2):966–86.
99. Schar D, Zhao C, Wang Y, Larsson DGJ, Gilbert M, Van Boeckel TP. Twenty-year trends in antimicrobial resistance from aquaculture and fisheries in Asia. *Nat Commun*. 2021 Sep 10;12(1):5384.

CHIKUNGUNYA

Claudia Cataldo (a), Elena Arsevska (b), Maria Bellenghi (a), Francesca Dagostin (c), Marco Di Luca (d), Flavia Riccardo (d), Kyla Serres (e), William Wint (f), Luca Busani (a)
(a) Centro di Riferimento per la Medicina di Genere, Istituto Superiore di Sanità, Rome
(b) UMR Animals, Health, Territories, Risks, and Ecosystems (Astre), Department of Biological Systems (Bios), French Agricultural Research and International Cooperation Organization for Development (CIRAD), Campus International de Baillarguet, Montpellier
(c) Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige, Trento
(d) Dipartimento Malattie Infettive, Istituto Superiore di Sanità, Rome
(e) Spatial Epidemiology Lab, Université Libre de Bruxelles, Brussels,
(f) Department of Biology, Environmental Research Group Oxford Ltd, Oxford

Biological, ecological and molecular features of the causative agent

Disease name

Chikungunya (CHIK).

Disease agent

Common scientific and Latin name

Chikungunya virus (CHIKV) is the etiological agent of chikungunya fever (CHIKF), an arthropod-borne disease transmitted mainly by mosquito species of *Aedes* genus, (*Ae. furcifer-taylori*, *Ae. africanus*, *Ae. luteocephalus*, and *Ae. neoafricanus,* enzootic vectors in African savannah and forest cycles, *Ae. aegypti and Ae. albopictus,* vectors in urban areas). The name 'chikungunya' derives from a word in the Kimakonde language, meaning "to become contorted" and refers to the contorted posture of infected patients suffering from severe joint pain (1).

Taxonomy

CHIKV is an RNA virus that belongs to the Semliki Forest antigenic group of the genus *Alphaviridae* (Family *Togaviridae)*, which includes other arthritogenic alphaviruses (as o'nyong-nyong, Ross River, Barmah Forest, and Mayaro viruses) (2). Phylogenetic analyses reveal three genotypes of CHIKV: West African genotype, East/Central/ South African (ECSA) genotype including the Indian Ocean Lineage (IOL), and the Asian genotype (3).

Disease agent characteristics

CHIKV genome consists of a single 11.8-kbp strand of positive sense RNA, which encodes a 2472 amino acid non-structural and a 1244 amino acid structural polyprotein (4). The polyproteins give rise to the four non-structural proteins (nsP1-4) that make up the viral replication machine, and five structural proteins. Each spherical viral particle is approximately 70 nm in diameter and is comprised of a strand of genomic RNA, encapsulated by capsid (C) proteins, surrounded by a host cell–derived lipid bilayer spiked with heterodimers of envelope proteins E1 and E2 (5). The

other two structural proteins, 6K and E3, are leader peptides for E1 and E2, respectively, and are not observed in abundance in the mature virion (5).

Physiochemical properties

CHIKV is sensitive to increasing temperatures and can be safely and quickly inactivated when treated at temperatures above 70°C for at least 1 minute. Furthermore, a superior virucidal effect of propanol-based disinfectants over ethanol-based solutions has been demonstrated (6). The efficacy of amotosalen and ultraviolet A light treatment, inactivates high levels of CHIKV has been demonstrated with a slog reduction factors LRF >5.0 log in both plasma and in platelet components. Additionally, other alphaviruses predicted to be important emerging agents have been successfully inactivated by amotosalen/UVA (7).

Priority level for EU

CHIKV is currently not endemic in the EU and the majority of the cases are travellers infected during international travel. However, two large outbreaks were reported in Italy in 2007 and 2017, and some autochthonous cases were reported in France in 2010, 2014 and 2017 (source ECDC, European Centre for Disease Prevention and Control). Local transmission in the EU is linked to importation of virus by infected travellers to areas with established presence of competent vectors (*Aedes albopictus* present in many countries in mainland Europe and *Aedes aegypti* in Madeira, Canary Islands, Cyprus and countries around the Black Sea) when environmental conditions favour vector activity. Considering the frequency of travellers between high incidence areas in the world and the European Union and the past experiences in Italy and France, there is a risk of future CHIKF outbreaks in continental Europe. This is why, this disease is prioritized in EU surveillance and the spread of *Ae. albopictus* and other invasive *Aedes* species is monitored in the EU/EEA (European Union/European Economic Area).

Distribution of the pathogen

CHIKV has been sporadically detected in Africa and Asia and, since 2004, has extended its geographic range causing outbreaks in the Indian Ocean, south-eastern Asia, Europe and the Americas. This global expansion has been possible because CHIKV established a transmission cycle in urban settings using anthropophilic vectors such as *Ae. albopictus* and *Ae. aegypti.*

To date, *Ae. albopictus* has been established as the main CHIKV vector of transmission in Europe. Nevertheless, *Ae. aegypti,* which was eradicated in Europe since the 1950s, has been detected again around the Black Sea in Southern Russia, Abkhazia, and Georgia in 2004 and north-eastern Turkey in 2015 (8) and more recently in Cyprus and the Canary Islands (Ref). In addition, other invasive mosquitoes, *Ae. koreicus* and *Ae. japonicus*, have been introduced and established in several European countries, *Ae. koreicus* is also able to experimentally transmit CHIKV (9). CHIKV outbreaks in Southeast Asia occurred in larger cities, where *Ae. aegypti* mosquitoes were implicated as the primary transmission vector (10).

The presence of competent vectors is the prerequisite for a possible transmission of CHIKV in a given area. When the environmental conditions are favourable, in areas where *Ae. albopictus* is established, viraemic travel-related cases may trigger local transmission of the virus as

demonstrated by the sporadic events of CHIKV transmission since 2007. No events of autochthonous transmission were reported at European level from 2017 to date (February 2023).

Table 1 describes the distribution of *Ae. albopictus* is for the years 2017-2022 and in Figure 1 the map of *Ae. albopictus* distribution, as of March 2024, is reported.

Table 1. 5 years monitoring of presence and abundance of *Ae. albopictus* in Europe

Country	2017	2018	2019	2020	2021	2022	CHIKV outbreak
Belgium	A	A	A	I	I	I	
Central France	I	I	I	E	E	E	
Central Spain	ND	ND	ND	I	I	E	
Italy	E	E	E	E	E	E	Emilia-Romagna 2007 Lazio and Calabria 2017
Netherland	I	I	I	I	I	I	
North France	A	A	A	A	A	A	
North Germany	A	A	A	A	A	A	
North Spain	ND	ND	ND	A**	A**	A**	
Portugal	A	A	A	A**	A***	A***	
South France	E	E*	E*	E	E	E	Montpellier 2014 Le Cannet-des-Maures 2017
South Germany	I	I	I	I	I****	I****	
Spain Mediterranean Coast	E	E	E	E	E	E	
UK	A	A	A	A	A	A	

A=Absent; ND=No Data; I=Introduced; E=Established; *in expansion; **some plot in the region of Galizia; ***some plot of established *Ae. albopictus* in the south and north of Portugal; ****some plot of established *Ae. albopictus*.

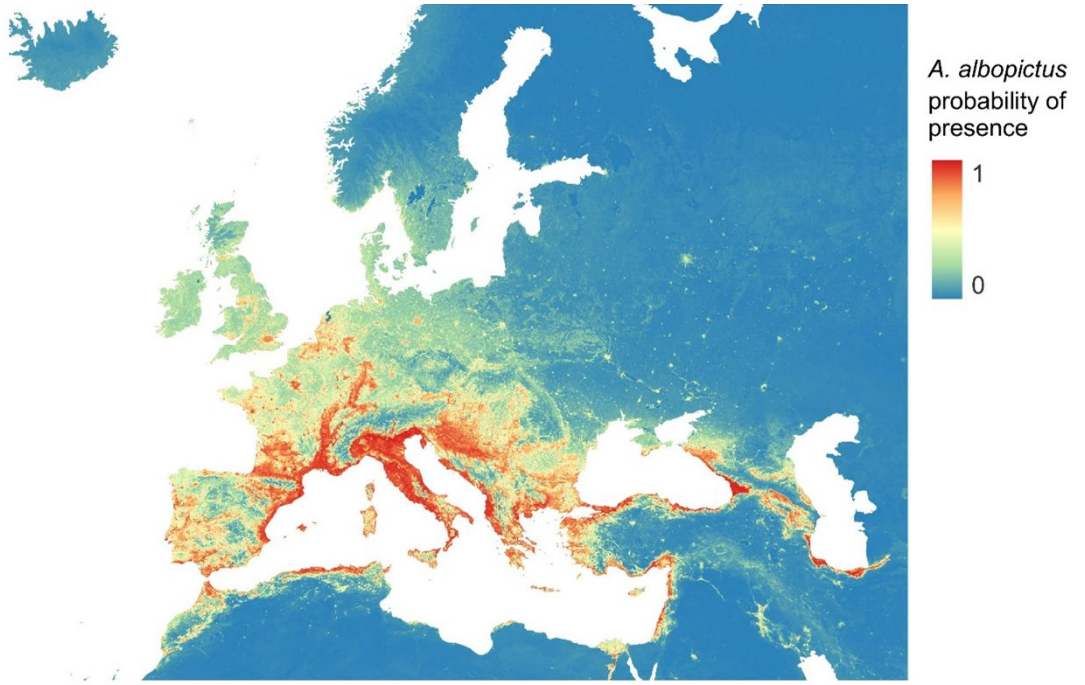

Figure 1. Current 1-km probability of presence of *Ae. albopictus* across Europe, produced using random forest and boosted regression trees analyses (source: updated by ERGO for E4Warning Project)

Ecology and transmission routes

CHIK is a climate sensitive disease, however its transmission is also affected by deforestation, population migration, disorderly occupation of urban areas, and precarious sanitary conditions that favour the amplification of both the virus and the mosquito vectors, and the transmission of the infection (11, 12).

Among factors that probably facilitated recent CHIKV emergence include:

1) availability of immunologically naïve human populations in vast geographic areas;
2) CHIKV reliance on peridomestic and anthropophilic mosquitoes as vectors;
3) increase in international travel,
4) genetic adaptation of CHIKV to a new mosquito vector, *Ae. albopictus*.

CHIKV transmission by *Ae. albopictus* was consistently associated with an aminoacid substitution in the E1 protein (E1-A226V) (13-15). Recently, effective transmission of strains without the A226V mutation was demonstrated in Italy, where *Ae. albopictus* was the implicated vector (16).

Drivers of the disease emergence and spread

Ecological drivers

The influence of climate change and variability on infectious disease spread and emergence should, however, not be viewed in isolation as it is expected to interact with other drivers, such as urbanization, land use, and human mobility.

Particularly, the unprecedented increase in human mobility, both at the local and global scales, is considered a major driver for the expansion of dengue and CHIK (17).

Non-human primates are believed to be the primary CHIKV reservoir hosts, and the 5–10-year periodicity of virus activity in a given locality is hypothesized to depend on oscillations in monkey herd immunity. In addition, the virus and/or neutralizing antibodies were detected also in birds, rodents and other small mammals, but their role as reservoir is questionable.

In Table 2 and in Table 3 the most important environmental and vector drivers, which are thought to impact on the spread of CHIKV are reported.

Table 2. List of individual CHIK environmental covariates reported in selected studies, in descending order of importance according to the number of references

Environmental drivers	n. of papers# (n. 3)	% of impact* (n. 9)
Temperature (incubation in experimental condition; average daily temperature)	4	44.4%
Time (season during year)	4	44.4%
Water (rainwater storage tanks)	1	11.1%

number of papers extracted, the number of references per covariates is higher because more covariates may have been extracted from a document;
*% calculated on the total number of references.

Table 3. List of CHIK vector covariates included in the selected studies in decreasing order of importance according to the number of references

Vector drivers	n. of papers# (n. 12)	% of impact* (n. 33)
Transmission (transmission/infection rate; viral titre)	14	42.4%
Abundance (mosquito density; n. of eggs/ovitrap)	10	30.3%
Activity (transmission rate; biting rate)	6	18.2%
Ecology (n. of breeding sites)	3	9%

number of papers extracted, the number of references per covariates is higher because more covariates may have been extracted from a document; * % calculated on the total number of references

Natural history of disease in humans and animals, including symptoms, morbidity and mortality

Brief history of the pathogen and disease

The earliest report of CHIKF described an outbreak of a dengue-like illness that occurred in 1952 to 1953 in the present region of Tanzania, where an estimated 60% to 80% of the population in this region developed symptoms (1).

Between the 1960s and 1990s, incidental human infection led to numerous, small-scale CHIKV outbreaks in countries throughout Central and Southern Africa, and Senegal, Guinea, and Nigeria in Western Africa (18). In 2004, a large-scale CHIKV epidemic erupted, sweeping down the coast of Kenya into islands on the Indian Ocean (Comoros, Mayotte, Seychelles, Re´ union, Madagascar, Sri Lanka, and the Maldives), India, Southeast Asia (Malaysia, Singapore, Thailand), and China (19). Although CHIKV infection in travellers returning to Europe had been reported previously, autochthonous transmission of CHIKV was observed for the first time in Italy in 2007 (20), and in France in 2009 (21). In December 2013, the first cases of locally transmitted CHIKV in the Americas were confirmed in St. Martin, followed rapidly by cases identified throughout the Caribbean and Latin America (22). Currently, CHIKV infection has been reported in different countries on all continents, except Antarctica (23). In some regions, especially in South America, the co-circulation of CHIKV with other arboviruses, such as DENV, ZIKV, Mayaro (MAYV) and yellow fever (YFV), requires rigorous epidemiological surveillance and differential diagnosis strategies (24).

Disease in humans

Following transmission, CHIKV replicates in the skin and then disseminates to the liver and joints, presumably through the blood (25). The incubation period is 2–4 days and is followed by a sudden onset of clinical disease. Symptoms of CHIKV infection include high fever, rigors, headache, photophobia and a petechial rash or maculopapular rash. In addition, most infected individuals complain of severe joint pain that is often incapacitating (26). Patients often report some stiffness in the distal joints, such as the interphalangeal joints of the hands and feet, ankles, and wrists, particularly upon awakening (27). Myalgia is more frequent in the arms, forearms, thighs, and calves and may compromise the daily activities of patients, particularly when associated with polyarthralgia/polyarthritis. The acute phase of CHIKV infection is usually self-limited and clinical manifestation lasts from a few days to a couple of weeks. The post-acute phase begins after the 21st day of clinical manifestations and continues for three months (28).

Only a small proportion of patients remain completely asymptomatic after two-three weeks following the onset of disease. Generally, most patients exhibit only transitory improvements in their clinical condition and relapses occur after a brief "healing" period. Moreover, persistent polyarthralgia or polyarthritis without any change in intensity has been reported by a considerable percentage of patients, which requires analgesic or anti-inflammatory medication to alleviate the pain. During the post-acute phase, the decompensation of pre-existing traumatic or degenerative arthropathies, such as osteoarthritis or tendinitis, occasionally calcified, may occur. Additionally, local manifestations, such as reactional oedema and nerve compression syndromes, particularly of the ulnar, medial, and tibial nerves, which produce cubital, carpal, and tarsal tunnel syndromes, respectively, have also been observed. Morning joint stiffness, neuropathic pain, and peripheral vascular phenomena, such as Raynaud syndrome, have also been described (28). Notably, during this phase, a set of non-specific clinical manifestations that are not always associated with CHIK usually occurs, which may be overlooked by health professionals. The most frequently reported manifestations are chronic fatigue, changes in skin colour (hypo- or hyperchromia), alopecia, decompensated endocrine and metabolic diseases, and decompensation of other pre-existing chronic diseases, such as systemic arterial hypertension, depression, and anxiety (28).

In contrast to the acute phase, the chronic phase of disease has not been extensively investigated. Recurrent joint pain, which can last for years in some cases, is experienced by 30–40% of those infected, although this is not thought to be a result of chronic infection, as infectious virus cannot be isolated from these patients. Radiographic studies are typically normal or show mild swelling, which is consistent with joint pain. It has been suggested that this joint pain is immune mediated. This has not been formally shown, although the presence of autoantibodies has been reported in one case of CHIKV infection with severe musculoskeletal complications (29). Other types of musculoskeletal manifestations may characterise the chronic phase, with the most frequent being tenosynovitis. Typically, two or more tendons are affected, the most common of which are the wrist, finger, and ankle extensors and flexors. Many patients with hypertrophic wrist tenosynovitis complain of nocturnal paraesthesia in the fingers.

During the more recent CHIKV outbreaks, total or partial alopecia on the head or body, predominately in female patients, and ophthalmological alterations, such as uveitis and retinitis, were described during the chronic phase of infection (30).

In new-borns, congenital infections may be accompanied by varying clinical signs, such as fever, lack of appetite, apnoea, skin manifestations, distal and cerebral oedema, encephalitis and haemorrhage (31) (32). Bullous lesions associated to CHIKV infection have also been reported in four-month-old babies, who had 20% of their body surface affected on the second day after the onset of fever (25). Deaths from CHIKV infection is a rare event, but some studies conducted in Brasil described an increased risk of mortality after infection, probably due to neurological affections, mainly in neonates, immunocompromised and elderly (32, 33).

Availability of preventive, therapeutic and control measures, including licensed or pipelines vaccines

Therapy in humans

There is presently no licensed targeted therapy for acute CHIKV infection. Treatment is primarily supportive care and includes the use of analgesic and anti-inflammatory medication, rehydration, and rest (34). Going more in depth the most common symptoms, fever and joint pain can be alleviated with the use of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) (35).

Licensed or pipelined vaccines

In November 2023 the Food and Drugs Administration (United States) approved the first CHIKV vaccine for individuals 18 years of age and older. It contains a live, weakened version of the virus (https://www.fda.gov/news-events/press-announcements/fda-approves-first-vaccine-prevent-disease-caused-chikungunya-virus). Other anti-CHIKV candidates that have been already tested in humans and/or animals include inactivated vaccines, attenuated vaccines, Virus Like Particle (VLP) vaccines, nucleic acid vaccines and chimeric vaccines (36).

Other prevention measures

Apart from the use of the recently approved vaccine, in the absence of therapeutic strategies, efficient vector control plays a crucial role in CHIKV prevention (37). Integrated anti-virus control is required and should include: a) epidemiological surveillance; b) environmental management focusing on educative actions to eliminate potential mosquito breeding sites and reduce standing water sites; c) chemical control using repellents (mainly for travellers and pregnant women) and insecticides, monitoring the vectors resistance; and d) biological control of eggs, *larvae* and mosquitoes (38) (24) (39) (40). In certain countries such as Germany, Italy, and Spain, tools or apps have been provided to citizens for reporting mosquito presence and biting incidents.

Transmission of CHIKV through transfusion and transplantation has not been reported, but preventive blood safety measures should be considered in case of donors from areas with ongoing viral circulation. Virus prevalence in blood donors ranged from 0 to 2% in areas of reported virus circulation (41, 42).

Disease specific recommendations

Individuals living in or travelling to endemic regions should consider the vaccination to prevent the disease. In addition, protective measures should always be taken against mosquito bites, especially during the day when mosquitoes are active, and for those groups, such as pregnant women and immunocompromised persons, for whom vaccination should be carefully considered (43).

Epidemiological situation at different spatial scales: past and current trends

The epidemiology of CHIK in Europe is characterized by relatively small outbreaks involving local transmission following the introduction of an imported index case. The first European outbreak wa436s reported in Northeastern Italy from June to September 2007. Four other reported outbreaks involving local transmission of CHIK by *Ae. albopictus* have been reported in Europe. Other outbreaks occurred in Italy in 2017 and three in France in 2010, 2014 and 2017, involving from two indigenous cases in France in 2010, up to 436 cases in Italy in 2017. From 2018 to 2020, 770 cases were notified to the ECDC, and 89% were in people who were exposed abroad. The number of cases notified does not present a clear trend, with great variability across countries and years (Figure 2). Considering the period from 2012 to 2020, the trend of CHIK in Europe was characterized by great variability in the number of cases and countries involved, with the highest peak in 2014, due to the high number of cases (788) notified in France, 98% of them travel-associated. United Kingdom, Spain and Germany are the other countries with high number of

cases, in the considered period, mostly travel-associated. Most of the cases reported in 2017 were from Italy, where an autochthonous outbreak occurred (Figure 3). The spatiotemporal dynamics of CHIK outbreaks remain unpredictable in Europe, with most of the cases imported from endemic areas by travellers, and some outbreaks in areas with high vector density.

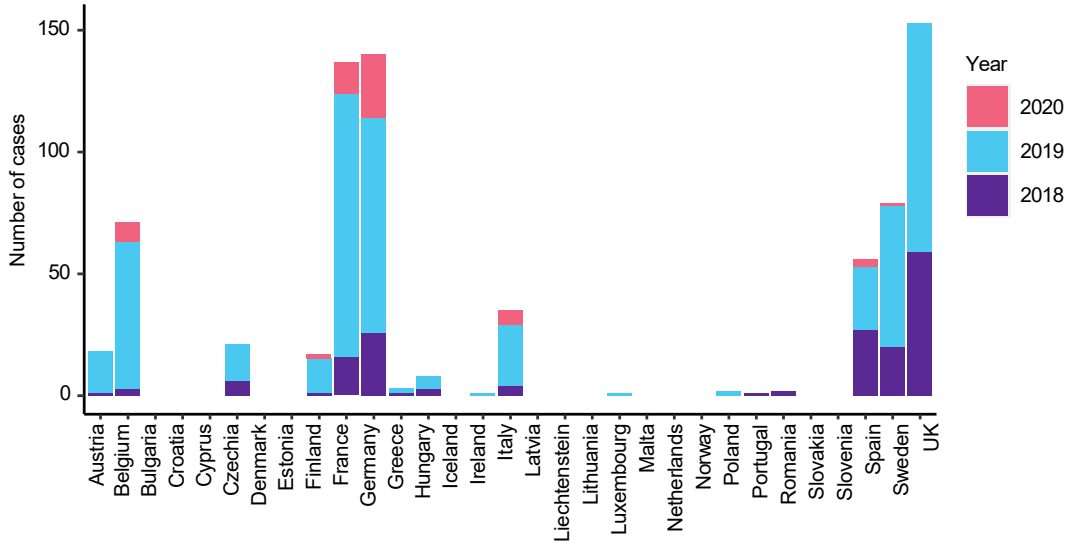

Figure 2. Number of reported cases of CHIKV infection in countries of the European Union and European Economic Area, 2018–2020 (85% travel-associated)
(data from http://atlas.ecdc.europa.eu/public/index.aspx)

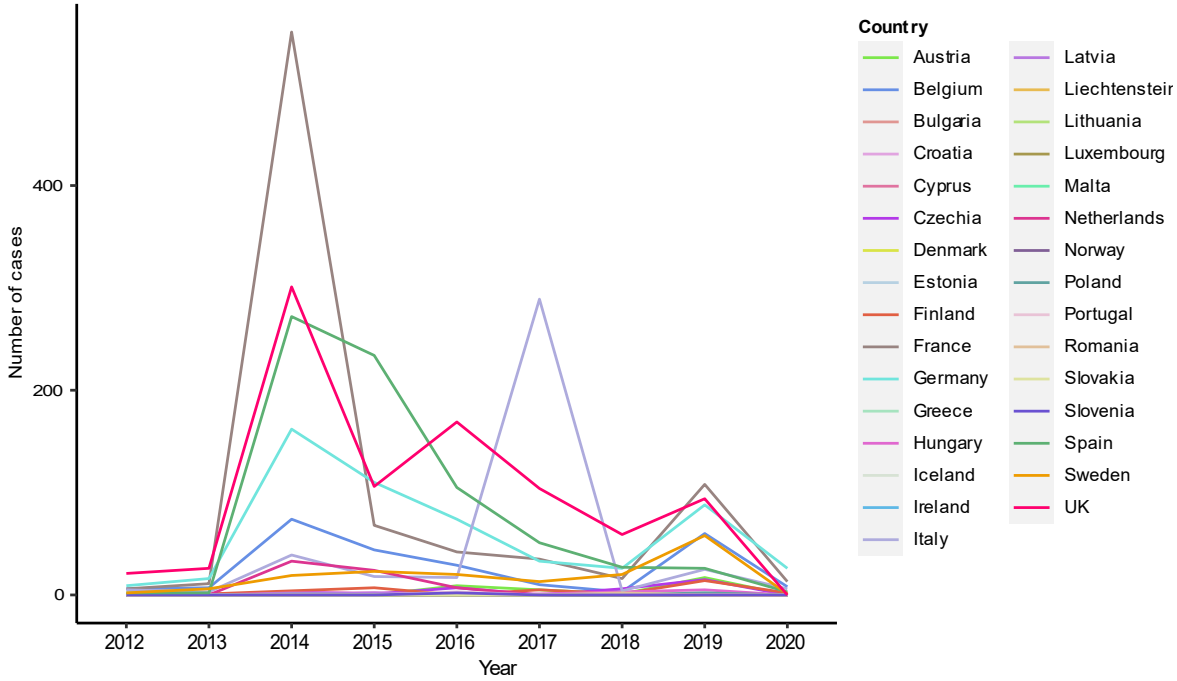

Figure 3. Number of reported cases of CHIK in countries of the European Union and European Economic Area, 2012-2021 (85% travel-associated)
(data from http://atlas.ecdc.europa.eu/public/index.aspx)

Sociological and demographical dimension affecting susceptibility and exposure, including gender

The occurrence of CHIKV infections, at EU level, arise for several factors. In temperate climate countries, the risk of infection increases in people, due to social and behavioural factors. Globalisation of travel through highly viraemic tourists travelling from endemic countries to non-endemic areas, in combination to risky behaviours that favour exposure to *Ae. albopictus* bites, such as staying outdoors during daytime and scarce adoption of protective behaviours (i.e., use of insect repellents) increase the risk of infection. The highest rates of infection occurred in the oldest, particularly in males (44, 45). Table 4 shows the most important human covariates related to the CHIV infections, and Figure 4 shows the notification rate of CHIKV infection by gender and age group.

Table 4. List of CHIK human covariates reported in the selected papers in decreasing order of importance according to the number of references

Human drivers		n. of papers#(n. 14)	% of impact*(n. 44)
Infection	(cumulative attack rate; average number of symptomatic day; n. of notified cases; CHIKV/antibodies detection)	27	61.36%
Gender	(rates/gender; risk factor/gender; n. of cases/gender)	8	18.2%
Age	(rates/age)	7	15.9%
Economic condition		1	2.27%
Human behaviour	(use of repellents, work or recreational activity)	1	2.27%

number of papers extracted, the number of references per covariates is higher because more covariates may have been extracted from a document; *% calculated on the total number of references

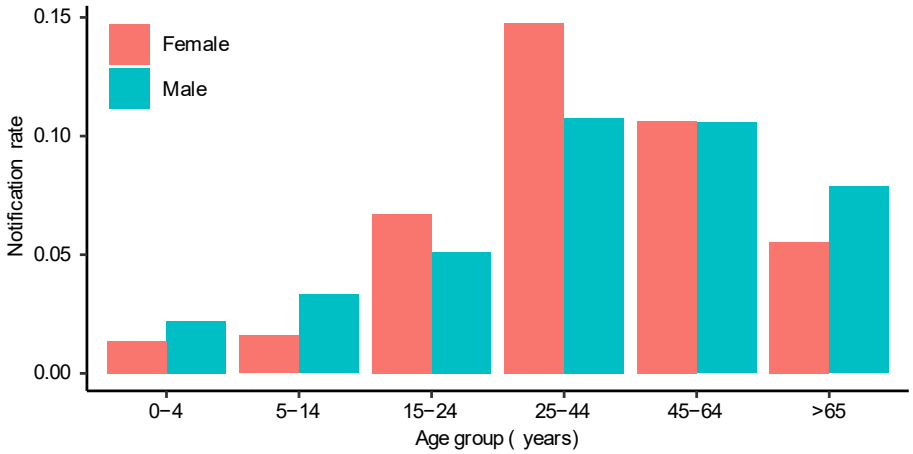

Figure 4. CHIKV notification rate by gender and age group in the European Union and European Economic Area, 2012-2021 (85% travel-associated) (data from The European Surveillance System (TESSy-ECDC)

Diagnostic procedures and notification systems used at local, national and European scale

Diagnosis

Individuals infected by arboviruses can present a wide range of similar clinical manifestations, such as rash, myalgia, exanthema, arthralgia, joint pain, headache, lymph node hypertrophy, neurological impairment and fever especially when Zika Virus (ZIKV) and Dengue Virus (DENV) are co-circulating in the same geographical region (46, 47).

In this context, variations in the clinical presentation of cases can give hints as to the viral aetiology; for instance, the salient and prolonged polyarthralgia, often accompanied by rash, is typically more indicative of CHIK, while haemorrhagic manifestations and myalgia are more commonly observed in DENV infections (48).

Since the variety and intensity of symptoms associated to CHIKV, DENV and ZIKV infections are so similar, laboratory analysis is necessary to confirm the respective viral aetiology as laboratory tests for specific diagnosis of CHIKV infection are based on virus isolation, viral RNA detection and serology (49). Molecular methods of CHIKV diagnosis – such as Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR), Reverse Transcriptase Loop Mediated Isothermal Amplification (RT-LAMP), quantitative RT-PCR (qRT-PCR) – have gained increasing importance. They are more sensitive and faster than viral isolation, and permit RNA detection from all CHIKV lineages with high specificity. Usually, serum samples collected up to seven days of symptom-onset are suitable for CHIKV detection by molecular diagnostic platforms (50, 51).

In later phases of infection, CHIKV detection is usually based on serological methods, such as ELISA (Enzyme-Linked Immunosorbent Assay) techniques which are useful to distinguish between acute or convalescent infections via detection of anti-CHIKV IgM or IgG antibodies. IgM can be detected from two/four days up to three months after the onset of illness, while IgG can be detected for several years (52).

Infrastructure capacity to identify pathogens for each Member State

CHIK is among the communicable diseases that according to the Commission Implementing Decision (EU) 2018/945 are covered by epidemiological surveillance. It means that EU Member States are required to establish national capacity of detection and reporting of human cases. The decision provides a case definition and laboratory criteria:

1. *Probable case*
    a. Detection of CHIK specific IgM antibodies in a single serum sample.
2. *Confirmed case* at least one of the following four*:*
    a. Isolation of CHIKV from a clinical specimen;
    b. Detection of CHIK viral nucleic acid from a clinical specimen;
    c. Detection of CHIK specific IgM antibodies in a single serum sample and confirmation and by neutralization;
    d. Seroconversion or four-fold antibody titre increase of CHIK specific antibodies in paired serum samples.

Diagnosis is routinely made by clinical microbiology laboratories, and there is no European-wide reference laboratory network or national laboratories in most EU countries.

Estimated influence of environmental change on the disease future trends

The current risk of CHIKV transmission in Europe is not primarily restricted by temperature, which allows extrinsic incubation of the virus, but rather by the distribution and density of competent vectors.

Some risk scenarios for Europe were developed, with highly suitable areas more widespread than previously assumed. Coastal areas of the Mediterranean Sea, in the western part of the Iberian Peninsula, and in Atlantic coastal areas of France are those at highest risk, but under a worst-case scenario, even large areas of western Germany and the Benelux states are considered potential areas of transmission (53). However, some parts of the regions of highest current suitability, eg. northern Italy near the Adriatic coast, are projected to experience a decline in suitability due to increased probabilities of summer droughts, which will reduce the habitat suitability for the vectors (54).

References

1. Robinson MC. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952-53. I. Clinical features. *Trans R Soc Trop Med Hyg* 1955;49:28-32.
2. Lwande OW, Obanda V, Bucht G, *et al.* Global emergence of Alphaviruses that cause arthritis in humans. *Infect Ecol Epidemiol* 2015;5:29853.
3. Weaver SC, Forrester NL. Chikungunya: evolutionary history and recent epidemic spread. *Antiviral Res* 2015;120:32-9.
4. Khan AH, Morita K, Parquet M del C, *et al.* Complete nucleotide sequence of chikungunya virus and evidence for an internal polyadenylation site. *J Gen Virol* 2002;83:3075-84.
5. Jose J, Snyder JE, Kuhn RJ. A structural and functional perspective of alphavirus replication and assembly. *Future Microbiol* 2009;4:837-56.
6. Franz S, Friesland M, Passos V, *et al.* Susceptibility of Chikungunya Virus to inactivation by heat and commercially and World Health Organization-recommended biocides. *J Infect Dis* 2018; 218: 1507–1510.
7. Lanteri MC, Santa-Maria F, Laughhunn A, *et al.* Inactivation of a broad spectrum of viruses and parasites by photochemical treatment of plasma and platelets using amotosalen and ultraviolet A light. *Transfusion* 2020; 60: 1319–1331.
8. Akiner MM, Demirci B, Babuadze G, *et al.* Spread of the invasive mosquitoes Aedes aegypti and Aedes albopictus in the Black Sea Region increases risk of Chikungunya, Dengue, and Zika outbreaks in Europe. *PLoS Negl Trop Dis* 2016; 10: e0004664.
9. Ciocchetta S, Prow NA, Darbro JM, *et al.* The new European invader *Aedes* (*Finlaya*) *koreicus*: a potential vector of chikungunya virus. *Pathog Glob Health* 2018; 112:107–114.
10. Vu DM, Jungkind D, Angelle Desiree LaBeaud null. Chikungunya Virus. *Clin Lab Med* 2017;37:371–382.
11. Jourdain F, Roiz D, Valk H de, *et al.* From importation to autochthonous transmission: drivers of chikungunya and dengue emergence in a temperate area. *PLoS Negl Trop Dis.* 2020;14(5).

12. Saager ES, Iwamura T, Jucker T, Murray KA. Deforestation for oil palm increases microclimate suitability for the development of the disease vector Aedes albopictus. *Sci Rep* 2023; 13: 9514.
13. Tsetsarkin KA, Vanlandingham DL, McGee CE, *et al.* A single mutation in Chikungunya virus affects vector specificity and epidemic potential. *PLoS Pathog* 2007; 3: 1895–1906.
14. De Lamballerie X, Leroy E, Charrel RN, *et al.* Chikungunya virus adapts to tiger mosquito via evolutionary convergence: a sign of things to come? *Virol J* 2008;5:33.
15. Schuffenecker I, Iteman I, Michault A, *et al.* Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. *PLoS Med* 2006; 3: e263.
16. Fortuna C, Toma L, Remoli ME, *et al.* Vector competence of Aedes albopictus for the Indian Ocean lineage (IOL) chikungunya viruses of the 2007 and 2017 outbreaks in Italy: a comparison between strains with and without the E1:A226V mutation. *Euro Surveill.* 2018;23(22):1800246. doi:10.2807/1560-7917.ES.2018.23.22.1800246.
17. Wilder-Smith A, Gubler DJ. Geographic expansion of dengue: the impact of international travel. *Med Clin North Am* 2008;92:1377-90, x.
18. Powers AM, Logue CH. Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus. *J Gen Virol* 2007;88:2363-77.
19. Kariuki Njenga M, Nderitu L, Ledermann JP, *et al.* Tracking epidemic Chikungunya virus into the Indian Ocean from East Africa. *J Gen Virol* 2008;89:2754-60.
20. Rezza G, Nicoletti L, Angelini R, *et al.* Infection with chikungunya virus in Italy: an outbreak in a temperate region. *The Lancet* 2007;370:1840-6.
21. Grandadam M, Caro V, Plumet S, *et al.* Chikungunya Virus, Southeastern France. *Emerg Infect Dis* 2011;17(5):910-3. doi:10.3201/eid1705.101873.
22. Fischer M, Staples JE, Arboviral Diseases Branch, National Center for Emerging and Zoonotic Infectious Diseases, CDC. Notes from the field: chikungunya virus spreads in the Americas - Caribbean and South America, 2013-2014. *MMWR Morb Mortal Wkly Rep* 2014; 63: 500–501.
23. CDC. *Areas at Risk for Chikungunya*. Atlanta, GA: Centers for Disease Control and Prevention; 2024.
24. Benelli G. Managing mosquitoes and ticks in a rapidly changing world – Facts and trends. *Saudi J Biol Sci* 2019; 26: 921–929.
25. Robin S, Ramful D, Zettor J, *et al.* Severe bullous skin lesions associated with Chikungunya virus infection in small infants. *Eur J Pediatr* 2010; 169: 67–72.
26. Mourya D, Mishra A. Chikungunya fever. *The Lancet* 2006; 368: 186–187.
27. Borgherini G, Poubeau P, Staikowsky F, *et al.* Outbreak of chikungunya on Reunion Island: early clinical and laboratory features in 157 adult patients. *Clin Infect Dis* 2007; 44: 1401–1407.
28. Simon F, Javelle E, Cabie A, *et al.* French guidelines for the management of chikungunya (acute and persistent presentations). November 2014. *Med Mal Infect* 2015; 45: 243–263.
29. Maek-A-Nantawat W, Silachamroon U. Presence of autoimmune antibody in chikungunya infection. *Case Rep Med* 2009;2009:840183.
30. Cunha RV da, Trinta KS. Chikungunya virus: clinical aspects and treatment - A Review. *Mem Inst Oswaldo Cruz* 2017;112:523-31.
31. Lyra PPR, Campos GS, Bandeira ID, *et al.* Congenital Chikungunya Virus Infection after an Outbreak in Salvador, Bahia, Brazil. *AJP Rep* 2016;6:e299-300.
32. Bandeira AC, Campos GS, Sardi SI, *et al.* Neonatal encephalitis due to Chikungunya vertical transmission: First report in Brazil. *IDCases* 2016;5:57-9.

33. Cerqueira-Silva T, Pescarini JM, Cardim LL, *et al.* Risk of death following chikungunya virus disease in the 100 Million Brazilian Cohort, 2015–18: a matched cohort study and self-controlled case series. *The Lancet Infect Dis.* 2024; May;24(5):504-513. doi: 10.1016/S1473-3099(23)00739-9.
34. Kaur P, Chu JJH. Chikungunya virus: an update on antiviral development and challenges. *Drug Discov Today* 2013;18:969-83.
35. Millsapps EM, Underwood EC, and Barr KL Development and application of treatment for Chikungunya fever. *Res Rep Trop Med*. 2022;13:55-66. doi: 10.2147/RRTM.S370046.
36. Gao S, Song S, Zhang L. Recent progress in vaccine development against Chikungunya Virus. *Front Microbiol* 2019;10:2881.
37. Huang Y-J, Higgs S, Vanlandingham D. Biological control strategies for mosquito vectors of arboviruses. *Insects* 2017;8:21.
38. Hemingway J, Ranson H. Insecticide resistance in insect vectors of human disease. *Annu Rev Entomol* 2000;45:371-91.
39. Benelli G. Plant-borne ovicides in the fight against mosquito vectors of medical and veterinary importance: a systematic review. *Parasitol Res* 2015;114:3201-12.
40. Islam J, Zaman K, Duarah S, *et al.* Mosquito repellents: An insight into the chronological perspectives and novel discoveries. *Acta Trop* 2017;167:216–230.
41. Giménez-Richarte Á, Salazar MO de, Arbona C, Giménez-Richarte MP, Collado M, Fernández PL *et al.* Prevalence of Chikungunya, Dengue and Zika viruses in blood donors: a systematic literature review and meta-analysis. *Blood Transfusion* 2022; 267–280.
42. Venturi G, Fabiani M, Amendola A, Marsili G, Benedetti E, Fiorentini C *et al.* Lack of Evidence of chikungunya virus infection among blood donors during the chikungunya outbreak in Lazio Region, Italy, 2017. *Viruses* 2022;14:619.
43. European Centre for Disease Prevention and Control. *Information for travellers to areas with chikungunya transmission*. Stockholm: ECDC. Available form: https://www.ecdc.europa.eu/en/chikungunya/threats-and-outbreaks/information-travellers; last visited 21 April 2022.
44. Rezza G, Nicoletti L, Angelini R, *et al.* Infection with chikungunya virus in Italy: an outbreak in a temperate region. *The Lancet* 2007;370:1840-6.
45. Moro ML, Rezza G, Grilli E, *et al.* Chikungunya virus in North-Eastern Italy: a seroprevalence survey. *Am J Trop Med Hyg* 2010;82:508-11.
46. Brito CAA de, Cordeiro MT. One year after the Zika virus outbreak in Brazil: from hypotheses to evidence. *Rev Soc Bras Med Trop* 2016;49: 537-43.
47. Hua C, Combe B. Chikungunya virus-associated disease. *Curr Rheumatol Rep* 2017;19:69.
48. Lee VJ, Chow A, Zheng X, *et al.* Simple clinical and laboratory predictors of chikungunya versus dengue infections in adults. *PLoS Negl Trop Dis* 2012 6:e1786.
49. Johnson BW, Russell BJ, Goodman CH. Laboratory diagnosis of chikungunya virus infections and commercial sources for diagnostic assays. *J Infect Dis* 2016;214:S471-S474.
50. Edwards CJ, Welch SR, Chamberlain J, *et al.* Molecular diagnosis and analysis of Chikungunya virus. *J Clin Virol* 2007; 39: 271–275.
51. Sharma S, Dash PK, Santhosh SR, *et al.* Development of a Quantitative Competitive Reverse Transcription Polymerase Chain Reaction (QC-RT–PCR) for detection and quantitation of Chikungunya virus. *Mol Biotechnol* 2010; 45: 49–55.
52. Grivard P, Le Roux K, Laurent P, *et al.* Molecular and serological diagnosis of Chikungunya virus infection. *Pathol Biol* 2007;55:490-4.

53. Tjaden NB, Cheng Y, Beierkuhnlein C, *et al.* Chikungunya Beyond the Tropics: Where and When Do We Expect Disease Transmission in Europe? *Viruses* 2021;13:1024.

54. Tjaden NB, Suk JE, Fischer D, *et al.* Modelling the effects of global climate change on Chikungunya transmission in the 21st century. *Sci Rep* 2017;7:3813.

CRIMEAN-CONGO HAEMORRHAGIC FEVER

Soushieta Jagadesh (a), Claudia Cataldo (b), Francesca Dagostin (c), Ferran Jori Massanas (d, e), Annapaola Rizzoli (c), Luciano Toma (f), William Wint (g), Luca Busani (b)
(a) International Society of Infectious Diseases, Boston (MA)
(b) Centro di Riferimento per la Medicina di Genere, Istituto Superiore di Sanità, Rome
(c) Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige, Trento
(d) Cirad, UMR Astre, Montpellier
(e) University of Montpellier, INRAE, Montpellier Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige, Trento
(f) Dipartimento Malattie Infettive, Istituto Superiore di Sanità, Rome
(g) Department of Biology, Environmental Research Group Oxford Ltd, Oxford

Biological, ecological and molecular features of the causative agent

Disease name

Crimean-Congo Haemorrhagic Fever (CCHF).

Disease agent

Common scientific and Latin name

Crimean-Congo hemorrhagic fever virus (CCHFV) is the etiological agent of CCHF, a tick-borne disease transmitted by Ixodid ticks mainly of the genus *Hyalomma* (in particular *H. marginatum, H. anatolicum, H. rufipes and H. asiaticum*) *that* are both reservoirs and vectors (1, 2). CCHFV was first identified in the Crimean region, Russia, in 1944 and was subsequently shown to be identical to the Congo virus identified in the Congo basin in 1956, giving the virus its current name (1, 3, 4).

Taxonomy

CCHFV belongs to the genus *Orthonairovirus* of the family *Nairoviridae* within the order *Bunyavirales* (5,6). In addition to CCHFV, the *Nairoviridae* family consists of arthropod-borne viruses causing enzoonotics such as Nairobi sheep disease virus (NSDV), Dugbe virus and Hazara virus (HAZV), with some rare cases in humans reported (7). The *Bunyaviridae* family contains over 350 named isolates classified within five genera, namely, *Hantavirus*, *Nairovirus*, *Orthobunyavirus*, *Phlebovirus*, and *Tospovirus* (8). *Bunyaviridae* family together with members of the *Arenaviridae* and *Orthomyxoviridae* families, are known as segmented negative-strand RNA viruses (sNSVs) attributed to their multiple genome segments (9).

Disease agent characteristics

CCHFV is an enveloped virus of 80-120 nanometres (nm) in diameter with a single negative-sense RNA (9, 10) with a three-segmented genome, small (S), medium (M), and large (L) RNA segments, which encode a viral nucleocapsid protein (NP), glycoprotein precursor, and

polymerase proteins, respectively (11). The genome size of CCHFV was found to be approximately 19.2 kilobases (kb) in length, consisting of a 1.7-2.1 kb S segment, a 4.4-6.3 kb M segment and a 11-1.4 kb L segment (9, 12). CCHFV NP possesses a racket-shaped overall structure with dimensions of 40 × 50 × 95 Å, and features two major parts: a "head" domain and a "stalk" domain (13). Although the NP and L proteins of CCHFV strains are conserved at approximately 95% or more, the CCHFV GPC is much less conserved, with divergent strains exhibiting less than 75% amino acid conservation (14, 15). The genetic diversity of CCHFV correlates with geography (15, 16). CCHFV is classified into seven clades/genotypes (I to VII): Africa-1 (genotype I), Africa-2 (genotype II), Africa-3, (genotype IIIa) and Africa 4 (genotype IIIb); Asia-1 (genotype IVa) and Asia-2 (genotype IVb); Europe-1 (genotype V), Europe-2 (genotype VI) and Europe-3 (genotype VII) (15) based on the S segment sequences with genotype IV divided further into two subgenotypes.

Physiochemical properties

CCHFV is classified as a biosafety level 4 (BSL4) pathogen (17). As an enveloped virus, CCHFV can be inactivated by suitable disinfectant solutions including chlorine-based disinfectants like 1% sodium hypochlorite (18, 19), 40% ethanol within 2 min (20), 10% aqueous solution of household bleach, 2% solution of glutaraldehyde (18), phenolic disinfectants (0.5%-3%) such as formalin and paraformaldehyde, and other disinfectants, such as hydrogen peroxide and peracetic acid (21). The virus gets inactivated by dry heat at 56°C for 30 minutes or 60°C for 15 minutes (22). Ultraviolet exposure (1,200 to 3,000 μW/cm^2) or low pH (less than 6) also inactivates the virus (1, 21). CCHFV is stable for upto10 days in blood kept at 40°C (23). The virus is stable under wet conditions for 7 hours at 37 °C, 11 days at 20 °C and 15 days at 4 °C (20) while under dry conditions, it is stable for at least 90 min, but less than 24 hours (24). Infectivity of CCHFV is also destroyed by boiling or autoclaving (25).

Priority level for European Union

CCHF is classified as a priority disease for the European Union (EU) because of its epidemic potential, its high case fatality ratio, its potential for nosocomial outbreaks, and the difficulties in treatment and prevention. CCHF in humans is a notifiable disease at the EU/EEA (European Economic Area) level (26) and it was included in the WHO R&D Blueprint priorities for research and product development in 2016 due of its epidemic potential and insufficient countermeasures against CCHFV (27, 28). Within the EU, CCHF is currently reported in Bulgaria, Spain, and Greece, but disease activity is documented in neighbouring countries Albania, Georgia, Kosovo, Russia, Ukraine, and Turkey (29). In Bulgaria, CCHF is considered endemic since the 1950's (30, 31). Imported cases have been reported in France (32), Greece (33), and Germany (34). In 2018, Greece reported its first and only autochthonous case of Crimean-Congo haemorrhagic fever (CCHF) (35, 36). Spain reported its first case in 2016 in the province of Ávila, Castile-León (37). A retrospective study observed that a case of CCHF had occurred in the same province in 2013 (38).

Distribution of the pathogen

The geographic distribution of CCHFV is directly linked with the distribution of *Hyalomma* ticks, having a 50° north latitude limit. The virus has been detected across a wide geographic

range in more than 50 countries of Asia, Europe, and Africa where it is associated either to outbreaks of haemorrhagic fever or only sporadic cases, making CCHF the most geographically widespread viral tick-transmitted haemorrhagic fever (3, 4, 39-41). The geographic distribution of CCHF spans from western China, across Southern Asia to the Middle East, Spain, the Balkans, and most of Africa (14, 42, 43) (Figure 1). CCHFV has been reported in Asia (Iran, Afghanistan, Pakistan, Iraq, United Arab Emirates, Kuwait, Oman, Saudi Arabia, China, Tajikistan, Uzbekistan, Kazakhstan, India) (44), Africa (South Africa, Egypt, Mauritania, Kenya, Sudan, Democratic Republic of Congo, Chad, Niger, Nigeria, Senegal, Uganda, Tanzania) (45), and Europe (Albania, Bulgaria, Turkey, Greece, Georgia, Russia, Kosovo, Spain, North Macedonia) (46). To date, CCHF has not been reported in northern Europe, Australia or in the Americas.

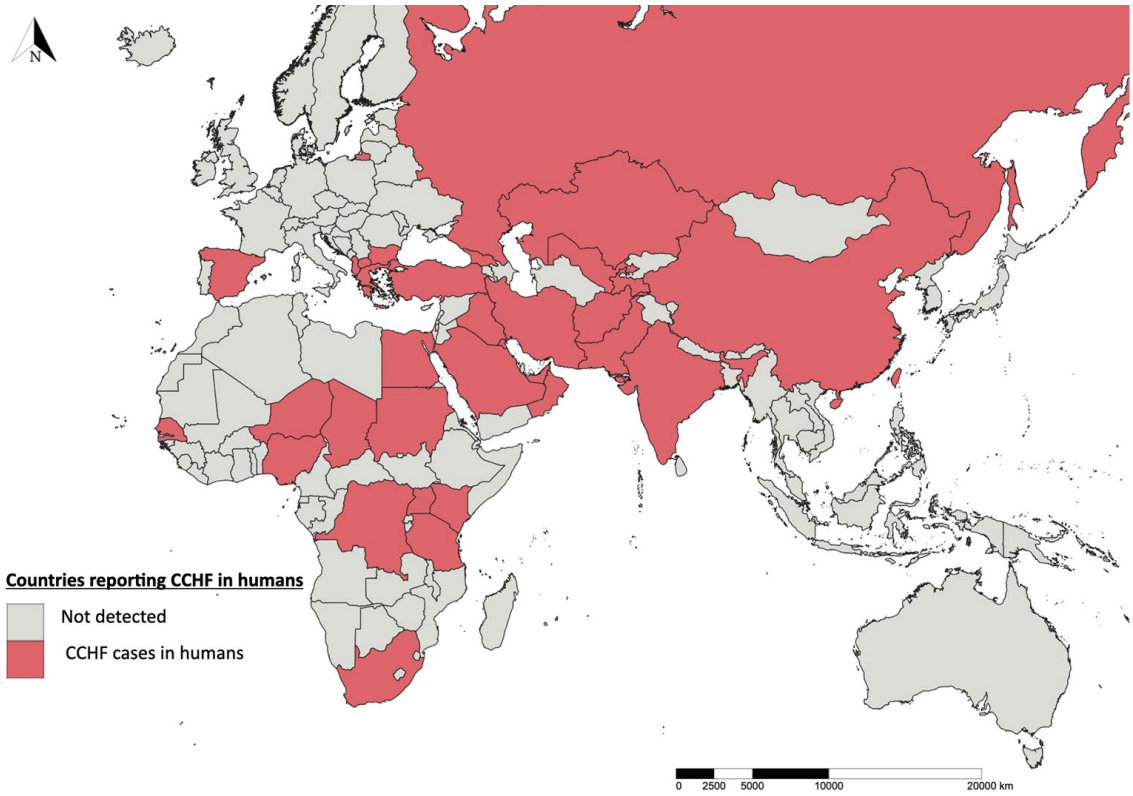

Figure 1. Distribution of CCHF in humans in 2020

The endemicity of CCHFV corresponds to the broad distribution of *Hyalomma* ticks, the predominant vector and reservoir of the virus (47-49) and are considered as crucial in maintaining endemic foci (1, 14, 43). The geographic distribution of CCHF is directly linked with the distribution of *Hyalomma* ticks, having a 50° north latitude limit. *Hyalomma marginatum* remains the main vector of CCHFV in Europe, it is found in Albania, Bulgaria, Cyprus, France, Greece, Italy, Kosovo, Moldavia, Portugal, Romania, Russia, Serbia, Spain, Turkey, and the Ukraine, and is well adapted to a wide range of abiotic conditions but it prefers rather arid localities with high summer temperatures (1) (Figure 2). *H. lusitanicum* ticks have been found to play an important role of CCHFV persistence in Spain (50,51) and Portugal (Figure 3).

CCHV has also been isolated from *Dermacentor marginatus* tick collected in wild boars in Spain (95).

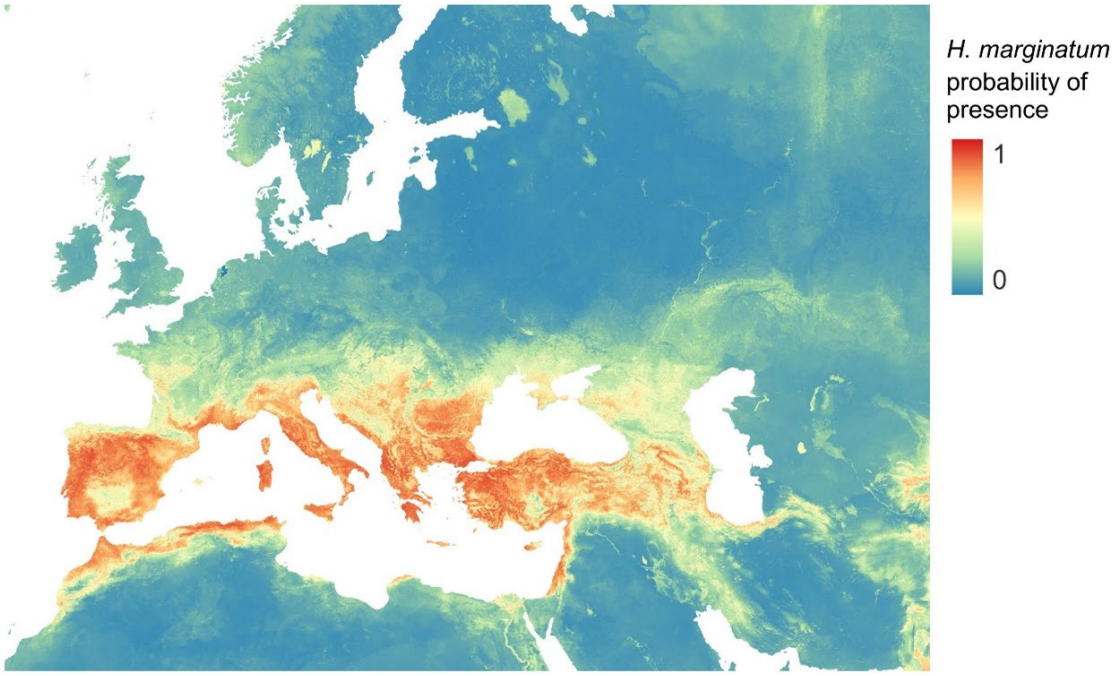

Figure 2. Current 1-km probability of presence of *H. marginatum* across Europe, produced using random forest and boosted regression trees analyses (source: updated by ERGO group)

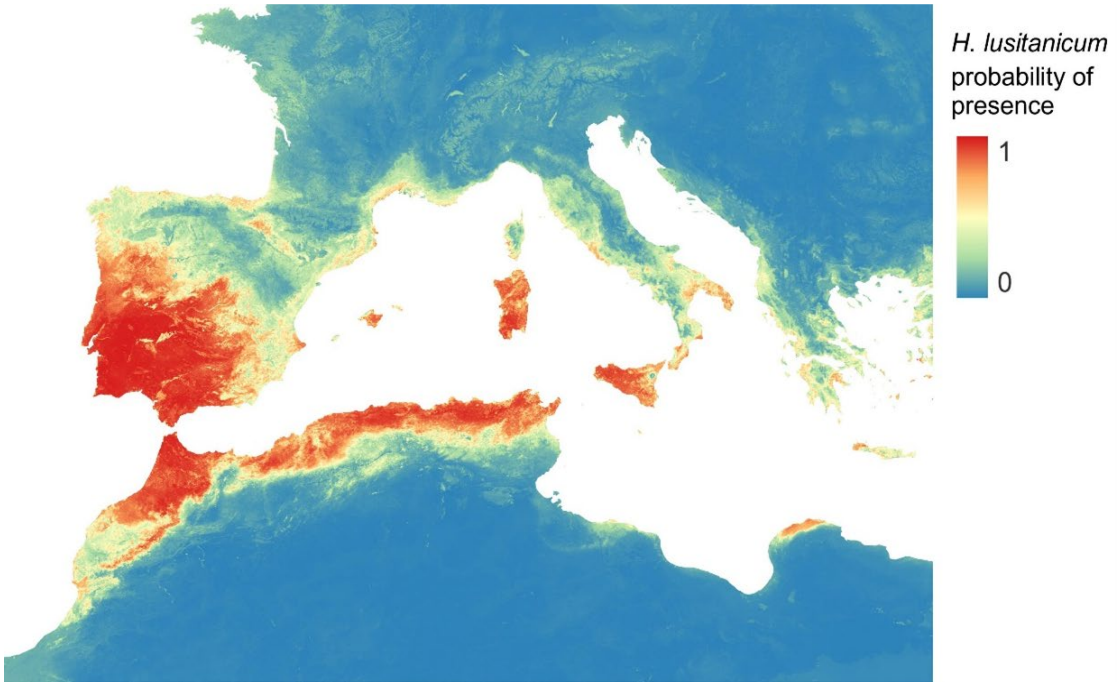

Figure 3. Current 1-km probability of presence of *H. lusitanicum* across Europe, produced using random forest and boosted regression trees analyses (source: updated by ERGO group)

Ecology and transmission routes

CCHFV is transmitted through an enzootic sylvatic cycle: tick – non-human vertebrate – tick. Hard bodied or ixodid ticks, mostly of the *Hyalomma* genus, are considered biological vectors, as well as reservoirs for CCHFV, as they can maintain the virus for several months or even years (1). CCHFV has been detected or isolated from additional tick species, but studies are needed to show whether they are competent virus vectors or merely coincidental unmaintained tick infection from recent feeding on an infected animal or co-feeding (feeding on an uninfected vertebrate host in proximity with an infected tick) (52). They are also able to transmit CCHFV from one generation to the next (vertical transmission), from one development stage to the other (transovarial transmission), from males to females during copulation (sexual transmission), or from one tick to other ticks feeding closely on a same non-viremic host (cofeeding) (43, 53). After infection, ticks remain infective during their lifespan.

The main vector of CCHFV seems to be *H. anatolicum* in Near East and Middle East, *H. asiaticum* from Central Asia to China, *H. rufipes* in Southern Russia and Africa and *H. marginatum* in the Europe and some parts of Asia and Africa. In Spain, CCHFV was detected in *H. lusitanicum* ticks before the identification of the first human case, and studies have observed that this tick species plays an important role in virus circulation in the country (50, 51).

In CCHF endemic areas, where the climatic and environmental factors are suitable for *H. marginatum* ticks (and their animal hosts), their population is increased in spring and summer, accounting for >30% of tick species in the area. *H. marginatum* needs the presence of vertebrate hosts to complete the blood meals required to molt from one development stage to the next. It is a ditropic tick meaning that it is a two-host species. The larvae and nymph stages feed on the same host species, which are small vertebrates such as lagomorphs, birds, hedgehogs, and rodents, whereas adult stages usually feed on large ungulates such as horses, cattle, sheep, goat, deer or wild boar, and occasionally humans (54).

Non-human vertebrate hosts are not symptomatic, but they can replicate the virus and be a source of infection for both ticks and humans (55) during the viremia phase, that lasts 2–15 days (2). CCHFV has been described vertebrate species and is found to circulate according to the geographical distribution of its tick vectors and different sedentary or migratory vertebrate hosts, which are amplifiers of virus (1). *Hyalomma* ticks feed on a variety of mammals including domestic ruminants such as sheep, goats and cattle, rodents, lagomorphs, and wild herbivores (56). Infection in mammals, although subclinical, produces sufficient viremia levels to enable CCHFV transmission to uninfected ticks (57).

Domestic animal species have been implicated in CCHFV transmission to humans. In endemic regions, sheep have been recognized as CCHFV reservoirs and have been epidemiologically linked to human cases (58-61).

In Uzbekistan, three CCHF cases were described in persons in contact with a sick cow (62). Similarly, the first patient in an epizootic of CCHFV in Mauritania became ill following butchering a goat (63).

Studies have shown that increased CCHFV IgG seropositivity in livestock often parallels reports of CCHF cases in humans with exposure to livestock (e.g., slaughterers, butchers, and farmers), particularly in those who handle blood and organs from infected livestock (31,64-68).

Cattle are noted to be the most sensitive indicator of low-level CCHFV circulation as they tend to be highly infested with *Hyalomma* spp. ticks, the numbers of which can be ten times higher than those found on small ruminants (69) (Table 1).

Table 1. List of European countries that reported seroprevalence for CCHFV in domestic animals

Animal	Country	Seroprevalence %	Assay
Cattle	Albania (ten regions) (70)	4.74	IgG ELISA
	Albania (Berat) (71)	4	IgG ELISA
	Albania (Gjirokastra) (53)	2.1	IgG ELISA
	Albania (Kolonje) (71)	7.4	IgG ELISA
	Albania (Rreshen) (53)	2.6	IgG ELISA
	Armenia (72)	4.2	AGDP
	Bulgaria (73)	33.2	AGDP
	Bulgaria (Aytos) (74)	71	IgG ELISA
	Hungary (75)	0.9	AGDP*
	Ireland (76)	1.9	RPHI
	Kosovo (77)	18.4	IgG ELISA
	Republic of North Macedonia (78)	14.6	IgG ELISA
	Russia (Astrakhan Oblast) (79)	5.1	AGDP
	Russia (Rostov Oblast) (80)	23	AGDP
	Russia (Rostov Oblast) (81)	2.8	AGDP
	Russia (Rostov oblast) (82)	0.5-17.0	AGDP
Goats	Albania (83)	20	IgG ELISA
	Bulgaria (73)	62.3	AGDP
	Bulgaria (Aytos) (74)	60	IgG ELISA
	Kosovo (77)	10	IgG ELISA
	Turkey (marmara) (84)	66	IgG ELISA
Horses	Bulgaria (73)	39	AGDP
	Russia (Astrakhan Oblast) (79)	3.1	AGDP
	Russia (Rostov Oblast) (80)	Pos	AGDP
Sheep	Bulgaria (73)	32.9	AGDP
	Bulgaria (Aytos) (74)	74	IgG ELISA
Donkeys	Bulgaria (73)	62.3	AGDP
	Bulgaria (Aytos) (74)	60	IgG ELISA
Camels	Russia (Astrakhan Oblast) (85)	1.4	AGDP
Misc. small livestock	Kosovo (excluding sheep) (59)	14	IgG ELISA

ELISA Enzyme-Linked Immunosorbent Assay
AGDP Agar gel Diffusion Precipitation

Considerable seroprevalence was consistently reported in wild animals as reported with hares (3-22%), buffalo (10-20%), and rhinoceroses (40-68%) (53). A substantial tick load of up to 40 larval and nymphal *H. marginatum* ticks has been described on hedgehog hosts (*Erinaceus europaeus*, *Hemiechinus auritus*) during the peak season of immature tick activity (1, 86). However, the role of hedgehogs in enzootic maintenance appears to be variable by species. *Hemiechinus auritus* developed viremia during experimental infection (87) and are considered a natural CCHFV reservoir by serving as a source of CCHFV for feeding ticks.

In contrast, in the same study, experimental infection in the European hedgehog (*E. europaeus*) did not produce detectable viremia, suggesting reduced susceptibility to infection or more efficient viral clearance.

Among the EU/EEA countries, seroprevalence studies have demonstrated the presence of antibodies to CCHFV in bats in France (88, 89), and in hares in Bulgaria (73) and Hungary (90) (Table 2). Many bird species are important hosts for *Hyalomma* ticks and can transport them over long distances (91, 93). However, most birds are resistant to infection except for ostriches (94).

Table 2. Wild animals testing positive for antibodies against CCHFV in Europe

Class	Common name	Species	Country (ref.)	Seroprevalence
Aves	Eurasian magpie	*Pica pica*	Russia (95)	1 animal
Mammalia	Red fox	*Vulpes vulpes*	Russia (95)	-
Mammalia	Bats	*Various spp.*	France (88, 89)	10.5% (2/19)
Mammalia	European hare	*Lepes europaeus*	Russia (95)	20%
Mammalia	European hare	*Lepes europaeus*	Hungary (90)	6% (12/198)
Mammalia	Hare	*Lepes spp.*	Bulgaria (73)	3% (1/33)
Mammalia	Wild boar	*Sus scrofa*	Spain (96)	19.4%
Mammalia	Red deer	*Cervus elaphus*	Spain (96 97)	25.4
Mammalia	Roe deer	*Capreolus capreolus*	Spain (98)	1.2% (1/79)
Mammalia	Iberian Ibex	*Capra hispanica*	Spain (98)	78% (66/84)
Mammalia	Mouflon	*Ovis musimon*	Spain (99)	100% (48/48)

CCHFV is transmitted to humans by bites from infected ticks or by direct contact with blood or tissues of infected ticks, viraemic patients or viraemic livestock. There have been a few reports of infection after consumption of raw meat (100-102). Drinking unpasteurized milk is also mentioned to be a risk factor in CCHFV transmission. CCHFV is usually inactivated in meat due to post-slaughter acidification (3).

Hospital-acquired infections can occur due to direct contact with blood or tissues of viremic patients or improperly sterilized medical devices (103). Human-to-human transmission usually occurs in hospital settings with a high risk of transmission to healthcare workers (HCWs) including doctors, nurses, laboratory staff, research scientists, emergency service staff and cleaning personnel (103). Several nosocomial outbreaks have been attributed to hospitalized patients acting as index cases (104-106).

Retrospective analysis from Turkey has demonstrated that needle stick injuries are the most frequent cause of nosocomial exposures, followed by ‘splash’ exposures to mucous membranes (107). Horizontal transmission of the CCHFV from a mother to her child (107), as well as intrauterine infection (109) have been reported. Transmission of CCHFV during aerosol-generating medical procedures (105) or sexual contact (110) may be possible.

Drivers of the disease emergence and spread

Ecological drivers

CCHF, as other vector-borne viral diseases, is influenced by dynamic factors such as climate change, alterations of land use, habitats fragmentation, loss of biodiversity, and introduction of new species that impact the distribution of the vector and hosts (91, 111, 112). Studies have observed increased incidence of CCHF with increasing mean temperature in endemic areas (113-115). The seasonal pattern and abundance of *H. marginatum* was found dependent on temperature (116, 117). High temperatures, especially in the spring and summer, tend to accelerate *H. marginatum* cycle by switching on its interstadial development (118), and increase host questing activity (3, 116, 118). Also, the risk of exposure to ticks for humans is higher in warmer temperature due to increased recreational and outdoor activities (119, 120). Areas regularly experiencing long periods of low rainfall and humidity were associated with increased occurrence of CCHF in Iran (121) and Senegal (122).

In temperate areas, the pattern of seasonality of CCHF cases reflects the period of the year with high tick activity; between spring and early autumn (123-126). Mild winters were followed by CCHF outbreaks in Kosovo in 2001 and in Turkey in 2004 (3).

The incidence of CCHF is higher in those areas characterized by a high proportion of grasslands, scrub, and herbaceous vegetation (savannah-type environment), the environmental niche for *Hyalomma* ticks (113, 127). Studies in Turkey and Greece found non-irrigated agricultural land cover (e.g., pasture and rangeland) to be associated with CCHF incidence (66, 128). Areas with a higher incidence of CCHF were those characterized by a highly fragmented habitat (113, 129, 130), supporting the hypothesis that a fragmented land structure may increase the risk of acquiring CCHF by favouring viral circulation and amplification though frequent at-risk contacts between ticks, humans, livestock and wildlife (129). Deforestation has been hypothesized to increase the risk of re-emergence of CCHF in Central Africa as the local CCHFV persistence is supported by the sylvatic natural cycle (48). Landscape modifications such as disruption of agricultural activities and expansion of the hare population infested with infected ticks, followed by the reintroduction of cattle and sheep, have been associated with the CCHF outbreaks in the former Soviet Union, Bulgaria, Kosovo, and Turkey (3).

The impact of biodiversity loss and its consequent dilution effect on CCHF remains relatively unknown as the disease characterized by a variety of different transmission and tick hosts. However, the rise in the wild boars and deer population densities could facilitate the spread of CCHFV through a parallel increase in tick numbers and dispersion across Europe (131). Migration of animals is strongly impacted by climate warming, and changes in host migratory patterns have important consequences for infectious diseases (132, 133). The CCHFV infection in livestock was found to be a strong positive predictor of CCHF incidence in humans in Iran (58) and Mauritania (134). However, in Bulgaria where vaccination coverage is high amongst at-risk populations (e.g., veterinarians and farm workers), livestock density was not found to be a significant driver of CCHF incidence in humans (113). Livestock transportation outside the safety regulations is also reported in the spread of CCHF (126).

Studies demonstrate a significant positive association between Normalized Difference Vegetation Index (NDVI) and CCHF incidence (113, 135), suggesting that the NDVI may proxy for tick seasonal activity. Socioeconomic and demographic factors influencing CCHF outbreaks include social disruption, conflict, and war (123-126).

Natural history of disease in humans and animals, including symptoms, morbidity, and mortality

Brief history of the pathogen and disease

CCHFV introduction to Central and South Asia dated back to the 12th century, which is compatible with historical references describing a disease, which is now believed to have been CCHF, around 1100 AD near Tajikistan in Middle Asia (1). In 20th century, CCHF was described for the first time among Soviet Union military personnel in Crimea during World War II (1944-45) and was named Crimea haemorrhagic fever. The virus was later isolated from blood and tissues of patients using intracerebral inoculation of suckling mice in 1967 (136). The virus responsible for Crimea haemorrhagic fever was found identical to Congo virus that caused febrile illness in Belgian Congo. Later, the two names were combined to new nomenclature of the CCHFV in 1969 (136). Following its description in 1967, cases were reported from former USSR (Crimea, Astrakhan, Rostov), Uzbekistan, Kazakhstan, Tajikistan, and Bulgaria in Eurasia (1, 136) and from Democratic Republic of the Congo, Uganda, and Mauritania in Africa (40, 138). Sporadic cases were reported in Iraq (139), the United Arab Emirates (UAE) (140), and Saudi Arabia (141) in the late 20th century. In Western Europe, the presence of the virus had only been detected indirectly by seroprevalence assays in the serum of two people from Southern Portugal in the early 1980s (142). At the start of 21st century, emergence of CCHF was reported from Pakistan (143), Iran (144), Bulgaria (123), Turkey (41, 145, 146), and India (147). In western Europe, the Greece reported its first autochthonous case in 2008 (35, 147) CCHFV was detected in ticks from deer captured in western Spain in 2010 (50), sheep from Portugal (149), and ticks from birds migrating from Morocco in 2013 (150) prior to detection of their first case in 2016 (37).

Phylogenetic studies have shown that there have been only two virus introductions to Europe (151). The first genotypic group was introduced to Europe via the Volga Delta region only a few hundred years ago and spread to the Balkans. The second introduction was via Turkey around a century ago. A third introduction occurred more recently when the African III genotype was introduced by migratory birds from West Africa in Spain (150).

Disease in humans

In humans, CCHF characterized by fever and haemorrhage, often with nonspecific signs and symptoms with case fatality rates (CFR) ranging from 5-30% (3,14). However, seroprevalence studies show that most CCHF cases (>80%) are asymptomatic or mild (60, 152). The disease presents in four distinct phases: incubation, pre-haemorrhagic, haemorrhagic and convalescence (1, 40, 41, 153). The incubation period is usually less than a week (range 1-9 days) and is dependent on the route of exposure and virus dose. The incubation period is the shortest following a tick bite/needlestick (usually 1-3 days) and slightly longer following exposure to blood, tissue and secretions of infected livestock and humans (5-6 days). The pre-haemorrhagic stage lasts about 2-4 days on average (range 1-7 days) and begins abruptly with nonspecific prodromal symptoms including fever (39-41°C), headache, myalgia, dizziness, neck pain and stiffness, backache, headache, sore eyes and photophobia (153). This may be accompanied by sore throat, abdominal pain, nausea, vomiting and non-bloody diarrhoea (1, 153). Hyperaemia/ cutaneous flushing of the face, neck and chest, congested sclera and conjunctivitis, and jaundice may also be noticed (1). In severe cases, neurological changes in mood and sensory perception have been reported. Somnolence may replace agitation (154, 155). In most patients, the pre-haemorrhagic

phase progresses to haemorrhagic phase. On examination, hypotension, relative bradycardia, tachypnea, and Hepatomegaly and splenomegaly may also be present (41, 145, 156, 157).

The haemorrhagic stage is usually short (approximately 2-3 days) but can be prolonged up to two weeks (153). Haemorrhagic manifestations range from petechiae to extended ecchymoses on mucous membranes and skin (1). These cutaneous signs are particularly pronounced with CCHF compared with other viral haemorrhagic fevers and have found to have a correlation between morbilliform eruptions, platelet count and favourable prognosis (158). Epistaxis, melena, haematemesis, haematuria and haemoptysis are common as is bleeding from injection sites (153, hyju154). Case reports of haemorrhage in other sites such as the vagina (159), uterus (160) and brain (161) have been reported. The haemorrhagic stage is pronounced in severe cases, with rapid progression to disseminated intravascular coagulation (DIC), overt bleeding, kidney, liver or pulmonary failure, and shock (104, 152 153, 162). Acute Respiratory Distress Syndrome (ARDS) and diffuse alveolar haemorrhage have also been reported during haemorrhagic manifestations (163, 164). Death usually occurs in the second week of illness (153). At this stage, laboratory tests demonstrate thrombocytopenia, leukopenia and elevated levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase and creatine phosphokinase (153, 155, 165-167) along with elevated levels of inflammatory cytokines (168, 169). Studies show that coagulation is affected, with prolonged prothrombin and activated partial thromboplastin times accompanied by a decrease in fibrinogen levels and an increase in the levels of fibrinogen degradation products (153, 155, 165, 167).

In survivors, convalescence generally begins around 9-10 days post-onset of illness (range 9-20 days) and is associated with a return to normal for laboratory parameters (40, 145, 53). This stage can be prolonged and may be associated with hypotension, tachycardia or bradycardia, polyneuritis, sweating, headache, dizziness, nausea, poor appetite, breathing difficulties, xerostomia, vision and hearing deficiencies, hair loss and memory loss among others are rarely permanent, but may persist for a year or more (1). However, there is insufficient evidence to describe the long-term complications, sequelae and disability associated with CCHF (170). Post-traumatic stress disorder and mild hearing loss have been reported (171, 172). The differential diagnosis of CCHF is broad and is dependent (Table 3) on patient's geographic origin (173).

Table 3. Differential diagnosis of CCHF

Criteria	Differential diagnosis of CCHF
Geographic origin	
Middle East	Alkhurma haemorrhagic fever and Rift Valley fever
Russia	Omsk haemorrhagic fever
India	Kyasanur forest disease
Europe and Asia	Hantaviral diseases
Africa	Lassa virus, Ebola virus, Marburg virus, Rift Valley fever and yellow fever
Asia and central Africa	Dengue
Tropical & subtropical	Malaria
Transmission	
Vector: Tick bite	*Rickettsia* spp., *Ehrlichia* spp., *Borrelia*, *Anaplasma* and *Babesia*
Clinical symptoms	
Symptomatology	Tularaemia, Q fever, viral hepatitis, influenza virus infection, meningococcal meningitis, leptospirosis, typhoid fever, sepsis due to staphylococci or Gram-negative bacilli, toxic shock syndrome, salmonellosis, shigellosis, psittacosis, trypanosomiasis, septic infection due to Yersinia pestis, rubella and measles

Disease in animals

Most non-human mammals acquire sub-clinical infections and are asymptomatic during and following the period of viremia (55). Most birds are resistant to infection except for ostriches (94).

Availability of preventive, therapeutic and control measures, including licensed or pipelines vaccines

Therapy in humans

Symptomatic treatment of CCHF during the pre-haemorrhagic phase includes antipyretics for fever, proton pump inhibitors to prevent gastrointestinal bleeding (3, 174, 175) and in women, progesterone to avoid menorrhagia (174). Intramuscular injections and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) to be avoided due to the repercussions for clotting (3). Suitable electrolyte replacement must be ensured, and hypotonic solutions are to be avoided (175).

In the haemorrhagic phase, CCHF requires close laboratory monitoring: complete blood count, alanine transaminase (ALT), aspartame aminotransferase (AST), total bilirubin, creatinine, prothrombin time, activated partial thromboplastin time (aPTT), and lactate dehydrogenase. If Disseminated Intravascular Coagulation (DIC) is suspected, d-dimer and arterial blood gas values should be obtained (174, 175).

The efficacy of glucocorticoids for the treatment of CCHF is inconclusive (175) as studies evaluating their usefulness are limited and consist of small case series in both adults and paediatric patients (176). These studies found that administration of high-dose methylprednisolone (20-30 mg/day) seems to promote early haematological recovery, reverse haemorrhagic lesions and decrease the need for transfusion of blood products. However, these results have been inconclusive due to patients simultaneously receiving ribavirin (176, 177). Transfusion with blood products such platelet concentrates and fresh plasma in the haemorrhagic phase, is effective in cases of complications massive haemorrhage, liver failure, thrombocytopenic thrombotic purpura, dilutional coagulopathy, DIC, INR 1.5 times above normal limits and decreased aPTT (174, 175). There is currently limited evidence regarding treatment with plasma or antibodies from survivors (178, 179) and monoclonal antibodies against viral proteins (180,181).

To date, there are no antiviral drugs with proven efficacy against CCHF virus (182, 183). One of the main problems for research on active drugs against CCHFV is the lack of animal model as CCHFV is not pathogenic in animals. Ribavirin is the antiviral drug with the most extensive experience of use in CCHF, with controversial data (153 154-156, 183, 184-189). It has demonstrated conflicting efficacy against CCHF virus (190-193). Data on its efficacy is poor in humans, based on observational studies (174, 177, 191), a single open-label clinical trial (194) and two meta-analyses (189, 195). The meta-analyses report that the data supporting the efficacy of ribavirin against CCHFV are poor owing to confounding factors in reported data sets and any benefit probably requires early treatment during the phase of high viremia (196). Favipiravir showed significant protective effects in lethally infected mice (192, 193, 197), preventing death and significantly reducing viral loads in key target tissues of CCHFV, even when be initiated days after infection, and in mice with advanced disease. This data suggests that favipiravir may be effective in patients presenting to health-care systems with advanced CCHF. However, further pre-clinical and clinical studies are needed in humans. Molnupiravir, recently used to treat SARS-CoV-2 infection in humans, exhibits efficacy against CCHFV *in vitro* with similar inhibitory

concentrations as favipiravir (197). However, molnupiravir failed to protect against CCHFV infection in lethally infected mice even when treatment was started before infection (197).

Licensed or pipelined vaccines

To date, multiple vaccine platforms have been evaluated in animal models for CCHFV such as inactivated virus preparations (198), subunit vaccines (199), VLP vaccines (200, 201), recombinant live-attenuated viruses (201-203), replication-deficient viral-vectored vaccines (204) and nucleic acid-based vaccines (205-208). However, clinical trials have not identified any vaccine with proven efficacy (182, 209). Clinical trials are difficult to conduct as outbreaks are sporadic with irregular numbers of cases (178). A vaccine derived from inactivated CCHFV, propagated in mouse brain, is used in Bulgaria (210).

Other prevention measures

Presently there are no vaccines available for animal protection. CCHF is on the diseases list of WOAH (World Organization for Animal Health). Imports into the EU of live animals are prohibited from endemic areas if the animal tests positive for CCHFV (Commission Regulation (EU) 206/2010).

To reduce the risk of introduction and spread of the diseases from infected countries to non-infected ones there are several measures provided by official regulations, like control of livestock movements from endemic countries (transborder transmission) (211, 212). However, as the infection in animals is asymptomatic, it is difficult to detect and control transmission of CCHFV from animal hosts.

The CCHFV tick vectors are relatively easy to control. Acaricides are useful for tick control when applied prior to animal slaughter, and a 14-day period of quarantine prior to slaughter has also been used (94, 212).

Measures to prevent the infection in humans are based on protection from tick bites. Depending on the geographical location and species, ticks are generally active between April and September in the North Hemisphere. Except during the egg stage, all other biological stages of ticks feed on blood from humans. At risk populations such as agricultural workers and others working with animals including those who live in rural endemic areas should consider basic protection measures (appropriate clothes) and use of tick repellents (4). Minimizing the risk of tick-borne disease transmission with mechanical methods can be performed with tools readily available in most regions (213, 214) such as with tweezers (82.5% success rate) was found to be superior to both lassoing (47.5% success rate) and card detachment (7.5% success rate) (214).

Occupations at high risk for CCHF include veterinarians, abattoir workers, and farmers. For risk reduction in veterinarians and abattoir workers, the use of standard infection control practices when handling potentially infectious blood or ticks was found effective (154). Creating public awareness, targeted at high-risk groups in endemic regions, is useful in reducing the exposure to the virus and controlling the spread of the disease (211, 212).

Disease specific recommendations

Reducing the risk of human-to-human transmission

To prevent nosocomial transmission of CCHFV, particularly among health-care workers, early detection and diagnosis of CCHF cases and adequate provision of PPE is essential together with

proper disposal of used instruments and equipment (syringes, needles) (215). However, poor compliance to the recommended PPE for CCHF has previously been reported (216, 217).

At the community level, prompt epidemiological investigation and contact tracing, and safe burial practices must be implemented for CCHF cases. (148, 218).

Epidemiological situation at different spatial scales: past and current trends

Since 2000, the incidence and geographic range of CCHF cases have markedly increased (44, 194). Turkey has reported approximately 900 new CCHF cases annually, with a total of 9,787 cases reported from 2002 to 2015 (107). CCHF is endemic in the Balkan region, in Kosovo, 228 cases were reported from 1995 to 2013 (77).

Among the EU/EEA countries, the first documented outbreak was reported in Bulgaria occurred during the agricultural collectivization in 1953 (1). After the introduction of a vaccination programme of high-risk groups of the population in 1974 (46), a drastic reduction of cases was observed from 1105 in 1953-1974 to 279 in 1975-1996 (218), to 196 in 1997-2008 (220).

Although the overall number of cases decreased over time, a major outbreak was observed in 2008, with a cluster of cases in the southwest part of Bulgaria, an area historically considered at low risk for CCHF outbreaks (219). In Bulgaria, over 1,500 cases have been reported since 1952 (221). Imported cases have been reported from France in 2004 (32), Germany in 2009 (34), and Greece in 2018 (33). Since its first outbreak in 2016, Spain has reported ten cases of CCHF (Table 4).

Table 4. Cases reported in EU/EEA countries since 2013.

Year	Country	No. of cases	Place of exposure	Transmission
2013	Bulgaria (221)	8	Shumen, Yambol, Haskovo, Kardjali and Blagoevgrad regions	Community
	Spain (37)	1	Ávila province, Castile-León	Community
2014	Bulgaria (222)	8	Haskovo, Kardjali, Blagoevgrad, Plovdiv and Burgas regions	Community
2015	Bulgaria (223)	4	Blagoevgrad, Haskovo and Yambol regions	Community
2016	Bulgaria (224)	4	Blagoevgrad, Kardjali and Yambol regions	Community
	Spain (37)	2	Ávila province, Castile-León	Community
			Madrid province	Nosocomial
2017	Bulgaria (225)	2	Kardjali and Haskovo region	Community
2018	Bulgaria (226)	6	Kardjali and Plovdiv regions	Community
	Spain (227)	2	Badajoz and Salamanca provinces	Community
2019	Bulgaria (228)	2	Kardjali region	Community
2020	Bulgaria (229)	1	Burgas region	Community
	Spain (230)	3	Salamanca province, Castile-León	Community
2021	Spain (231)	2	Salamanca and León provinces	Community
2022	Spain (232)	2	León province, Castile-León	Community

Sociological and demographical dimension affecting susceptibility and exposure, including gender

Human-animal interactions differ across cultures and some interactions may result in social and cultural practices such as the movement of potentially infected animals increasing the risk of CCHFV exposure and linked to CCHF outbreaks (212, 234, 235). The practice of livestock sacrifice plays a major role in festivals like the Hajj and Eid-al-Adha resulting in the contact of large numbers of people with potentially infectious animal blood and body fluids. During Eid-al-Adha, nearly eight million animals are sacrificed each year in Pakistan alone (235) and two million small ruminants, and 750,000 cattle are slaughtered in Turkey, accounting for 25% of all annual slaughtering in that country (235).

Behaviour patterns providing exposure to multifactorial risk factors were gender-based. Men were primarily associated with herding and farming activities, including sleeping outside during seasonal migrations and were more at risk for exposure to CCHFV (236). Seroprevalence studies from Turkey demonstrated anti-CCHFV IgG seropositivity higher in male populations (237, 238). Also, in Spain most of the cases were males, while in Greece, females were more at risk (49) (239). Several studies have shown that increased age was an important risk factor for CCHFV transmission (44, 49, 237, 240-242) and poor prognosis (243). Rural populations are more at risk in terms of exposure to ticks and CCHF.

People living in settlements in rural areas, employed in agricultural activities or in contact with animals (farmers, veterinarians, hunters) are at higher risk of exposure to ticks and CCHF (238, 241, 244-246)

Social disruption, conflict, and war have been the major factors influencing a considerable number of outbreaks in the community and the nosocomial setting (123-126) reported from 2000 to 2008 in South-Eastern Europe (247); in Bulgaria in 2002, 2003 (123), and 2008 (248) and in Albania and Kosovo in 2001.

Diagnostic procedures and notification systems used at local, national and European scale

Diagnosis

Early and accurate diagnosis of CCHF is critical for the patient prognosis, early treatment with ribavirin (196), and prevention of nosocomial infection (215). Direct diagnosis by detection of CCHFV RNA using molecular methods or detection of CCHFV antigen and isolation of the virus (BSL 4 laboratory needed) is used in the viremic phase i.e., first week of illness up to day 16 (249). Molecular assays offer a rapid, sensitive and specific diagnosis of CCHF (249). However, the efficacy of molecular methods is affected by high genetic variability of CCHFV strains (250). To mitigate this, it is recommended that nucleic acid amplification tests (NAAT) be used in combination with serological assays, which are less impacted by minor variations (251), for highest detection sensitivity (252-254).

Indirect diagnosis by detection of CCHFV-specific IgM and IgG antibodies from the fifth day of clinical symptoms is accomplished using serological methods such as ELISA (Enzyme-Linked Immunosorbent Assay) or indirect immunofluorescence. These assays target the CCHFV N protein, which induces nearly, strong and long-lasting immune response in humans (194). CCHF infection is confirmed by detection of IgM antibodies or four-fold increase in IgG antibody titres

in serial serum samples (154, 255, 256). Antibody response is often absent or delayed in severe cases. To date, there are no rapid detection tests for CCHF in development.

CCHF in humans is a notifiable disease at the EU/EEA level (26). All EU/EEA countries have passive surveillance in humans implemented (257). Country specific CCHFV surveillance systems are categorized into five levels based on the incidence of cases, potential for disease transmission to humans, and presence of surveillance systems (221). Level 1 countries are those in which human CCHF cases are reported annually and the virus is endemic (Bulgaria among the EU/EEA countries); Level 2 countries have sporadic autochthonous human cases (Spain and Greece); Level 3 countries have no documented human cases but ecologic data (Portugal and Hungary) with possible human infections; Level 4 countries have the exclusively presence of *Hyalomma* ticks, suggesting the need of seroprevalence surveys (Italy, France, Germany, Austria) and Level 5 countries are ones for which no information is available. Some countries, have in place early detection of exotic ticks and pathogens, some, like in Italy, are based on migratory birds' ticks testing, while others, like in the Netherlands, rely on citizen's reporting of exotic ticks' bites in humans and animals (93, 258).

Infrastructure capacity to identify pathogens for each Member State

Virus isolation is rarely used for CCHF diagnosis as BSL4 laboratories are required and none of the European BSL4 laboratories are situated in CCHF endemic areas. Currently, the routine laboratory diagnosis of CCHF is based on the combination of the detection of the viral genome and CCHFV specific IgM and IgG as per the international recommendations (4, 253).

There is no official, agreed-upon case definition for CCHF in the EU (148), 22/27 EU/EEA countries use the generic case definition for viral haemorrhagic fevers defined according to the Commission Implementing Decision (EU) 2018/945 of 22 June 2018 for CCHF surveillance.

Bulgaria, Greece, Germany, and Spain have their own case definitions for CCHF which considers as suspect case any patient high fever and one of the following symptoms: severe headache, myalgias, nausea, vomiting, and/or diarrhoea and history of tick bite or history of contact with tissues, blood, or other biological fluids from a possibly infected animal (e.g., abattoir workers, livestock owners, veterinarians) or healthcare workers with a history of exposure to a suspect, probable, or laboratory-confirmed CCHF case, within 14 days prior to the onset of symptoms.

A probable CCHF case is a suspected with thrombocytopenia AND haemorrhagic manifestation. A confirmed CCHF case is laboratory-confirmed by ELISA or ImmunoFluorescence Assay (IFA) for specific antibodies, by RT-PCR for CCHF virus genome or virus isolation (254).

The EU definition of viral haemorrhagic fevers (VHF) is based on clinical criteria (fever or haemorrhagic manifestations), laboratory criteria (virus isolation or detection of specific virus nucleic acid in a clinical specimen and genotyping), and epidemiological criteria (travel or exposure to a case of VHF within the last 21 days).

The case classification includes probable case (clinical criteria with an epidemiological link) and confirmed case (clinical and the laboratory criteria) (2018/945/EU).

Estimated influence of environmental change on the disease future trends

Environmental factors and human behaviours are among the most important drivers influencing the lifecycle of *Hyalomma* ticks (220). According to ecological models ran by the World Health Organization Eastern Mediterranean Region, increasing temperature and decreasing rainfall will expand the suitable habitat for *Hyalomma* ticks and will subsequently increase the risk of CCHF (44).Studies predict that trend toward warmer temperatures in central and northern Europe might permit CCHFV to expand outside its current geographic range, through the introduction of infected *Hyalomma* or other reservoir ticks by migratory birds or the international livestock trade (36, 118, 259, 260) A recent study forecasted that the number of countries that have yet to record CCHF have areas that are environmentally suitable for the disease, especially those with Mediterranean coastlines (France, Italy, Southern Balkans) (261). For this reason, future seroprevalence studies in animals should focus on CCHFV presence in Spain, Southern France, Italy, Hungary, and Slovakia (221). Different domestic and wild animal species can be infected by CCHFV, and seroprevalence studies conducted in areas without circulation of the disease have demonstrated seropositivity in cattle, sheep and goats, potentially due to undetected virus circulation between the abundant population of tick vectors and the domestic animals (53, 262).

References

1. Hoogstraal H. The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. *J Med Entomol*. 1979 May 22;15(4):307–417.
2. Spengler JR, Estrada-Peña A, Garrison AR, Schmaljohn C, Spiropoulou CF, Bergeron É, *et al.* A chronological review of experimental infection studies of the role of wild animals and livestock in the maintenance and transmission of Crimean-Congo hemorrhagic fever virus. *Antiviral Res*. 2016 Nov;135:31–47.
3. Ergönül O. Crimean-Congo haemorrhagic fever. *Lancet Infect Dis*. 2006 Apr;6(4):203–14.
4. Whitehouse CA. Crimean-Congo hemorrhagic fever. *Antiviral Res*. 2004 Dec;64(3):145–60.
5. Adams MJ, Lefkowitz EJ, King AMQ, Harrach B, Harrison RL, Knowles NJ, *et al.* Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2017). *Arch Virol*. 2017 Aug 1;162(8):2505–38.
6. King AMQ, Lefkowitz EJ, Mushegian AR, Adams MJ, Dutilh BE, Gorbalenya AE, *et al.* Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2018). *Arch Virol*. 2018 Sep 1;163(9):2601–31.
7. Burt FJ, Spencer DC, Leman PA, Patterson B, Swanepoel R. Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia. *Epidemiol Infect*. 1996 Jun;116(3):353–61.
8. Schmaljohn C, Nichol ST, Bunyaviridae KD, Howley P. *Fields Virol.* 2007;1:1741–89.
9. Carter SD, Surtees R, Walter CT, Ariza A, Bergeron É, Nichol ST, *et al.* Structure, function, and evolution of the Crimean-Congo hemorrhagic fever virus nucleocapsid protein. *J Virol*. 2012 Oct;86(20):10914–23.
10. Flick R, Whitehouse CA. Crimean-Congo hemorrhagic fever virus. *Curr Mol Med*. 2005 Dec;5(8):753–60.

11. Wei PF, Luo YJ, Li TX, *et al.* Serial expression of the truncated fragments of the nucleocapsid protein of CCHFV and identification of the epitope region. *Virol Sin.* 2010;25(1):45-51. doi:10.1007/s12250-010-3067-7.
12. Duh D, Nichol ST, Khristova ML, Saksida A, Hafner-Bratkovic I, Petrovec M, *et al.* The complete genome sequence of a Crimean-Congo hemorrhagic fever virus isolated from an endemic region in Kosovo. *Virol J*. 2008 Jan 15;5:7.
13. Guo Y, Wang W, Ji W, Deng M, Sun Y, Zhou H, *et al.* Crimean-Congo hemorrhagic fever virus nucleoprotein reveals endonuclease activity in bunyaviruses. *Proc Natl Acad Sci U S A*. 2012 Mar 27;109(13):5046–51.
14. Bente DA, Forrester NL, Watts DM, McAuley AJ, Whitehouse CA, Bray M. Crimean-Congo hemorrhagic fever: history, epidemiology, pathogenesis, clinical syndrome and genetic diversity. *Antiviral Res*. 2013 Oct;100(1):159–89.
15. Deyde VM, Khristova ML, Rollin PE, Ksiazek TG, Nichol ST. Crimean-Congo hemorrhagic fever virus genomics and global diversity. *J Virol*. 2006 Sep;80(17):8834–42.
16. Zhou Z, Deng F, Han N, Wang H, Sun S, Zhang Y, *et al.* Reassortment and migration analysis of Crimean-Congo haemorrhagic fever virus. *J Gen Virol*. 2013 Nov;94(Pt 11):2536–48.
17. Weidmann M, Avsic-Zupanc T, Bino S, Bouloy M, Burt F, Chinikar S, *et al.* Biosafety standards for working with Crimean-Congo hemorrhagic fever virus. *J Gen Virol*. 2016 Nov;97(11):2799–808.
18. Appannanavar SB, Mishra B. An update on crimean congo hemorrhagic Fever. *J Glob Infect Dis*. 2011 Jul;3(3):285–92.
19. Smither S, Phelps A, Eastaugh L, Ngugi S, O'Brien L, Dutch A, *et al.* Effectiveness of four disinfectants against Ebola virus on different materials. *Viruses*. 2016 Jul 7;8(7):185.
20. Hardestam J, Simon M, Hedlund KO, Vaheri A, Klingström J, Lundkvist A. Ex vivo stability of the rodent-borne Hantaan virus in comparison to that of arthropod-borne members of the Bunyaviridae family. *Appl Environ Microbiol*. 2007 Apr;73(8):2547–51.
21. Krauss H, Weber A, Appel M, Enders B, Isenberg HD, Schiefer HG, *et al.* Zoonoses: infectious diseases transmissible from animals to humans. ASM press Washington, DC; 2003.
22. Mushtaq A, El-Azizi M, Khardori N. Category C Potential bioterrorism agents and emerging pathogens. *Infect Dis Clin North Am*. 2006 Jun;20(2):423–41.
23. Stramer SL, Hollinger FB, Katz LM, Kleinman S, Metzel PS, Gregory KR, *et al.* Emerging infectious disease agents and their potential threat to transfusion safety. Transfusion (Paris). 2009 Aug;49 Suppl 2:1S-29S.
24. Bartolini B, Gruber CE, Koopmans M, Avšič T, Bino S, Christova I, *et al.* Laboratory management of Crimean-Congo haemorrhagic fever virus infections: perspectives from two European networks. *Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull*. 2019 Jan;24(5).
25. Manuguerra JC. Chapter 3.1.5.- Crimean-Congo haemorrhagic fever. In: World Organization for Animal Health (Ed.). *Animal Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 12th edition*. Paris: WOAH; 2023. p. 1-9 Available from: https://www.woah.org/fileadmin/Home/fr/Health_standards/tahm/3.01.05_CCHF.pdf
26. Europe. Commission implementing decision (EU) 2018/ 945 - of 22 June 2018 - on the communicable diseases and related special health issues to be covered by epidemiological surveillance as well as relevant case definitions. *Official Journal of the European Union L*170 6/7/2018.
27. Mehand MS, Al-Shorbaji F, Millett P, Murgue B. The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development efforts. *Antiviral Res*. 2018 Nov;159:63–7.

28. Word Health Organization. *Prioritizing diseases for research and development in emergency contexts*. Geneva: WHO. Available from: https://www.who.int/activities/prioritizing-diseases-for-research-and-development-in-emergency-contexts; last accessed 11 October 2023.
29. European Centre for Disease Prevention and Control. *Factsheet about Crimean-Congo haemorrhagic fever*. Stockholm: ECDC. Available from: https://www.ecdc.europa.eu/en/crimean-congo-haemorrhagic-fever/facts/factsheet; last accessed 11 October 2023.
30. Papa A, Sidira P, Tsatsaris A. Spatial cluster analysis of Crimean-Congo hemorrhagic fever virus seroprevalence in humans, Greece. *Parasite Epidemiol Control*. 2016;1(3):211–8.
31. Christova I, Gladnishka T, Taseva E, Kalvatchev N, Tsergouli K, Papa A. Seroprevalence of Crimean-Congo hemorrhagic fever virus, Bulgaria. *Emerg Infect Dis*. 2013 Jan;19(1):177–9.
32. Jauréguiberry S, Tattevin P, Tarantola A, Legay F, Tall A, Nabeth P, *et al.* Imported Crimean-Congo hemorrhagic Fever. *J Clin Microbiol*. 2005 Sep;43(9):4905–7.
33. Papa A, Markatou F, Maltezou HC, Papadopoulou E, Terzi E, Ventouri S, *et al.* Crimean-Congo haemorrhagic fever in a Greek worker returning from Bulgaria, June 2018. *Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull*. 2018 Aug;23(35).
34. Conger NG, Paolino KM, Osborn EC, Rusnak JM, Günther S, Pool J, *et al.* Health care response to CCHF in US soldier and nosocomial transmission to health care providers, Germany, 2009. *Emerg Infect Dis*. 2015 Jan;21(1):23–31.
35. Papa A, Dalla V, Papadimitriou E, Kartalis GN, Antoniadis A. Emergence of Crimean-Congo haemorrhagic fever in Greece. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis*. 2010 Jul;16(7):843–7.
36. Maltezou H, Papa A. Crimean-Congo hemorrhagic fever: Risk for emergence of new endemic foci in Europe? *Travel Med Infect Dis*. 2010 May;8(3):139–43.
37. Negredo A, de la Calle-Prieto F, Palencia-Herrejón E, Mora-Rillo M, Astray-Mochales J, Sánchez-Seco MP, *et al.* Autochthonous Crimean-Congo hemorrhagic fever in Spain. *N Engl J Med*. 2017 Jul 13;377(2):154–61.
38. Negredo A, Sánchez-Ledesma M, Llorente F, Pérez-Olmeda M, Belhassen-García M, González-Calle D, *et al.* Retrospective identification of early autochthonous case of Crimean-Congo hemorrhagic fever, Spain, 2013. *Emerg Infect Dis*. 2021 Jun;27(6):1754–6.
39. Charrel RN, Attoui H, Butenko AM, Clegg JC, Deubel V, Frolova TV, *et al.* Tick-borne virus diseases of human interest in Europe. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis*. 2004 Dec;10(12):1040–55.
40. Swanepoel R, Shepherd AJ, Leman PA, Shepherd SP, McGillivray GM, Erasmus MJ, *et al.* Epidemiologic and clinical features of Crimean-Congo hemorrhagic fever in Southern Africa. *Am J Trop Med Hyg*. 1987 Jan;36(1):120–32.
41. Bakir M, Ugurlu M, Dokuzoguz B, Bodur H, Tasyaran MA, Vahaboglu H, *et al.* Crimean-Congo haemorrhagic fever outbreak in Middle Anatolia: a multicentre study of clinical features and outcome measures. *J Med Microbiol*. 2005 Apr;54(Pt 4):385–9.
42. Spengler JR, Bergeron É, Spiropoulou CF. Crimean-Congo hemorrhagic fever and expansion from endemic regions. *Curr Opin Virol*. 2019 Feb;34:70–8.
43. Gargili A, Estrada-Peña A, Spengler JR, Lukashev A, Nuttall PA, Bente DA. The role of ticks in the maintenance and transmission of Crimean-Congo hemorrhagic fever virus: A review of published field and laboratory studies. *Antiviral Res*. 2017 Aug;144:93–119.
44. Al-Abri SS, Abaidani IA, Fazlalipour M, Mostafavi E, Leblebicioglu H, Pshenichnaya N, *et al.* Current status of Crimean-Congo haemorrhagic fever in the World Health Organization Eastern Mediterranean Region: issues, challenges, and future directions. *Int J Infect Dis IJID Off Publ Int Soc Infect Dis*. 2017 May;58:82–9.

45. Temur AI, Kuhn JH, Pecor DB, Apanaskevich DA, Keshtkar-Jahromi M. Epidemiology of Crimean-Congo Hemorrhagic Fever (CCHF) in Africa - Underestimated for decades. *Am J Trop Med Hyg*. 2021 Apr 26;104(6):1978–90.
46. Maltezou H, Andonova L, Andraghetti R, Bouloy M, Ergonul O, Jongejan F, *et al.* Crimean-Congo hemorrhagic fever in Europe: current situation calls for preparedness. *Euro Surveill*. 2010 Mar 11;15(10):48–51.
47. AvŠiČ-Županc T. Epidemiology of Crimean-Congo Hemorrhagic Fever in the Balkans. In: Ergonul O, Whitehouse CA (Ed.). *Crimean-Congo Hemorrhagic Fever: a global perspective*. Dordrecht: Springer Netherlands; 2007. p. 75–88. https://doi.org/10.1007/978-1-4020-6106-6_7
48. Grard G, Drexler JF, Fair J, Muyembe JJ, Wolfe ND, Drosten C, *et al.* Re-emergence of Crimean-Congo hemorrhagic fever virus in Central Africa. *PLoS Negl Trop Dis*. 2011 Oct;5(10):e1350.
49. Papa A, Tzala E, Maltezou HC. Crimean-Congo hemorrhagic fever virus, Northeastern Greece. *Emerg Infect Dis*. 2011 Jan;17(1):141–3.
50. Estrada-Peña A, Palomar AM, Santibáñez P, Sánchez N, Habela MA, Portillo A, *et al.* Crimean-Congo hemorrhagic fever virus in ticks, Southwestern Europe, 2010. *Emerg Infect Dis*. 2012 Jan;18(1):179–80.
51. Sánchez-Seco MP, Sierra MJ, Estrada-Peña A, Valcárcel F, Molina R, de Arellano ER, *et al.* Widespread Detection of Multiple Strains of Crimean-Congo Hemorrhagic Fever Virus in Ticks, Spain. *Emerg Infect Dis*. 2022 Feb;28(2):394–402.
52. Nasirian H. Ticks infected with Crimean-Congo hemorrhagic fever virus (CCHFV): A decision approach systematic review and meta-analysis regarding their role as vectors. *Travel Med Infect Dis*. 2022 Jun;47:102309.
53. Spengler JR, Bergeron É, Rollin PE. Seroepidemiological Studies of Crimean-Congo hemorrhagic fever virus in domestic and wild animals. *PLoS Negl Trop Dis*. 2016 Jan;10(1):e0004210.
54. Apanaskevich DA. (Host-parasite relationships of the genus Hyalomma Koch, 1844 (Acari, Ixodidae) and their connection with microevolutionary process). *Parazitologiia*. 2004 Dec;38(6):515–23.
55. Spengler J, Estrada-Pena A. Host preferences support the prominent role of Hyalomma ticks in the ecology of Crimean-Congo hemorrhagic fever. *PLoS Negl Trop Dis*. 2018 Feb;12(2).
56. Nalca A, Whitehouse CA. Crimean-Congo Hemorrhagic Fever Virus Infection among Animals. In: Ergonul O, Whitehouse CA (Ed.). *Crimean-Congo Hemorrhagic Fever: a global perspective*. Dordrecht: Springer Netherlands; 2007. p. 155–65. https://doi.org/10.1007/978-1-4020-6106-6_13
57. Swanepoel R, Paweska JT. Chapter 26 - Crimean-Congo haemorrhagic fever. In: Palmer SR, *et al.* (Ed.). *Oxford textbook of zoonoses: biology, clinical practice, and public health control, 2 edn.* Oxford: Oxford University Press; 2011. p. 287-293. https://doi.org/10.1093/med/9780198570028.001.0001.
58. Mostafavi E, Haghdoost A, Khakifirouz S, Chinikar S. Spatial analysis of Crimean Congo hemorrhagic fever in Iran. *Am J Trop Med Hyg*. 2013 Dec;89(6):1135–41.
59. Humolli I, Dedushaj I, Zupanac TA, Muçaj S. Epidemiological, serological and herd immunity of Crimean-Congo haemorrhagic fever in Kosovo. *Med Arh. 2010;64(2):91–3.*
60. Papa A, Sidira P, Kallia S, Ntouska M, Zotos N, Doumbali E, *et al.* Factors associated with IgG positivity to Crimean-Congo hemorrhagic fever virus in the area with the highest seroprevalence in Greece. *Ticks Tick-Borne Dis*. 2013 Sep;4(5):417–20.
61. Yen YC, Kong LX, Lee L, Zhang YQ, Li F, Cai BJ, *et al.* Characteristics of Crimean-Congo hemorrhagic fever virus (Xinjiang strain) in China. *Am J Trop Med Hyg*. 1985 Nov;34(6):1179–82.
62. Chumakov M, Vafakulov KB, Zavodova TI, Karmysheva VYa, Maksumov SS, Mart'yanova L I, Rodin V I, Sukharenko SN. Cases of Crimean hemorrhagic fever transmission in Uzbekistan through

contacts with the blood of a sick cow and a patient and also by tick bites. *Trudy Instituta Poliomielita i Virusnykh Entsefalitov Akademii Meditsinskikh Nauk SSSR* 1974;22:(2):29-34.

63. Nabeth P, Cheikh DO, Lo B, Faye O, Vall IOM, Niang M, *et al.* Crimean-Congo hemorrhagic fever, Mauritania. *Emerg Infect Dis*. 2004 Dec;10(12):2143–9.
64. Mustafa ML, Ayazi E, Mohareb E, Yingst S, Zayed A, Rossi CA, *et al.* Crimean-Congo hemorrhagic fever, Afghanistan, 2009. *Emerg Infect Dis*. 2011 Oct;17(10):1940–1.
65. Chinikar S, Ghiasi SM, Moradi M, Goya MM, Shirzadi MR, Zeinali M, *et al.* Geographical distribution and surveillance of Crimean-Congo hemorrhagic fever in Iran. *Vector Borne Zoonotic Dis Larchmt N*. 2010 Oct;10(7):705–8.
66. Sargianou M, Panos G, Tsatsaris A, Gogos C, Papa A. Crimean-Congo hemorrhagic fever: seroprevalence and risk factors among humans in Achaia, Western Greece. *Int J Infect Dis IJID Off Publ Int Soc Infect Dis*. 2013 Dec;17(12):e1160-1165.
67. Lwande OW, Irura Z, Tigoi C, Chepkorir E, Orindi B, Musila L, *et al.* Seroprevalence of Crimean Congo hemorrhagic fever virus in Ijara District, Kenya. *Vector Borne Zoonotic Dis Larchmt N*. 2012 Sep;12(9):727–32.
68. Sharifi-Mood B, Metanat M, Alavi-Naini R. Prevalence of Crimean-Congo hemorrhagic fever among high risk human groups. *Int J High Risk Behav Addict*. 2014 Mar;3(1):e11520.
69. Camicas JL, Wilson M, Cornet JP, Digoutte JP, Calvo MA, Adam F, *et al.* Ecology of ticks as potential vectors of Crimean-Congo hemorrhagic fever virus in Senegal: epidemiological implications. In Springer; 1991. p. 303–22.
70. Lugaj A, Mertens M, Groschup MH, Bërxholi K. Serological survey of CCHFV in cattle in 10 regions of Albania. *Int J Res Appl Nat Soc Sci* I. 2014;2: p. 55–60.
71. Lugaj A, Koni M, Mertens M, Groschup M, Berxholi K. Serological survey of Crimean-Congo hemorrhagic fever virus in cattle in Berat and Kolonje, Albania. Albanian *J Agric Sci.* 2014;
72. Matevosyan KS, Semashko I, Rubin S, Chumakov M. Antibody for CHF virus in human and cattle blood sera in the Armenian SSR (Russian). *Tr Inst Polio Virus Entsef Akad Med Nauk* SSSR. 1974;22(1):173–5.
73. Vasilenko S, Katsarov G, Mikhailov A, Teckharova M, Levi V, Levi S. Crimean hemorrhagic fever (CHF) in Bulgaria. *Tr Inst Polio Virus Entsef*. 1971;19:100–11.
74. Barthel R, Mohareb E, Younan R, Gladnishka T, Kalvatchev N, Moemen A, *et al.* Seroprevalance of Crimean-Congo haemorrhagic fever in Bulgarian livestock. *Biotechnol Biotechnol Equip*. 2014 May 4;28(3):540–2.
75. Horváth LB. Incidence of antibodies to Crimean haemorrhagic fever in animals (author's transl). *Acta Microbiol Acad Sci Hung*. 1975;22(1):61–3.
76. Hassanein KM, el-Azazy OM, Yousef HM. Detection of Crimean-Congo haemorrhagic fever virus antibodies in humans and imported livestock in Saudi Arabia. *Trans R Soc Trop Med Hyg*. 1997 Oct;91(5):536–7.
77. Fajs L, Humolli I, Saksida A, Knap N, Jelovšek M, Korva M, *et al.* Prevalence of Crimean-Congo hemorrhagic fever virus in healthy population, livestock and ticks in Kosovo. *PloS One*. 2014;9(11):e110982.
78. Mertens M, Vatansever Z, Mrenoshki S, Krstevski K, Stefanovska J, Djadjovski I, *et al.* Circulation of Crimean-Congo Hemorrhagic Fever Virus in the former Yugoslav Republic of Macedonia revealed by screening of cattle sera using a novel enzyme-linked immunosorbent assay. *PLoS Negl Trop Dis*. 2015 Mar;9(3):e0003519.
79. Berezin V, Chumakov M, Rubin S, Stolbov D, Butenko A, Bashkirtsev V. Contribution to the ecology of Crimean hemorrhagic fever virus in the lower Volga River (NAMRU-T836). *Arboviruses*. 1969;2:120–2.

80. Badalov M. *Results of serological investigation of the rural population and domestic animals in Rostov Oblast in connection with the problem of prevention*. 1969

81. Karinskaya GA, Chumakov MP, Butenko AM, Badalov ME, Rubin SG. Investigation of Antibodies to Crimean Hemorrhagic Fever Virus in Animal Blood Samples from Rostov Oblast. In: Chumakov MP (Ed.). *A Translation of "Crimean Hemorrhagic Fever": Papers from the Third Regional Workshop at Rostov-on-Don in May 1970*. Annapolis, MD: Entomological Society of America; 1974. p. 147. https://doi.org/10.4182/KGVP3013.9-3.147

82. Kuchin VV, Yanovich TD, Butenko AM, Kirsanova KS. Serological examination for antibodies to chf virus in domestic animals of Rostov Oblast. In: Chumakov MP (Ed.). *A Translation of "Crimean Hemorrhagic Fever": Papers from the Third Regional Workshop at Rostov-on-Don in May 1970*. Annapolis, MD: Entomological Society of America; 1974. p. 149.

83. Papa A, Velo E, Papadimitriou E, Cahani G, Kota M, Bino S. Ecology of the Crimean-Congo hemorrhagic fever endemic area in Albania. *Vector Borne Zoonotic Dis Larchmt N*. 2009 Dec;9(6):713–6.

84. Dinçer E, Uyar Y, Özkul AA. Crimean-Congo hemorrhagic fever infection in domestic animals in Marmara region, Western Turkey. *Ankara Üniv Vet Fak Derg.* 2014 61(1), 49-53.

85. Berezin V, Chumakov M, Stolbov D, Butenko A. On the problem of natural hosts of Crimean hemorrhagic fever virus in Astrakhan region. (Engl NAMRU3-T912). *Tr Inst Polio Virusn Entsefalitov Akad Med Nauk SSSR*. 1971;19:210–6.

86. Berezin V, Povalishina T, Ermakova R, Stolbov D, naval medical research unit no 3 cairo (egypt) dept of medical zoology. On the role of birds in feeding immature stages of Hyalomma plumbeum plumbeum ticks-vectors of hemorrhagic fever of the Crimean type in foci of the Volga Delta. (Engl NAMRU3-Tl98). *Tr Inst Polio Virusn Entsefalitov Akad Med Nauk SSSR.* 1965;7:296–303.

87. Blagoveshchenskaya N, Donets MA, Zarubina LV, Kondratenko VF, Kuchin VV. Study of susceptibility to Crimean hemorrhagic fever (CHF) virus in European and long-eared hedgehogs. *Tezisy Konf. Vop. Med. Virus* 1975 2: 269-270).

88. Portillo A, Palomar AM, Santibáñez P, Oteo JA. Epidemiological aspects of Crimean-Congo hemorrhagic fever in Western Europe: what about the future? *Microorganisms*. 2021 Mar 21;9(3):649. doi:10.3390/microorganisms9030649

89. Tkachenko E, Khanun K, Berezin V. Serological investigation of human and animal sera in agar gel diffusion and precipitation (AGDP) test for the presence of antibodies of Crimean hemorrhagic fever and Grand Arbaud viruses. (Engl NAMRU3-T620). *Mater*. 1969;16.

90. Németh V, Oldal M, Egyed L, Gyuranecz M, Erdélyi K, Kvell K, *et al.* Serologic evidence of Crimean-Congo hemorrhagic fever virus infection in Hungary. *Vector Borne Zoonotic Dis Larchmt N*. 2013 Apr;13(4):270–2.

91. Capek M, Literak I, Kocianova E, Sychra O, Najer T, Trnka A, *et al.* Ticks of the *Hyalomma marginatum* complex transported by migratory birds into Central Europe. *Ticks Tick-Borne Dis*. 2014 Sep;5(5):489–93.

92. Jameson LJ, Morgan PJ, Medlock JM, Watola G, Vaux AGC. Importation of *Hyalomma marginatum*, vector of Crimean-Congo haemorrhagic fever virus, into the United Kingdom by migratory birds. *Ticks Tick-Borne Dis*. 2012 Apr;3(2):95–9.

93. Mancuso E, Toma L, Polci A, d'Alessio SG, *et al.* Crimean-Congo hemorrhagic fever virus genome in tick from migratory bird, Italy. *Emerg Infect Dis* 2019; 25: 1418–1420..

94 Swanepoel R, Leman PA, Burt FJ, Jardine J, Verwoerd DJ, Capua I, *et al.* Experimental infection of ostriches with Crimean-Congo haemorrhagic fever virus. *Epidemiol Infect*. 1998 Oct;121(2):427–32.

95 Zarubinsky Vy, Klisenko G, Kuchin V, Timchenko V, Shanoyan N. Application of the indirect hemagglutination inhibition test for serological investigation of Crimean hemorrhagic fever focus

in Rostov Oblast. (Engl NAMRU3-T1145). *Sb Tr Inst Virus Im Ivanov Akad Med Nauk SSSR*. 1975;2:73–7.

96 Moraga-Fernández A, Ruiz-Fons F, Habela MA, Royo-Hernández L, Calero-Bernal R, Gortazar C, *et al.* Detection of new Crimean-Congo haemorrhagic fever virus genotypes in ticks feeding on deer and wild boar, Spain. *Transbound Emerg Dis*. 2021 May;68(3):993–1000.

97 Cuadrado-Matías R, Baz-Flores S, Peralbo-Moreno A, Herrero-García G, Risalde MA, Barroso P, *et al.* Determinants of Crimean-Congo haemorrhagic fever virus exposure dynamics in Mediterranean environments. *Transbound Emerg Dis*. 2022 Nov;69(6):3571–81.

98 Espunyes J, Cabezón O, Pailler-García L, Dias-Alves A, Lobato-Bailón L, Marco I, *et al.* Hotspot of Crimean-Congo Hemorrhagic fever virus seropositivity in wildlife, Northeastern Spain. *Emerg Infect Dis*. 2021 Sep;27(9):2480–4.

99 Carrera-Faja L, Cardells J, Pailler-García L, Lizana V, Alfaro-Deval G, Espunyes J, *et al.* Evidence of prolonged Crimean-Congo Hemorrhagic fever virus endemicity by retrospective serosurvey, Eastern Spain. *Emerg Infect Dis*. 2022 May;28(5):1031–4.

100 Fazlalipour M, Baniasadi V, Mirghiasi SM, Jalali T, Khakifirouz S, Azad-Manjiri S, *et al.* Crimean-Congo Hemorrhagic fever due to consumption of raw meat: case reports from East-North of Iran. *Jpn J Infect Dis*. 2016 May 20;69(3):270–1.

101 Ziapour SP, Enayati A, Nikookar SH, Sahraei-Rostami F, Kheiri S, Charkameh A, *et al.* Eating raw liver, a potential risk factor of crimean-Congo hemorrhagic fever (CCHF) occurrence in high-risk occupations in Nur County, Northern Iran. *Int J Infect Dis*. 2016 Apr 1;45:195.

102 Mood BS, Metanat M, Shahri SH, Mardani M, Hashemi S, Jahani FF. Crimean-Congo hemorrhagic fever following consumption of uncooked liver: case series study. *Arch Clin Infect Dis*. 2011;6(3):128–30.

103 Tsergouli K, Karampatakis T, Haidich AB, Metallidis S, Papa A. Nosocomial infections caused by Crimean-Congo haemorrhagic fever virus. *J Hosp Infect.* 2020 May;105(1):43–52.

104 Jamil B, Hasan RS, Sarwari AR, Burton J, Hewson R, Clegg C. Crimean-Congo hemorrhagic fever: experience at a tertiary care hospital in Karachi, Pakistan. *Trans R Soc Trop Med Hyg*. 2005 Aug;99(8):577–84.

105 Pshenichnaya NY, Nenadskaya SA. Probable Crimean-Congo hemorrhagic fever virus transmission occurred after aerosol-generating medical procedures in Russia: nosocomial cluster. *Int J Infect Dis IJID Off Publ Int Soc Infect Dis*. 2015 Apr;33:120–2.

106 Chinikar S, Shayesteh M, Khakifirouz S, Jalali T, Rasi Varaie FS, Rafigh M, *et al.* Nosocomial infection of Crimean-Congo haemorrhagic fever in eastern Iran: case report. *Travel Med Infect Dis*. 2013 Aug;11(4):252–5.

107 Leblebicioglu H, Ozaras R, Irmak H, Sencan I. Crimean-Congo hemorrhagic fever in Turkey: Current status and future challenges. *Antiviral Res*. 2016 Feb;126:21–34.

108 Saijo M, Tang Q, Shimayi B, Han L, Zhang Y, Asiguma M, *et al.* Possible horizontal transmission of Crimean-Congo hemorrhagic fever virus from a mother to her child. *Jpn J Infect Dis*. 2004 Apr;57(2):55–7.

109 Ergonul O, Celikbas A, Yildirim U, Zenciroglu A, Erdogan D, Ziraman I, *et al.* Pregnancy and Crimean-Congo haemorrhagic fever. *Clin Microbiol Infect*. 2010 Jun;16(6):647–50.

110 Pshenichnaya NY, Sydenko IS, Klinovaya EP, Romanova EB, Zhuravlev AS. Possible sexual transmission of Crimean-Congo hemorrhagic fever. *Int J Infect Dis.* 2016;45:109-11. doi:10.1016/j.ijid.2016.02.1008

111 Estrada-Pena A, Venzal JM. Climate niches of tick species in the Mediterranean region: modeling of occurrence data, distributional constraints, and impact of climate change. *J Med Entomol*. 2007;44(6):1130–8.

112 Estrada-Peña A, Jameson L, Medlock J, Vatansever Z, Tishkova F. Unraveling the ecological complexities of tick-associated Crimean-Congo hemorrhagic fever virus transmission: A gap analysis for the western palearctic. *Vector-Borne Zoonotic Dis*. 2012;12(9):743–52.

113 Vescio FM, Busani L, Mughini-Gras L, Khoury C, Avellis L, Taseva E, *et al.* Environmental correlates of Crimean-Congo haemorrhagic fever incidence in Bulgaria. *BMC Public Health*. 2012 Dec 27;12:1116.

114 Nili S, Khanjani N, Jahani Y, Bakhtiari B. The effect of climate variables on the incidence of Crimean Congo Hemorrhagic Fever (CCHF) in Zahedan, Iran. *BMC Public Health*. 2020 Dec 9;20(1):1893.

115 Duygu F, Sari T, Kaya T, Tavsan O, Naci M. The relationship between Crimean-Congo hemorrhagic fever and climate: does climate affect the number of patients? *Acta Clin Croat*. 2018 Sep;57(3):443–8.

116 Estrada-Peña A, Martínez Avilés M, Muñoz Reoyo MJ. A population model to describe the distribution and seasonal dynamics of the tick *Hyalomma marginatum* in the Mediterranean Basin. *Transbound Emerg Dis*. 2011 Jun;58(3):213–23.

117 Estrada-Peña A. Climate, niche, ticks, and models: what they are and how we should interpret them. *Parasitol Res*. 2008 Dec;103 Suppl 1:S87-95.

118 Gray J, Dautel H, Estrada-Peña A, Kahl O, Lindgren E. Effects of climate change on ticks and tick-borne diseases in Europe. *Interdiscip Perspect Infect Dis* 2009: 593232. 2009;

119 Randolph SE, Asokliene L, Avsic-Zupanc T, Bormane A, Burri C, Gern L, *et al.* Variable spikes in tick-borne encephalitis incidence in 2006 independent of variable tick abundance but related to weather. *Parasit Vectors*. 2008 Dec 9;1(1):44.

120 Randolph SE. To what extent has climate change contributed to the recent epidemiology of tick-borne diseases? *Vet Parasitol*. 2010 Feb 10;167(2–4):92–4.

121 Ansari H, Shahbaz B, Izadi S, Zeinali M, Tabatabaee SM, Mahmoodi M, *et al.* Crimean-Congo hemorrhagic fever and its relationship with climate factors in southeast Iran: a 13-year experience. *J Infect Dev Ctries*. 2014 Jun 11;8(6):749–57.

122 Wilson ML, LeGuenno B, Guillaud M, Desoutter D, Gonzalez JP, Camicas JL. Distribution of Crimean-Congo hemorrhagic fever viral antibody in Senegal: environmental and vectorial correlates. *Am J Trop Med Hyg*. 1990 Nov;43(5):557–66.

123 Papa A, Christova I, Papadimitriou E, Antoniadis A. Crimean-Congo hemorrhagic fever in Bulgaria. *Emerg Infect Dis*. 2004 Aug;10(8):1465–7.

124 Papa A, Bino S, Llagami A, Brahimaj B, Papadimitriou E, Pavlidou V, *et al.* Crimean-Congo hemorrhagic fever in Albania, 2001. *Eur J Clin Microbiol Infect Dis*. 2002 Aug;21(8):603–6.

125 Papa A, Bozovi B, Pavlidou V, Papadimitriou E, Pelemis M, Antoniadis A. Genetic detection and isolation of Crimean-Congo hemorrhagic fever virus, Kosovo, Yugoslavia. *Emerg Infect Dis*. 2002 Aug;8(8):852–4.

126 Vorou R, Pierroutsakos IN, Maltezou HC. Crimean-Congo hemorrhagic fever. *Curr Opin Infect Dis*. 2007 Oct;20(5):495–500.

127 Emelyanova I. Seasonal changes and host adaptability of ticks of the species Hyalomma marginatum in the Stavropol territory. *Zh Mikrobiol Epidemiol Immunobiol*. 2005;4:115.

128 Terzi O, Sisman A, Canbaz S, Sisman Y. An evaluation of spatial distribution of Crimean-Congo hemorrhagic fever with geographical information systems (GIS), in Samsun and Amasya region. *J Med Plants Res*. 2011;5(5):848–54.

129 Estrada-Peña A, Vatansever Z, Gargili A, Ergönul Ö. The trend towards habitat fragmentation is the key factor driving the spread of Crimean-Congo haemorrhagic fever. *Epidemiol Infect*. 2010;138(8):1194–203.

130 Estrada-Peña A, Zatansever Z, Gargili A, Aktas M, Uzun R, Ergonul O, *et al.* Modeling the spatial distribution of crimean-congo hemorrhagic fever outbreaks in Turkey. *Vector Borne Zoonotic Dis Larchmt* N. 2007 Winter;7(4):667–78.

131 Ruiz-Fons F, Fernandez-de-Mera I, Acevedo P, Hofle U, Vicente J, de la Fuente J, *et al.* Ixodid ticks parasitizing Iberian red deer (Cervus elaphus hispanicus) and European wild boar (Sus scrofa) from Spain: Geographical and temporal distribution. *Vet Parasitol*. 2006 Aug 31;140(1–2):133–42.

132 Harvell D, Altizer S, Cattadori IM, Harrington L, Weil E. Climate change and wildlife diseases: When does the host matter the most? *Ecology*. 2009;90(4):912–20.

133 Jensen PM, Jespersen JB. Five decades of tick–man interaction in Denmark – an analysis. *Exp Appl Acarol*. 2005 Feb 1;35(1):131–46.

134 Gonzalez JP, LeGuenno B, Guillaud M, Wilson ML. A fatal case of Crimean-Congo haemorrhagic fever in Mauritania: virological and serological evidence suggesting epidemic transmission. *Trans R Soc Trop Med Hyg*. 1990 Aug;84(4):573–6.

135 Estrada-Peña A, Vatansever Z, Gargili A, Buzgan T. An early warning system for Crimean-Congo haemorrhagic fever seasonality in Turkey based on remote sensing technology. *Geospatial Health*. 2007 Nov;2(1):127–35.

136 Watts D, Ksiazek T, Linthicum K, Hoogstraal H. Crimean-Congo hemorrhagic fever. In: Monath TP (Ed.). *The arboviruses: epidemiology and ecology*. Boca Raton, FL: CRC Press;1988. Chapter 22.

137 Simpson DI, Knight EM, Courtois G, Williams MC, Weinbren MP, Kibukamusoke JW. Congo virus: a hitherto undescribed virus occurring in Africa. I. Human isolations--clinical notes. *East Afr Med J.* 1967 Feb;44(2):86–92.

138 Saluzzo JF, Digoutte JP, Camicas JL, Chauvancy G. Crimean-Congo haemorrhagic fever and Rift Valley fever in south-eastern Mauritania. *Lancet Lond Engl*. 1985 Jan 12;1(8420):116.

139 Al-Tikriti SK, Al-Ani F, Jurji FJ, Tantawi H, Al-Moslih M, Al-Janabi N, *et al.* Congo/Crimean haemorrhagic fever in Iraq. *Bull World Health Organ*. 1981;59(1):85–90.

140 Schwarz TF, Nsanze H, Ameen AM. Clinical features of Crimean-Congo haemorrhagic fever in the United Arab Emirates. *Infection*. 1997 Dec;25(6):364–7.

141 el-Azazy OM, Scrimgeour EM. Crimean-Congo haemorrhagic fever virus infection in the western province of Saudi Arabia. *Trans R Soc Trop Med Hyg.* 1997 Jun;91(3):275–8.

142 Filipe AR, Calisher CH, Lazuick J. Antibodies to Congo-Crimean haemorrhagic fever, Dhori, Thogoto and Bhanja viruses in southern Portugal. *Acta Virol.* 1985 Jul;29(4):324–8.

143 Sheikh AS, Sheikh AA, Sheikh NS, Rafi-U-Shan, Asif M, Afridi F, *et al.* Bi-annual surge of Crimean-Congo haemorrhagic fever (CCHF): a five-year experience. *Int J Infect Dis*. 2005 Jan;9(1):37–42.

144 Sharifi-Mood B, Alavi-Naini R, Metanat M. Ten years after the beginning of Crimean-Congo hemorrhagic fever outbreak in Iran: A promising report. *Iranian Journal of Clinical Infectious Diseases* 2009;4(3):189-193.

145 Ergönül O, Celikbaş A, Dokuzoguz B, Eren S, Baykam N, Esener H. Characteristics of patients with Crimean-Congo hemorrhagic fever in a recent outbreak in Turkey and impact of oral ribavirin therapy. *Clin Infect Dis*. 2004 Jul 15;39(2):284–7.

146 Karti SS, Odabasi Z, Korten V, Yilmaz M, Sonmez M, Caylan R, *et al.* Crimean-Congo hemorrhagic fever in Turkey. *Emerg Infect Dis*. 2004 Aug;10(8):1379–84.

147 Patel AK, Patel KK, Mehta M, Parikh TM, Toshniwal H, Patel K. First Crimean-Congo hemorrhagic fever outbreak in India. *J Assoc Physicians India*. 2011 Sep;59:585–9.

148 Maltezou HC, Maltezos E, Papa A. Contact tracing and serosurvey among healthcare workers exposed to Crimean-Congo haemorrhagic fever in Greece. *Scand J Infect Dis*. 2009;41(11–12):877–80.

149 Mesquita J, Cruz R, Esteves F, Santos C, Pousa H, Coelho C, *et al.* Crimean-Congo hemorrhagic fever virus circulating among sheep of Portugal: a nationwide serosurvey assessment. *Trop Anim Health Prod*. 2022 Aug;54(4).

150 Palomar AM, Portillo A, Santibáñez P, Mazuelas D, Arizaga J, Crespo A, *et al.* Crimean-Congo hemorrhagic fever virus in ticks from migratory birds, Morocco. *Emerg Infect Dis*. 2013 Feb;19(2):260–3.

151 Sherifi K, Cadar D, Muji S, Robaj A, Ahmeti S, Jakupi X, *et al.* Crimean-Congo hemorrhagic fever virus clades V and VI (Europe 1 and 2) in ticks in Kosovo, 2012. *PLoS Negl Trop Dis*. 2014 Sep;8(9):e3168.

152 Bodur H, Akinci E, Ascioglu S, Öngürü P, Uyar Y. Subclinical infections with Crimean-Congo hemorrhagic fever virus, Turkey. *Emerg Infect Dis*. 2012 Apr;18(4):640–2.

153 Swanepoel R, Gill DE, Shepherd AJ, Leman PA, Mynhardt JH, Harvey S. The clinical pathology of Crimean-Congo hemorrhagic fever. *Rev Infect Dis*. 1989 Jun;11 Suppl 4:S794-800.

154 Ergonul O, Celikbas A, Baykam N, Eren S, Dokuzoguz B. Analysis of risk-factors among patients with Crimean-Congo haemorrhagic fever virus infection: severity criteria revisited. *Clin Microbiol Infect*. 2006 Jun;12(6):551–4.

155 Cevik MA, Erbay A, Bodur H, Gülderen E, Baştuğ A, Kubar A, *et al.* Clinical and laboratory features of Crimean-Congo hemorrhagic fever: predictors of fatality. *Int J Infect Dis IJID Off Publ Int Soc Infect Dis*. 2008 Jul;12(4):374–9.

156 Elaldi N, Bodur H, Ascioglu S, Celikbas A, Ozkurt Z, Vahaboglu H, *et al.* Efficacy of oral ribavirin treatment in Crimean-Congo haemorrhagic fever: a quasi-experimental study from Turkey. *J Infect*. 2009 Mar;58(3):238–44.

157 Ozkurt Z, Kiki I, Erol S, Erdem F, Yilmaz N, Parlak M, *et al.* Crimean-Congo hemorrhagic fever in Eastern Turkey: clinical features, risk factors and efficacy of ribavirin therapy. *J Infect*. 2006 Mar;52(3):207–15.

158 Akyol M, Ozçelik S, Engin A, Hayta SB, Biçici F. Cutaneous manifestations of Crimean-Congo haemorrhagic fever: morbilliform eruptions may reflect a favorable outcome and not low platelet levels. *Eur J Dermatol*. 2010 Aug;20(4):523–4.

159 Qaderi S, Hatami H, Omid AM, Sayad J. Vaginal bleeding as a sign of Crimean-Congo hemorrhagic fever infection: a case report. *J Med Case Reports.* 2022 Feb 22;16(1):76.

160 Hatami H, Qaderi S, Omid AM. Investigation of Crimean-Congo hemorrhagic Fever in patients admitted in Antani hospital, Kabul, Afghanistan, 2017-2018. Int *J Prev Med*. 2019;10:117.

161 Kleib AS, Salihy SM, Ghaber SM, Sidiel BW, Sidiya KC, Bettar ES. Crimean-Congo Hemorrhagic Fever with Acute Subdural Hematoma, Mauritania, 2012. *Emerg Infect Dis*. 2016 Jul;22(7):1305–6.

162 Papa A, Maltezou HC, Tsiodras S, Dalla VG, Papadimitriou T, Pierroutsakos I, *et al.* A case of Crimean-Congo haemorrhagic fever in Greece, June 2008. *Euro Surveill*. 2008;13(33):18952. doi:10.2807/ese.13.33.18952-en

163 Sannikova IV, Pacechnikov VD, Maleev VV. (Respiratory lesions in Congo-Crimean hemorrhagic fever). *Ter Arkh*. 2007;79(11):20–3.

164 Doganci L, Ceyhan M, Tasdeler NF, Sarikayalar H, Tulek N. Crimean Congo hemorrhagic fever and diffuse alveolar haemorrhage. *Trop Doct*. 2008 Oct;38(4):252–4.

165 Bastug A, Kayaaslan B, Kazancioglu S, Aslaner H, But A, Akinci E, *et al.* Crimean-Congo Hemorrhagic fever: prognostic factors and the association of leukocyte counts with mortality. *Jpn J Infect Dis*. 2016;69(1):51–5.

166 Aksoy F, Yilmaz G, Kaya S, Karahan SC, Koksal I. The prognostic importance of platelet indices in patients with Crimean-Congo Hemorrhagic Fever. Open Forum *Infect Dis*. 2017 Oct 4;4(Suppl 1):S352–3.

167 Dokuzoguz B, Celikbas AK, Gök ŞE, Baykam N, Eroglu MN, Ergönül Ö. Severity scoring index for Crimean-Congo hemorrhagic fever and the impact of ribavirin and corticosteroids on fatality. *Clin Infect Dis*. 2013 Nov;57(9):1270–4.

168 Papa A, Tsergouli K, Çağlayık DY, Bino S, Como N, Uyar Y, *et al.* Cytokines as biomarkers of Crimean-Congo hemorrhagic fever. *J Med Virol*. 2016 Jan;88(1):21–7.

169 Saksida A, Duh D, Wraber B, Dedushaj I, Ahmeti S, Avsic-Zupanc T. Interacting roles of immune mechanisms and viral load in the pathogenesis of Crimean-Congo hemorrhagic fever. *Clin Vaccine Immunol CVI*. 2010 Jul;17(7):1086–93.

170 Leblebicioglu H, Sunbul M, Barut S, Buyuktuna SA, Ozkurt Z, Yapar D, *et al.* Multi-center prospective evaluation of discharge criteria for hospitalized patients with Crimean-Congo hemorrhagic fever. *Antiviral Res*. 2016 Sep;133:9–13.

171 Gul S, Gul EU, Yesilyurt M, Ozturk B, Kuscu F, Ergonul O. Health-related quality of life and the prevalence of post-traumatic stress disorder among Crimean-Congo hemorrhagic fever survivors. *Jpn J Infect Dis*. 2012;65(5):392–5.

172 Engin A, Yildirim A, Kunt T, Bakir M, Dokmetas I, Ozdemir L. Clinical investigation of the transient evoked otoacoustic emission test in Crimean-Congo hemorrhagic fever. *Int J Infect Dis*. 2008 Mar;12(2):162–5.

173 Ergonul O. Crimean-Congo hemorrhagic fever virus: new outbreaks, new discoveries. *Curr Opin Virol*. 2012 Apr;2(2):215–20.

174 Leblebicioglu H, Bodur H, Dokuzoguz B, Elaldi N, Guner R, Koksal I, *et al.* Case management and supportive treatment for patients with Crimean-Congo hemorrhagic fever. *Vector Borne Zoonotic Dis*. 2012 Sep;12(9):805–11.

175 Ergonul O. Treatment of Crimean-Congo hemorrhagic fever. *Antiviral Res*. 2008 Apr;78(1):125–31.

176 Sharifi-Mood B, Alavi-Naini R, Metanat M, Mohammadi M, Shakeri A, Amjadi A. Efficacy of high-dose methylprednisolone in patients with Crimean-Congo haemorrhagic fever and severe thrombocytopenia. *Trop Doct*. 2013 Apr;43(2):49–53.

177 Dilber E, Cakir M, Erduran E, Koksal I, Bahat E, Mutlu M, *et al.* High-dose methylprednisolone in children with Crimean-Congo haemorrhagic fever. *Trop Doct*. 2010 Jan;40(1):27–30.

178 Keshtkar-Jahromi M, Kuhn JH, Christova I, Bradfute SB, Jahrling PB, Bavari S. Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies. *Antiviral Res*. 2011 May;90(2):85–92.

179 Fels JM, Maurer DP, Herbert AS, Wirchnianski AS, Vergnolle O, Cross RW, *et al.* Protective neutralizing antibodies from human survivors of Crimean-Congo hemorrhagic fever. *Cell*. 2021 Jun 24;184(13):3486-3501.e21.

180 Bertolotti-Ciarlet A, Smith J, Strecker K, Paragas J, Altamura LA, McFalls JM, *et al.* Cellular localization and antigenic characterization of crimean-congo hemorrhagic fever virus glycoproteins. *J Virol*. 2005 May;79(10):6152–61.

181 Golden JW, Shoemaker CJ, Lindquist ME, Zeng X, Daye SP, Williams JA, *et al.* GP38-targeting monoclonal antibodies protect adult mice against lethal Crimean-Congo hemorrhagic fever virus infection. *Sci Adv*. 2019 Jul;5(7):eaaw9535.

182 Hawman DW, Feldmann H. Crimean-Congo haemorrhagic fever virus. *Nat Rev Microbiol*. 2023 Jul;21(7):463–77.

183 de la Calle-Prieto F, Martín-Quirós A, Trigo E, Mora-Rillo M, Arsuaga M, Díaz-Menéndez M, *et al.* Therapeutic management of Crimean-Congo haemorrhagic fever. *Enfermedades infecciosas y microbiologia clinica (English ed.).* 2018;36(8): 517–522.

184 Ergonul O. Evidence supports ribavirin use in Crimean-Congo hemorrhagic fever. *Int J Infect Dis.* 2014 Dec;29:296.

185 Ceylan B, Calıca A, Ak O, Akkoyunlu Y, Turhan V. Ribavirin is not effective against Crimean-Congo hemorrhagic fever: observations from the Turkish experience. *Int J Infect Dis*. 2013 Oct;17(10):e799-801.

186 Espy N, Pérez-Sautu U, Ramírez de Arellano E, Negredo A, Wiley MR, Bavari S, *et al.* Ribavirin had demonstrable effects on the Crimean-Congo Hemorrhagic Fever Virus (CCHFV) population and load in a patient with CCHF Infection. *J Infect Dis*. 2018 May 25;217(12):1952–6.

187 Koksal I, Yilmaz G, Aksoy F, Aydin H, Yavuz I, Iskender S, *et al.* The efficacy of ribavirin in the treatment of Crimean-Congo hemorrhagic fever in Eastern Black Sea region in Turkey. *J Clin Virol Off Publ Pan Am Soc Clin Virol*. 2010 Jan;47(1):65–8.

188 Tasdelen Fisgin N, Ergonul O, Doganci L, Tulek N. The role of ribavirin in the therapy of Crimean-Congo hemorrhagic fever: early use is promising. *Eur J Clin Microbiol Infect Dis*. 2009;28(8):929-933. doi:10.1007/s10096-009-0728-2.

189 Ascioglu S, Leblebicioglu H, Vahaboglu H, Chan KA. Ribavirin for patients with Crimean-Congo haemorrhagic fever: a systematic review and meta-analysis. *J Antimicrob Chemother*. 2011 Jun;66(6):1215–22.

190 Bente DA, Alimonti JB, Shieh WJ, Camus G, Ströher U, Zaki S, *et al.* Pathogenesis and immune response of Crimean-Congo hemorrhagic fever virus in a STAT-1 knockout mouse model. *J Virol*. 2010 Nov;84(21):11089–100.

191 Ranadheera C, Valcourt EJ, Warner BM, Poliquin G, Rosenke K, Frost K, *et al.* Characterization of a novel STAT 2 knock-out hamster model of Crimean-Congo hemorrhagic fever virus pathogenesis. *Sci Rep*. 2020 Jul 23;10(1):12378.

192 Oestereich L, Rieger T, Neumann M, Bernreuther C, Lehmann M, Krasemann S, *et al.* Evaluation of antiviral efficacy of ribavirin, arbidol, and T-705 (favipiravir) in a mouse model for Crimean-Congo hemorrhagic fever. *PLoS Negl Trop Dis*. 2014 May;8(5):e2804.

193 Hawman DW, Haddock E, Meade-White K, Williamson B, Hanley PW, Rosenke K, *et al.* Favipiravir (T-705) but not ribavirin is effective against two distinct strains of Crimean-Congo hemorrhagic fever virus in mice. *Antiviral Res*. 2018 Sep;157:18–26.

194 Papa A, Mirazimi A, Köksal I, Estrada-Pena A, Feldmann H. Recent advances in research on Crimean-Congo hemorrhagic fever. *J Clin Virol.* 2015;64:137-143. doi:10.1016/j.jcv.2014.08.029

195 Johnson S, Henschke N, Maayan N, Mills I, Buckley BS, Kakourou A, *et al.* Ribavirin for treating Crimean Congo haemorrhagic fever. *Cochrane Database Syst Rev*. 2018 Jun 5;6(6):CD012713.

196 Arab-Bafrani Z, Jabbari A, Mostakhdem Hashemi M, Arabzadeh AM, Gilanipour A, Mousavi E. Identification of the crucial parameters regarding the efficacy of ribavirin therapy in Crimean-Congo haemorrhagic fever (CCHF) patients: a systematic review and meta-analysis. *J Antimicrob Chemother*. 2019 Dec 1;74(12):3432–9.

197 Wang Q, Cao R, Li L, Liu J, Yang J, Li W, *et al. In vitro* and in vivo efficacy of a novel nucleoside analog H44 against Crimean-Congo hemorrhagic fever virus. *Antiviral Res*. 2022 Mar;199:105273.

198 Pavel STI, Yetiskin H, Kalkan A, Ozdarendeli A. Evaluation of the cell culture based and the mouse brain derived inactivated vaccines against Crimean-Congo hemorrhagic fever virus in transiently immune-suppressed (IS) mouse model. *PLoS Negl Trop Dis*. 2020 Nov;14(11):e0008834.

199 Kortekaas J, Vloet RP, McAuley AJ, *et al.* Crimean-Congo Hemorrhagic Fever Virus subunit vaccines induce high levels of neutralizing antibodies but no protection in STAT1 knockout mice. *Vector Borne Zoonotic Dis*. 2015;15(12):759-764. doi:10.1089/vbz.2015.1855

200 Scholte FEM, Spengler JR, Welch SR, Harmon JR, Coleman-McCray JD, Freitas BT, *et al.* Single-dose replicon particle vaccine provides complete protection against Crimean-Congo hemorrhagic fever virus in mice. *Emerg Microbes Infect.* 2019;8(1):575–8.

201 Hinkula J, Devignot S, Åkerström S, Karlberg H, Wattrang E, Bereczky S, *et al.* Immunization with DNA plasmids coding for Crimean-Congo Hemorrhagic fever virus capsid and envelope proteins and/or virus-like particles induces protection and survival in challenged mice. *J Virol*. 2017 May 15;91(10).

202 Rodriguez SE, Cross RW, Fenton KA, Bente DA, Mire CE, Geisbert TW. Vesicular stomatitis virus-based vaccine protects mice against Crimean-Congo hemorrhagic fever. *Sci Rep*. 2019 May 23;9(1):7755.

203 Buttigieg KR, Dowall SD, Findlay-Wilson S, Miloszewska A, Rayner E, Hewson R, *et al.* A novel vaccine against Crimean-Congo Haemorrhagic Fever protects 100% of animals against lethal challenge in a mouse model. *PloS One.* 2014;9(3):e91516.

204 Zivcec M, Safronetz D, Scott DP, Robertson S, Feldmann H. Nucleocapsid protein-based vaccine provides protection in mice against lethal Crimean-Congo hemorrhagic fever virus challenge. *PLoS Negl Trop Dis*. 2018 Jul;12(7):e0006628.

205 Garrison AR, Shoemaker CJ, Golden JW, Fitzpatrick CJ, Suschak JJ, Richards MJ, *et al.* A DNA vaccine for Crimean-Congo hemorrhagic fever protects against disease and death in two lethal mouse models. *PLoS Negl Trop Dis*. 2017 Sep;11(9):e0005908.

206 Hawman DW, Ahlén G, Appelberg KS, Meade-White K, Hanley PW, Scott D, *et al.* A DNA-based vaccine protects against Crimean-Congo haemorrhagic fever virus disease in a Cynomolgus macaque model. *Nat Microbiol.* 2021 Feb;6(2):187–95.

207 Appelberg S, John L, Pardi N, Végvári Á, Bereczky S, Ahlén G, *et al.* Nucleoside-modified mRNA vaccines protect IFNAR(-/-) mice against Crimean-Congo Hemorrhagic Fever virus infection. *J Virol*. 2022 Feb 9;96(3):e0156821.

208 Tipih T, Heise M, Burt FJ. Immunogenicity of a DNA-based sindbis replicon expressing Crimean-Congo Hemorrhagic Fever virus nucleoprotein. *Vaccines.* 2021 Dec 16;9(12).

209 Spengler JR, Bente DA. Therapeutic intervention in Crimean-Congo hemorrhagic fever: where are we now? *Future Virol*. 2015;10(3):203–6.

210 Papa A, Papadimitriou E, Christova I. The Bulgarian vaccine Crimean-Congo haemorrhagic fever virus strain. *Scand J Infect Dis*. 2011 Mar;43(3):225–9.

211 Aslam S, Latif MS, Daud M, Rahman ZU, Tabassum B, Riaz MS, *et al.* Crimean-Congo hemorrhagic fever: risk factors and control measures for the infection abatement. *Biomed Rep*. 2016 Jan;4(1):15–20.

212 Leblebicioglu H, Sunbul M, Memish ZA, Al-Tawfiq JA, Bodur H, Ozkul A, *et al.* Consensus report: preventive measures for Crimean-Congo Hemorrhagic Fever during Eid-al-Adha festival. *Int J Infect Dis IJID Off Publ Int Soc Infect Dis*. 2015 Sep;38:9–15.

213 Coleman N, Coleman S. Methods of tick removal: A systematic review of the literature. *AMJ*. 2017;10:53–62.

214 Akin Belli A, Dervis E, Kar S, Ergonul O, Gargili A. Revisiting detachment techniques in human-biting ticks. *J Am Acad Dermatol*. 2016 Aug;75(2):393–7.

215 Fletcher TE, Gulzhan A, Ahmeti S, Al-Abri SS, Asik Z, Atilla A, *et al.* Infection prevention and control practice for Crimean-Congo hemorrhagic fever-A multi-center cross-sectional survey in Eurasia. *PloS One*. 2017;12(9):e0182315.

216 Gozel MG, Dokmetas I, Oztop AY, Engin A, Elaldi N, Bakir M. Recommended precaution procedures protect healthcare workers from Crimean-Congo hemorrhagic fever virus. *Int J Infect Dis IJID Off Publ Int Soc Infect Dis*. 2013 Nov;17(11):e1046-1050.

217 Rahnavardi M, Rajaeinejad M, Pourmalek F, Mardani M, Holakouie-Naieni K, Dowlatshahi S. Knowledge and attitude toward Crimean-Congo haemorrhagic fever in occupationally at-risk Iranian healthcare workers. *J Hosp Infect*. 2008 May;69(1):77–85.

218 Centers for Disease Control and Prevention and World Health Organization. *Infection control for viral haemorrhagic fevers in the African health care setting*. Atlanta, GA: CDC; 1998

219 Christova I, Di Caro A, Papa A, Castilletti C, Andonova L, Kalvatchev N, *et al.* Crimean-Congo hemorrhagic fever, Southwestern Bulgaria. *Emerg Infect Dis*. 2009 Jun;15(6):983–5.

220 Leblebicioglu H. Crimean-Congo haemorrhagic fever in Eurasia. *Int J Antimicrob Agents*. 2010 Nov;36 Suppl 1:S43-46.

221 Spengler JR, Bente DA, Bray M, Burt F, Hewson R, Korukluoglu G, Mirazimi A, Weber F, Papa A. Second international conference on crimean-congo hemorrhagic fever. *Antiviral Res*. 2018 Feb;150:137-147. doi: 10.1016/j.antiviral.2017.11.019.

222 National Center of Infectious and Parasitic Diseases. *Crimean-Congo haemorrhagic fever.* Sofia: NCIPD; 2013: Available from: https://www.ncipd.org/index.php?option=com_biuletin&view=view&layout=enversion&year=2013&month=50&lang=en; last visited 15 October 2023.

223 National Center of Infectious and Parasitic Diseases. *Crimean-Congo haemorrhagic fever*. Sofia. 2014: NCIPD. Available from: https://www.ncipd.org/index.php?option=com_biuletin&view=view&layout=enversion&year=2014&month=52&lang=en; last visited 15 October 2023.

224 National Center of Infectious and Parasitic Diseases. *Crimean-Congo haemorrhagic fever*. Sofia: NCIPD; 2015. Available from: https://www.ncipd.org/index.php?option=com_biuletin&view=view&layout=enversion&month=52&year=2015&lang=en; last visited 15 October 2023.

225 National Center of Infectious and Parasitic Diseases. *Crimean-Congo haemorrhagic fever.* Sofia: NCIPD; 2016. Available from: https://www.ncipd.org/index.php?option=com_biuletin&view=view&layout=enversion&month=52&year=2016&lang=en; last visited 15 October 2023.

226 National Center of Infectious and Parasitic Diseases. *Crimean-Congo haemorrhagic fever*. Sofia: NCIPD; 2017. Available from: https://www.ncipd.org/index.php?option=com_biuletin&view=view&layout=enversion&month=52&year=2017&lang=en; last visited 15 October 2023.

227 National Center of Infectious and Parasitic Diseases. *Crimean-Congo haemorrhagic fever*. Sofia: NCIPD; 2018. Available from: https://www.ncipd.org/index.php?option=com_biuletin&view=view&layout=enversion&month=52&year=2018&lang=en; last visited 15 October 2023.

228 Monsalve Arteaga L, Muñoz Bellido JL, Negredo AI, García Criado J, Vieira Lista MC, Sánchez Serrano JÁ, *et al.* New circulation of genotype V of Crimean-Congo haemorrhagic fever virus in humans from Spain. *PLoS Negl Trop Dis*. 2021 Feb;15(2):e0009197.

229 National Center of Infectious and Parasitic Diseases. *Crimean-Congo haemorrhagic fever.* Sofia: NCIPD; 2019. Available from: https://www.ncipd.org/index.php?option=com_biuletin&view=view&layout=enversion&month=52&year=2019&lang=en; last visited 15 October 2023.

230 National Center of Infectious and Parasitic Diseases. *Crimean-Congo haemorrhagic fever*. Sofia: NCIPD; 2020. Available from:

https://www.ncipd.org/index.php?option=com_biuletin&view=view&layout=enversion&month=52&year=2020&lang=en; last visited 15 October 2023.

231 Junta de Castilla y León. *Salud Pública confirma un caso de fiebre hemorrágica de Crimea-Congo en Salamanca*. Comunicación 11 de junio de 2020. Available from: https://comunicacion.jcyl.es/web/jcyl/Comunicacion/es/Plantilla100Detalle/1281372051501/NotaPrensa/1284960397145/Comunicacion; last visited 15 October 2023.

232 Junta de Castilla y León. *Salud Pública confirma un caso de fiebre hemorrágica de Crimea-Congo en El Bierzo*. Comunicación 10 de junio de 2021. Available from: https://comunicacion.jcyl.es/web/jcyl/Comunicacion/es/Plantilla100Detalle/1284877983892/NotaPrensa/1285065354547/Comunicacion; last visited 15 October 2023.

233 Junta de Castilla y León. *Salud Pública confirma un caso de fiebre hemorrágica de Crimea-Congo en El Bierzo*. Comunicación 20 de julio de 2022. Available from: https://comunicacion.jcyl.es/web/jcyl/Comunicacion/es/Plantilla100Detalle/1281372051501/Comunicado/1285190509128/Comunicacion; last visited 15 October 2023.

234 Mallhi TH, Khan YH, Tanveer N, Khan AH, Qadir MI. Commentary: surveillance of Crimean-Congo haemorrhagic fever in Pakistan. *Front Public Health.* 2017;5:132.

235 Mallhi TH, Khan YH, Sarriff A, Khan AH. Crimean-Congo haemorrhagic fever virus and Eid-Ul-Adha festival in Pakistan. *Lancet Infect Dis*. 2016 Dec;16(12):1332–3.

236 Chapman LE, Wilson ML, Hall DB, LeGuenno B, Dykstra EA, Ba K, *et al.* Risk factors for Crimean-Congo hemorrhagic fever in rural northern Senegal. *J Infect Dis*. 1991 Oct;164(4):686–92.

237 Yagci-Caglayik D, Korukluoglu G, Uyar Y. Seroprevalence and risk factors of Crimean-Congo hemorrhagic fever in selected seven provinces in Turkey. *J Med Virol*. 2014 Feb;86(2):306–14.

238 Çıtıl R, Eğri M, Önder Y, Duygu F, Bulut YE, Yaşayancan Ö, *et al.* Determination of seroprevalence and risk factors of Crimean-Congo Haemorrhagic Fever (CCHF) in the endemic region in Turkey: a population-based cross-sectional study. *J Trop Med*. 2021;2021:9945089.

239 Lorenzo Juanes HM, Carbonell C, Sendra BF, López-Bernus A, Bahamonde A, Orfao A, *et al.* Crimean-Congo Hemorrhagic Fever, Spain, 2013-2021. *Emerg Infect Dis*. 2023 Feb;29(2):252–9.

240 Aydin H, Uyanik MH, Karamese M, Sozdutmaz I, Timurkan MO, Gulen A, *et al.* Serological investigation of occupational exposure to zoonotic Crimean-Congo hemorrhagic fever Infection. *Eurasian J Med*. 2020 Jun;52(2):132–5.

241 Koksal I, Yilmaz G, Aksoy F, Erensoy S, Aydin H. The seroprevalance of Crimean-Congo haemorrhagic fever in people living in the same environment with Crimean-Congo haemorrhagic fever patients in an endemic region in Turkey. *Epidemiol Infect*. 2014 Feb;142(2):239–45.

242 Sidira P, Maltezou HC, Haidich AB, Papa A. Seroepidemiological study of Crimean-Congo haemorrhagic fever in Greece, 2009-2010. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis*. 2012 Feb;18(2):E16-19.

243 Akinci E, Bodur H, Sunbul M, Leblebicioglu H. Prognostic factors, pathophysiology and novel biomarkers in Crimean-Congo hemorrhagic fever. *Antiviral Res*. 2016 Aug;132:233–43.

244 Nasirian H. Crimean-Congo hemorrhagic fever (CCHF) seroprevalence: A systematic review and meta-analysis. *Acta Trop*. 2019 Aug;196:102–20.

245 Karakecili F, Cikman A, Aydin M, Binay UD, Kesik OA, Ozcicek F. Evaluation of epidemiological, clinical, and laboratory characteristics and mortality rate of patients with Crimean-Congo hemorrhagic fever in the northeast region of Turkey. *J Vector Borne Dis*. 2018 Sep;55(3):215–21.

246 Christova I, Panayotova E, Trifonova I, Taseva E, Hristova T, Ivanova V. Country-wide seroprevalence studies on Crimean-Congo hemorrhagic fever and hantavirus infections in general population of Bulgaria. *J Med Virol.* 2017 Oct;89(10):1720–5.

247 Vorou R, Papavassiliou V, Tsiodras S. Emerging zoonoses and vector-borne infections affecting humans in Europe. *Epidemiol Infect*. 2007 Nov;135(8):1231–47.

248 Kunchev A, Kojouharova M. Probable cases of Crimean-Congo-haemorrhagic fever in Bulgaria: a preliminary report. *Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull*. 2008 Apr 24;13(17):18845.

249 Burt FJ, Leman PA, Smith JF, Swanepoel R. The use of a reverse transcription-polymerase chain reaction for the detection of viral nucleic acid in the diagnosis of Crimean-Congo haemorrhagic fever. *J Virol Methods*. 1998 Feb;70(2):129–37.

250 Gruber CEM, Bartolini B, Castilletti C, Mirazimi A, Hewson R, Christova I, *et al.* Geographical variability affects CCHFV detection by RT-PCR: a tool for in-silico evaluation of molecular assays. *Viruses*. 2019 Oct 16;11(10).

251 Vanhomwegen J, Alves MJ, Zupanc TA, Bino S, Chinikar S, Karlberg H, *et al.* Diagnostic assays for Crimean-Congo hemorrhagic fever. *Emerg Infect Dis*. 2012 Dec;18(12):1958–65.

252 Mertens M, Schmidt K, Ozkul A, Groschup M. The impact of Crimean-Congo hemorrhagic fever virus on public health. *Antiviral Res*. 2013 May;98(2):248–60.

253 Drosten C, Kümmerer BM, Schmitz H, Günther S. Molecular diagnostics of viral hemorrhagic fevers. *Antiviral Res*. 2003 Jan;57(1–2):61–87.

254 Fernandez-Garcia MD, Negredo A, Papa A, Donoso-Mantke O, Niedrig M, Zeller H, *et al.* European survey on laboratory preparedness, response and diagnostic capacity for Crimean-Congo haemorrhagic fever, 2012. *Euro Surveill*. 2014 Jul 3;19(26):20844.

255 Burt FJ, Leman PA, Abbott JC, Swanepoel R. Serodiagnosis of Crimean-Congo haemorrhagic fever. *Epidemiol Infect*. 1994 Dec;113(3):551–62.

256 Shepherd AJ, Swanepoel R, Gill DE. Evaluation of enzyme-linked immunosorbent assay and reversed passive hemagglutination for detection of Crimean-Congo hemorrhagic fever virus antigen. *J Clin Microbiol*. 1988 Feb;26(2):347–53.

257 Schüpbach G, Silva LC, Buzzell-Hatav A. Surveillance plan proposal for early detection of zoonotic pathogens in ruminants. *EFSA Support Publ.* 2023;20(2):7887E.

258 Uiterwijk M, Ibáñez-Justicia A, van de Vossenberg B, Jacobs F, Overgaauw P, Nijsse R, *et al.* Imported Hyalomma ticks in the Netherlands 2018–2020. *Parasites & Vectors.* 2021;14:244.

259 Heyman P, Cochez C, Hofhuis A, van der Giessen J, Sprong H, Porter S, *et al.* A clear and present danger: tick-borne diseases in Europe. *Expert Rev Anti Infect Ther*. 2010 Jan;8(1):33–50.

260 Mild M, Simon M, Albert J, Mirazimi A. Towards an understanding of the migration of Crimean-Congo hemorrhagic fever virus. *J Gen Virol*. 2010 Jan;91(Pt 1):199–207.

261 Messina JP, Wint GRW. The Spatial Distribution of Crimean-Congo haemorrhagic fever and its potential vectors in Europe and beyond. *Insects.* 2023 Sep 17;14(9).

262 Grech-Angelini S, Lancelot R, Ferraris O, Peyrefitte CN, Vachiery N, Pédarrieu A, *et al.* Crimean-Congo Hemorrhagic Fever virus antibodies among livestock on Corsica, France, 2014-2016. *Emerg Infect Dis*. 2020 May;26(5):1041–4.

DENGUE

Ewy Ortega (a, b), Pachka Hammami (a), Elena Arsevska (a), Maria Bellenghi (c), Claudia Cataldo (c), Francesca Dagostin (d), Marco Di Luca (e), Claudia Fortuna (e), Flavia Riccardo (e), William Wint (f), Busani Luca (c)

(a) UMR Animals, Health, Territories, Risks, and Ecosystems (Astre), Department of Biological Systems (Bios), French Agricultural Research and International Cooperation Organization for Development (CIRAD), Campus International de Baillarguet, Montpellier
(b) Ministère de l'agriculture et de l'alimentation, Direction générale de l'alimentation (DGAL), Paris
(c) Centro di Riferimento per la Medicina di Genere, Istituto Superiore di Sanità, Rome
(d) Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige, Trento
(e) Dipartimento Malattie Infettive, Istituto Superiore di Sanità, Rome
(f) Department of Biology, Environmental Research Group Oxford Ltd, Oxford

Biological, ecological and molecular features of the causative agent

Disease name

Dengue (DEN).

Disease agent

Dengue virus (DENV).

Common, scientific and Latin name

Dengue virus (DENV) is the causal agent responsible for infections of dengue (DEN) a disease with a wide spectrum of symptoms, from extremely mild (unnoticeable) to severe cases and fatalities. DEN is an arthropod-borne viral disease transmitted by mosquito species of the *Aedes* genus, predominantly by *Ae. aegypti* and to a lesser extent by *Ae. albopictus*. Also known as 'break-bone fever', dengue fever was initially identified as a "water poison" associated with flying insects. The name 'dengue' derives from the Swahili phrase Ka-dinga pepo, meaning "cramp-like seizure" and came into general use only after the 1828 epidemic in Cuba (before that, it was also named Dunga) (1).

Taxonomy

DENV belongs to the genus *Flavivirus* and family *Flaviridae*. The dengue virus complex includes four genetically and antigenically related but distinct antigenic groups (serotypes) labelled DENV 1 to 4. For each serotype, four to six genetic groups (genotypes) were identified (2,3). DENV-1 includes five genotypes (I, II, III, IV, and V), DENV-2 six (Asian I, Asian II, Cosmopolitan, American, American/Asian and Sylvatic), DENV-3 four (I, II, III, and V) and DENV-4 four (I, II, III, Sylvatic) (4). Based on phylogenetic analysis of the envelope (*E*) gene sequences, each genotype can further be subdivided into multiple lineages (5). In 2013 a fifth variant (DENV-5) was isolated in a human. However, this serotype was found only in Sarawak forests in Malaysia where it mostly circulates among non-human primates (6).

Disease agent characteristics

DENV is an enveloped virus. Its genome is composed of a positive-sense, single-stranded RNA of approximately 10-11 kbp, which encodes seven non-structural genes (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) and three structural genes: capsid (C, 100 amino acids), precursor membrane (prM, 75 amino acids), and envelope (E, 495 amino acids). All of them are translated as a single polypeptide chain embedded in endoplasmic reticulum membranes and flanked by two untranslated (non-coding) regions at the 3' and 5' ends (7–9).

Physiochemical properties

Similar to other Flaviviruses, DENVs are stable at slightly alkaline pH (8.0) and low temperatures (especially at -60°C or below) and for at least 6 h in liquid aerosol suspension at room temperature and 23–80% humidity. On the other hand, ultra-low temperatures preserve infectivity almost indefinitely and once freeze-dried they survive almost indefinitely at room temperature (10).

DENV can be effectively inactivated by temperatures higher than 56°C for at least 30 minutes, and ultraviolet light irradiation of at least 45 minutes at 75 cm distance from the source (11). Similar to other Flaviviruses, DENV is readily inactivated by gamma-irradiation, and organic solvents and detergents, including 3–8% formaldehyde, 2% glutaraldehyde, 2-3% hydrogen peroxide, 500-5000 ppm available chlorine, alcohol, 1% iodine, and phenol iodophors (12). Non-ionic detergents, such as Triton X, inactivates DENV in plasma at 31°C within 10 min limiting the risk of transmission of all DENV with transfusion (13).

Priority level for EU

The risk of introduction of DENV in the European Union (EU) is high, especially because of population susceptibility, effect of climate change in temperate areas, presence of *Ae. albopictus* mosquito vector and the increased international trade and air travel, enhancing both the risk of other competent mosquito vectors, such as *Ae. aegypti*, being introduced and the importation of infected travellers (particularly around the Mediterranean coast) (14). These covariates favouring the coexistence of the three components of the epidemiological triangle necessary for local dengue transmission: host, vector, and virus.

DENV is currently not endemic in continental Europe and most of the DEN cases are travellers infected outside of mainland Europe. Nevertheless, the DEN expansion and the number of local outbreaks increased over the last decades with more than 200 autochthonous cases reported in Europe over the past 13 years (15,16). Considering the frequency of travellers coming from high-incidence areas, as well as the past experiences of local outbreaks in Croatia, Israel, France, Italy and Spain, the occurrence of DENF outbreaks in continental Europe is a rare, sporadic but not unexpected event (15).

Vigilance must be maintained, and prevention and control strategies should be carefully thought out, as the risk of DEN outbreaks in continental Europe is present in regions with well-established competent mosquito vector populations such as around the Mediterranean and in some continental regions (see section below: 'Distribution of the pathogen'). With the further spread of the competent vectors in Europe, more regions could become at risk.

Distribution of the pathogen

DENV circulation is endemic in more than 128 tropical and subtropical countries, mainly in Central and South America, Southeast Asia and the Western Pacific, and establishing epidemic cycles in parts of Africa, the Eastern Mediterranean and North America. Lately, its geographic expansion has spread to non-endemic countries (15).

The fast and large-scale spread of the disease seems to be related to demographic and social changes, including global population growth, urbanization, lack of effective mosquito control and increased travel between endemic and dengue-free areas.

International movements, initially for military and war purposes and then for trade and tourism, enable the virus and its vectors to be transported from endemic to dengue-free zones (17). The increase in these movements plays a vital role in the spread of DENV (18).

The occurrence of DEN outbreaks in Europe depends on the presence of competent vectors (Figure 1 and Figure 2). To date, *Ae. albopictus* is considered the main DENV vector in Europe. It is currently present in 13 countries and 337 regions across all the Mediterranean regions of Europe and in some continental regions in Spain, France, Italy, Switzerland, Slovenia, Greece, Croatia, Bosnia-Herzegovina, Montenegro, Albania, Monaco, Bulgaria and the Netherlands. *Ae. aegypti* was eradicated from the European continent in the 1950s; in recent decades it has established in Madeira, and areas bordering the Black Sea (Georgia, Southern Russia and Turkey); in 2010 it was sporadically detected in the Netherlands, but promptly eradicated and more recently, in 2022, it was introduced in the Canary Islands and Cyprus (19).

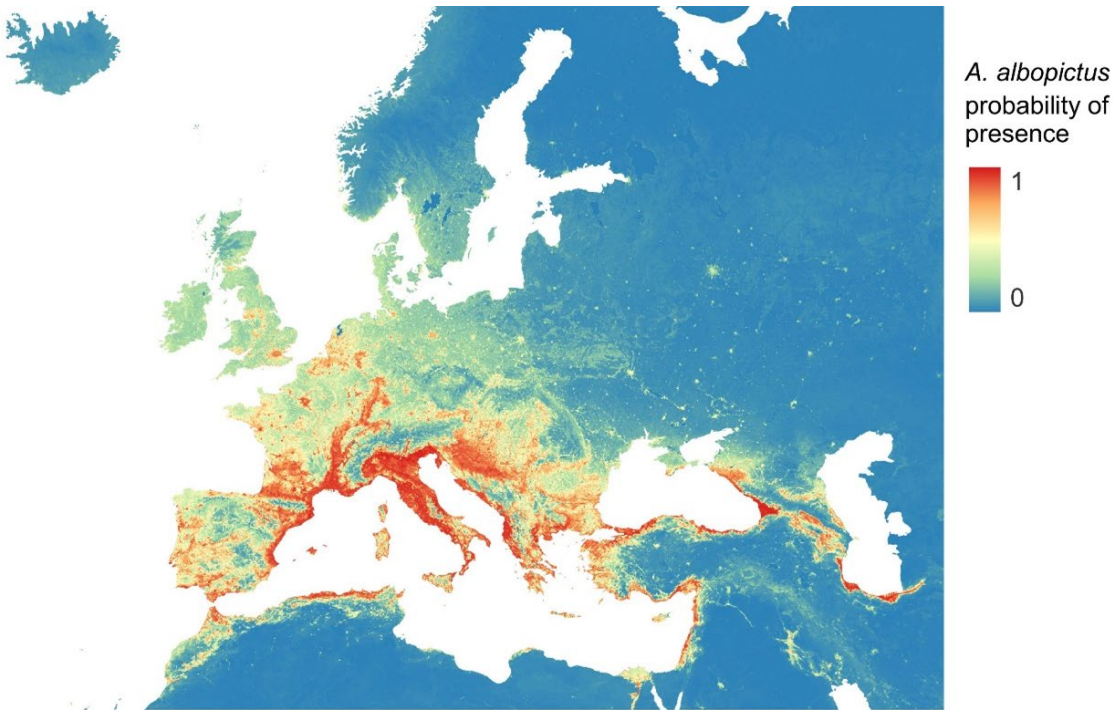

Figure 1. Current 1-km probability of presence of *Ae. albopictus* across Europe, produced using random forest and boosted regression trees analyses (source: updated by ERGO for E4Warning Project).

Figure 2. Current 1-km probability of presence of *Ae. aegypti* across Europe, produced using random forest and boosted regression trees analyses (source: updated by ERGO for E4Warning Project)

Ecology and transmission routes

DENV is mainly transmitted by horizontal transmission between infected and susceptible vector (*Aedes* spp.) and the host. However, vertical transmission within the vector population (from an infected female mosquito to her offspring) has also been reported for *Ae. aegypti*, both experimentally and under natural conditions (20, 21). Within the human population, other modes of transmission from the bite of infected mosquitoes, although rare, have already been described or suggested, namely from mother to child (vertical transmission), blood transfusion, organ transplantation and exposure via needle stick or in the laboratory. Recently, sexual transmission was considered in few cases, pointing out also this possible but apparently rare mode of transmission CIT: a DENV transmission is maintained in the sylvatic (enzootic) cycle and urban endemic cycle which involve non-human primates in sylvatic habitat and humans in urban settings, respectively.

There is evidence that suggests other animals than non-human primates may play a role in both the sylvatic and urban endemic cycles as potential secondary hosts. DENV and/or DENV antibodies have been detected in different animal species including bats, birds, bovids, dogs, horses, pigs, rodents, marsupials and other small animals, however, their role as amplifying reservoirs is not confirmed yet (26) In vector, the incubation period lasts approximately two weeks. Once the virus is disseminated to the salivary glands, it can be injected into susceptible hosts during blood meals. Immediate mechanical transmission was also suggested, e.g., the transfer of the viruses from an infected host to a susceptible one occurring in a short period between the two feeding events (17).

Drivers of the disease emergence and spread

Ecological drivers

DENV spread is affected by multiple factors including the distribution of the competent vectors. Mosquito population dynamics (spatial and temporal densities) is itself driven by multiple components which partly depend on ecological drivers, such as temperature, precipitation or relative humidity.

Environmental conditions including land cover and urbanization also play a critical role in *Aedes* spp. establishment, favouring the vector settlement and proliferation after its introduction. Indeed, some urban environments, such as gardens, terraces and green spaces serving are conducive to their establishment and breeding near the human population.

Due to its high level of eco-physiological plasticity, *Ae. albopictus* has adapted to temperate environments following the evolution of eggs towards the diapause process (24). Moreover, the temperature can also affect vector competence accelerating the DENV replication in the vector at hight temperatures (25).

Natural history of disease in humans and animals, including symptoms, morbidity and mortality

Brief history of the pathogen and disease

While clinical reports referring to dengue-like symptoms were published since the Jin Dynasty (265 to 420 A.D.), the first isolations of DENV occurred in 1943 and 1945 in Japan and Hawaii respectively. Current knowledge suggests that the common ancestor of the four DENV serotypes appeared around 1,000 years ago, and that transmission to humans occurred much later, only a few hundred years ago.

From the 1940s to date, DENV largely spread all over the world mainly to tropical Asia, the Pacific Islands and the Americas, but also in many parts of Africa. Each year 100-400 million DENV infections are reported worldwide (27). It became hyperendemic in tropical and subtropical regions worldwide, mostly in urban and semi-urban areas (17, 28, 29). In recent decades, the number of DENV infections has increased. Since 2010, several outbreaks of autochthonous infections have been reported in Europe, mostly involving DENV-1 and 2 (30).

Disease in humans

In the human host, the four serotypes can cause a wide range of symptoms from asymptomatic or paucisymptomatic (50-90%) to severe infections possibly leading to death. Following the bite of an infectious mosquito and an incubation period, an individual may or may not develop symptoms of DEN. Viremia begins toward the end of a 3 to 14 day incubation period and persists until around the time fever abates, which is typically 4 to 7 day and can go up to 10 days (2, 31).

The clinical pattern of the disease is characterized by 3 phases: febrile (3 to 7 days), critical (2 to 3 days) and recovery (2 to 3 days). The febrile phase is characterized by high fever accompanied by a range of symptoms that differ from individual to individual, from headaches, malaise, nausea, vomiting, myalgia, and abdominal pain to febrile convulsions. The critical phase is characterized by various complications. Haemorrhagic manifestations and haematological abnormalities can

occur in severe forms. Finally, the recovery phase is characterized by the loss of symptoms with the recovery of vascular permeability, although some organ dysfunctions may persist for several weeks (2, 4, 32).

In 2009 the World Health Organization (WHO) proposed a classification of DENV infection according to levels of severity: DEN and severe DEN. The clinical signs of DEN vary from asymptomatic infection (the majority of cases) to classical dengue fever, with high temperature, headaches, malaise, nausea, vomiting, myalgia, and abdominal pain. n. Dengue haemorrhagic fever and dengue shock syndrome are associated with the severe form and, as mentioned, can cause death (33). The clinical manifestations and severity of DENV infection depend on various factors such as the genetics of the virus and the host. For instance, some studies have shown that individuals with African ancestry are less likely to develop a severe form of DEN than those of European and Asian ancestry (34). Potential previous infection with a different DENV serotype or ZIKV (Zika Virus) seems to be one of the major risk factors for severe disease (22-24). An increased risk of severe forms was even highlighted in infants when anti-DENV antibodies were acquired by the mother (2). Age is also considered a risk factor, with children having a higher risk of severe forms (2,4). Finally, the serotypes DENV-2 and DENV-4 are associated with more severe or even fatal forms of the disease, even so, all 4 serotypes are capable of causing it (2).

Following the infection, the host's immune system produces antibodies specific to the serotype. These antibodies confer long-term immunity against that serotype, however, subsequent infections with new serotypes can result in cross-reactivity, leading to more serious infections, it is known as Antibody-Dependent Enhancement (ADE) (4, 32, 35). In a primary infection, anti-DEN IgM levels rise after 3 to 5 days of fever. Over the following 2 to 3 months, IgM levels decrease and are replaced by IgG, which are maintained throughout life and offered a life-long protection against repeated infection with this serotype, but not against reinfection with a different serotype. In secondary infection, the increase in IgM is much less marked than in primary infection and IgG levels rise rapidly to a high peak (2). Adults may experience profound fatigue for several weeks after recovery. The neutralizing antibodies inducted by the first infection can protect against other infections form the same serotype, but cross-protection from other serotypes is not long-term (22, 23).

Availability of preventive, therapeutic and control measures, including licensed or pipelines vaccines

Therapy in humans

The WHO provides guidelines on the clinical management of DEN from febrile phase to recovery. Depending on the disease severity, treatment generally includes rehydration and antipyretics and/or analgesics. In the critical phase and severe haemorrhagic forms, the therapy often includes meticulous fluid resuscitation and platelet transfusions. N-acetylcysteine and antibiotics may be indicated in specific cases. Finally, the benefit of steroids or immunoglobulins is not yet unanimous (36, 37). While there is no licensed targeted therapy or specific antiviral drugs for DENV infection, treatment in the early stage of infection with aspecific antivirals has also been tested to reduce viremia and the likelihood of developing a severe form of DENV infection (32, 38) Antiviral peptide inhibitors have also been used, with an aim to interrupt the DENV life cycle by affecting the functions of viral proteins, thus reducing viremia. Similarly, the use of neutralizing antibodies has been shown to play an important role in inhibiting the functional site of the DENV envelope (E) protein from interacting with the host cells (32). According to

some hypotheses, individuals with high viremia are more at risk of developing severe forms, thus all the above-mentioned treatments aim at preventing the progression of the disease into severe forms (38).

Licensed or pipelined vaccines

Partly due to the cross-reaction/antibody dependent enhancement occurring in case of secondary infection with a different serotype, the development of vaccines for DENV has been very challenging.

In 2015, Dengvaxia® was the first authorized licensed vaccine, but following an increase in hospitalization in the vaccinated cohort 3 years after the vaccination, it is now recommended to individuals with previous dengue fever infection and is not available in non-endemic countries (22).

Recently, in October 2022, the Qdenga® vaccine has been approved by the European Medicines Agency (EMA) (39). Inducing antibody responses against all four serotypes but with higher vaccine efficacy documented against DENV 2 followed by DENV 1. Other vaccines are in development (40-43).

Other prevention measures

The circulation of DENV is mainly linked to the vector, vector control is essential for both the prevention and control of DENV infection.

Effective vector control can include epidemiological and entomological surveillance, educational actions to eliminate mosquito breeding sites and to encourage the use of personal protective equipment such as appropriate clothing, chemical vector control using repellents and insecticides, monitoring vector resistance, and biological control of eggs, larvae and adults.

Other strategies, such as Sterile Insect Technique (SIT) or *Wolbachia*-infected mosquitoes (*Wolbachia*-mediated suppression and *Wolbachia* replacement method), can also be explored to reduce vector density (44,45). In certain countries such as Germany, Italy, and Spain, tools or apps have been provided to citizens for reporting mosquito presence and biting incidents.

Disease specific recommendations

Individuals living in or travelling to endemic regions should take protective measures against mosquito bites, especially during the day, when the mosquitoes are active.

The tetravalent live, attenuated vaccine Qdenga® is recommended for seropositive travellers before travel to an endemic country, for dengue naïve travellers aged from 4 to 16 years old, and for naïve travellers aged from 17 to 60 years old only for long trips in endemic areas.

Due to a lack of studies, Qdenga® is not recommended for individuals older than 60 years old (39). The dengue tetravalent vaccine (CYD-TDV; Dengvaxia®, Sanofi-Pasteur) is recommended using the three-dose series scheduling for individuals from 9 to 45 years old with previous dengue infection in endemic countries.

The trials for the live attenuated tetravalent dengue vaccine, Qdenga® (TAK-003), are still being processed, and it is currently only approved for use in Indonesia for individuals within the indicated age range of 6 to 45 years (42).

Epidemiological situation, at different spatial scales: past and current trends

DENV transmission is not endemic in mainland Europe and the majority of the cases are travellers infected in endemic regions. The epidemiology of locally transmitted DENV infection in Europe is characterized by relatively small outbreaks following the introduction of an imported index case. The first European epidemic was reported in Spain in 1793. From 1793 to 1945, other outbreaks were reported in different European countries (Spain, Cyprus, Gibraltar, Greece, Portugal, Austria, etc.) (47). In 2010, the first autochthonous DENV infections were diagnosed in France and Croatia with low numbers of cases (48,49). From 2008 to 2021, 25,755 cases, about 90% travel-associated, were reported in 28 different countries to the ECDC. During the summer of 2022, 65 autochthonous cases of dengue fever were identified in mainland France, across nine outbreaks (16). From August to Novembre 2023, at least four local transmission events of DENV were documented in different parts of Italy. During the same year local DENV transmission events were also reported in France and Spain.

In recent years the number of reported locally acquired DENV infections in Europe has increased. This could be due to many factors, including, among others, improved sensitivity of surveillance systems, increased climate suitability for DENV transmission, widespread establishment of *Ae. albopictus*, post-COVID-increase of international human mobility (with patterns that differ from country to country due to different preferred holiday destinations and privileged exchanges with overseas territories).The presence of sporadic local transmission events of DENV in continental Europe highlights the importance of continuous integrated surveillance of imported DENV infections, the timely activation of targeted vector control to reduce the risk of infection of local competent mosquito vectors present in Europe and subsequent onward local transmission (50).

Sociological and demographic dimension affecting susceptibility and exposure, including gender

Human behaviour influences the introduction of both dengue virus and vectors and the local exposure of the susceptible populations.

Tourism in endemic areas increases the risk of importation and emergence in EU/EEA (European Union/European Economic Area).

The exposure to mosquito bites and, consequently, the risk of DENV infection, are driven by multiple factors.

In Europe, the influence of sociological components remains understudied, and to date, no clear correlation was identified between the risk of *Ae. albopictus* exposure and age or gender (51). Environmental covariates, such as mosquito densities or changes in land use, like increase of green areas in urban settings, were highlighted as important drivers of bite exposure.

Diagnostic procedures and notification systems used at local, national and European scale

Diagnosis

DENV infection can cause a wide range of clinical presentations, from asymptomatic and mild disease to life-threatening haemorrhagic fever and shock. Given the wide range of symptoms, clinical diagnosis for DENV infection is not straightforward and early and accurate laboratory diagnosis are essential for appropriate patient management. However, cross-reactivity of antibody responses occurring in case of co-infections with different flaviviruses such as ZIKV and chikungunya virus or different DENV serotypes makes virus detection, serological conversion and establishment of differential diagnosis particularly challenging. To date, laboratory diagnosis includes virus isolation, molecular amplification of DENV RNA with reverse transcriptase polymerase chain reaction (RT-PCR), immunoassays to capture the DENV viral protein NS1 and serological approaches (52). In 2009, DENF case definition criteria were redefined by the WHO (52).

Infrastructure capacity to identify pathogens for each Member State

DEN is among the communicable diseases that according to the Commission Implementing Decision (EU) 2018/945 are covered by epidemiological surveillance (53). It means that EU Member States must establish national capacity for detecting and reporting human cases. The decision provides a case definition and clinical and laboratory criteria:

- *Clinical criteria*
  Fever
- *Laboratory criteria*
  A. Probable case:
  Detection of dengue-specific IgM antibodies in a single serum sample
  B. Confirmed case:
  At least one of the following five:
  1. Isolation of a DENV from a clinical specimen;
  2. Detection of dengue viral nucleic acid from a clinical specimen;
  3. Detection of dengue viral antigen from a clinical specimen;
  4. Detection of dengue specific IgM antibodies in a single serum sample AND confirmation by neutralization;
  5. Seroconversion or four-fold antibody titre increase of dengue-specific antibodies in paired serum samples
- *Epidemiological criteria*
  History of travel to, or residence in an area with documented ongoing transmission of dengue, within the two weeks prior to the onset of symptoms
  Case classification:
  A. Possible case:
  Not applicable.

B. Probable case:
   Any person meeting the clinical and the epidemiological criteria, and the laboratory criteria for a probable case,

C. Confirmed case:
   Any person meeting the laboratory criteria for a confirmed case. Regarding travel history and locally acquired case definition, an autochthonous case is defined as any case developing infection without a travel history within 15 days prior to the onset of symptoms in a given study area. In contrast to an imported case where the individual has a travel history (18).

In the EU, diagnosis is routinely made by clinical microbiology laboratories, and there is no European-wide reference laboratory network or national laboratories in most EU countries.

Estimated influence of environmental change on the disease future trends

The major climatic covariates identified for DENV infection are temperature, rainfall, and relative humidity. Except for the temperature, which affects the extrinsic incubation of the virus, those risk factors mainly affect the vector distribution. In continental Europe, temperature increase could favour northward colonization of competent vectors *Ae. albopictus* and, possibly, the re-establishment of *Ae. aegypti* increasing the risk of dengue emergence as predicted by many predictive models (54). Inversely, the risk could decrease if climate change is negatively affecting the climatic suitability for *Ae. aegypti* (55). Modelling studies are predicting a general increase in the risk of *Aedes*-borne diseases in Europe with climate change. However, not all studies agree on the future of the areas currently most at risk. Bouzid *et al.* (56), predict an intensification of the risk in the coastal areas of the Mediterranean and Adriatic, as well as the north-eastern part of Italy (56), on the other hand, Tjaden *et al.* (57), predict that future summer droughts in northern Italy could reduce the habitat suitability for the vectors and consequently the risk of *Aedes*-borne disease (57).

References

1. Gupta N, Srivastava S, Jain A, Chaturvedi UC. Dengue in India. *Ind Med Gaz*. 2012;136(3):373-390.
2. Murugesan A, Manoharan M. Dengue virus. *Emerging and Reemerging Viral Pathogens*. 2020;281-359. https://doi.org/10.1016/B978-0-12-819400-3.00016-8.
3. Rico-Hesse R. Microevolution and virulence of dengue viruses. *Adv Virus Res.* 2003;59:315-341.
4. Harapan H, Michie A, Sasmono RT, Imrie A. Dengue: a minireview. *Viruses*. 2020;12(8):829. https://doi.org/10.3390/v12080829.
5. Holmes EC, Twiddy SS. The origin, emergence and evolutionary genetics of dengue virus. *Infect Genet Evol*. 2003;3(1):19-28. https://doi.org/10.1016/S1567-1348(03)00004-2.
6. Mustafa MS, Rasotgi V, Jain S, Gupta V. Discovery of fifth serotype of dengue virus (DENV-5): A new public health dilemma in dengue control. *Med J Armed Forces India*. 2015;71(1):67-70. https://doi.org/10.1016/j.mjafi.2014.09.011.
7. Pollett S, Melendrez M, Maljkovic Berry I, Duchêne S, Salje H, Cummings D, *et al.* Understanding dengue virus evolution to support epidemic surveillance and counter-measure development. *Infect Genet Evol*. 2018;62:279-295. https://doi.org/10.1016/j.meegid.2018.04.032.

8. Kuhn RJ, Zhang W, Rossmann MG, Pletnev SV, Corver J, Lenches E, *et al.* Structure of dengue virus: implications for flavivirus organization, maturation, and fusion. *Cell*. 2002;108(5):717-725. https://doi.org/10.1016/s0092-8674(02)00660-8.
9. El Sahili A, Lescar J. Dengue virus non-structural protein 5. *Viruses*. 2017;9(4):91. https://doi.org/10.3390/v9040091.
10. Elveborg S, Monteil V, Mirazimi A. Methods of inactivation of highly pathogenic viruses for molecular, serology or vaccine development purposes. *Pathogens*. 2022;11(2):271. https://doi.org/10.3390/pathogens11020271.
11. Idris F, Muharram SH, Zaini Z, Diah S. Effectiveness of physical inactivation methods of dengue virus: heat- versus UV-inactivation. *BioRxriv*. 2018 Sept. https://doi.org/10.1101/427666.
12. Gritsun TS, Lashkevich VA, Gould EA. Tick-borne encephalitis. *Antiviral Res*. 2003;57(1-2):129-146. https://doi.org/10.1016/S0166-3542(02)00206-1.
13. Burnouf T, Chou ML, Cheng LH, Li ZR, Wu YW, El-Ekiaby M, *et al.* Dengue virus inactivation by minipool TnBP/Triton X-45 treatment of plasma and cryoprecipitate. *Vox Sang*. 2013;104(1):1-6. https://doi.org/10.1111/j.1423-0410.2012.01621.x.
14. Jelinek T. Trends in the epidemiology of dengue fever and their relevance for importation to Europe. *Euro Surveill*. 2009;14(25):19250. https://doi.org/10.2807/ese.14.25.19250-en.
15. Ahmed AM, Mohammed AT, Vu TT, Khattab M, Doheim MF, Ashraf Mohamed A, *et al.* Prevalence and burden of dengue infection in Europe: A systematic review and meta-analysis. *Revi Med Virol*. 2020;30(2):e2093. https://doi.org/10.1002/rmv.2093.
16. Cochet A, Calba C, Jourdain F, Grard G, Durand GA, Guinard A, *et al.* Autochthonous dengue in mainland France, 2022: geographical extension and incidence increase. *Euro Surveill*. 2022;27(44). https://doi.org/10.2807/1560-7917.ES.2022.27.44.2200818.
17. Silva NM, Santos NC, Martins IC. Dengue and zika viruses: epidemiological history, potential therapies, and promising vaccines. *Trop Med Infect Dis*. 2020;5(4):150. https://doi.org/10.3390/tropicalmed5040150.
18. Jourdain F, Roiz D, de Valk H, Noël H, L'Ambert G, Franke F, *et al.* From importation to autochthonous transmission: Drivers of chikungunya and dengue emergence in a temperate area. *PLoS Negl Trop Dis*. 2020;14(5):e0008320. https://doi.org/10.1371/journal.pntd.0008320.
19. Giunti G, Becker N, Benelli G. Invasive mosquito vectors in Europe: From bioecology to surveillance and management. *Acta Trop*. 2023;239:106832. https://doi.org/10.1016/j.actatropica.2023.106832.
20. Martins VEP, Alencar CH, Kamimura MT, Araújo FM de C, Simone SGD, Dutra RF, *et al.* Occurrence of natural vertical transmission of dengue-2 and dengue-3 viruses in Aedes aegypti and Aedes albopictus in Fortaleza, Ceará, Brazil. *PLoS One*. 2012;7(7):e41386. https://doi.org/10.1371/journal.pone.0041386.
21. Lequime S, Paul RE, Lambrechts L. Determinants of arbovirus vertical transmission in mosquitoes. *PLoS Pathog*. 2016;12(5):e1005548. https://doi.org/10.1371/journal.ppat.1005548.
22. Lin DCD, Weng SC, Tsao PN, Chu JJH, Shiao SH. Co-infection of dengue and Zika viruses mutually enhances viral replication in the mosquito Aedes aegypti. *Parasit & Vectors*. 2023;16(1):160. https://doi.org/10.1186/s13071-023-05778-1.
23. Stettler K, Beltramello M, Espinosa DA, Graham V, Cassotta A, Bianchi S, *et al.* Specificity, cross-reactivity, and function of antibodies elicited by Zika virus infection. *Science*. 2016;353(6301):823-826. https://doi.org/10.1126/science.aaf8505.
24. Navero-Castillejos J, Benitez R, Torner N, Muñoz J, Camprubí-Ferrer D, Peiró-Mestres A, *et al.* Molecular characterization of imported and autochthonous dengue in Northeastern Spain. *Viruses*. 2021;13(10):1910. https://doi.org/10.3390/v13101910.

25. Liu Z, Zhang Z, Lai Z, Zhou T, Jia Z, Gu J, *et al.* Temperature increase enhances Aedes albopictus competence to transmit dengue virus. *Front in Microbiol*. 2017;8. https://www.frontiersin.org/articles/10.3389/fmicb.2017.02337

26. Gwee SXW, St John AL, Gray GC, Pang J. Animals as potential reservoirs for dengue transmission: A systematic review. *One Health*. 2021;12:100216. https://doi.org/10.1016/j.onehlt.2021.100216.

27. Zeng Z, Zhan J, Chen L, Chen H, Cheng S. Global, regional, and national dengue burden from 1990 to 2017: A systematic analysis based on the global burden of disease study 2017. *eClinicalMedicine*. 2021;32. https://doi.org/10.1016/j.eclinm.2020.100712.

28. Messina JP, Brady OJ, Scott TW, Zou C, Pigott DM, Duda KA, *et al.* Global spread of dengue virus types: mapping the 70 year history. *Trends Microbiol*. 2014;22(3):138-146. https://doi.org/10.1016/j.tim.2013.12.011.

29. Weaver SC, Vasilakis N. Molecular evolution of dengue viruses: contributions of phylogenetics to understanding the history and epidemiology of the preeminent arboviral disease. *Infect Genet Evol*. 2009;9(4):523-540. https://doi.org/10.1016/j.meegid.2009.02.003.

30. Buchs A, Conde A, Frank A, Gottet C, Hedrich N, Lovey T, *et al.* The threat of dengue in Europe. *New Microbes New Infect*. 2022;49-50:101061. https://doi.org/10.1016/j.nmni.2022.101061.

31. Guzman MG, Gubler DJ, Izquierdo A, Martinez E, Halstead SB. Dengue infection. *Nat Rev Dis Primers*. 2016;2(1):1-25. https://doi.org/10.1038/nrdp.2016.55.

32. Kok BH, Lim HT, Lim CP, Lai NS, Leow CY, Leow CH. Dengue virus infection - a review of pathogenesis, vaccines, diagnosis and therapy. *Virus Res*. 2023;324:199018. https://doi.org/10.1016/j.virusres.2022.199018.

33. World Health Organization. *Dengue haemorrhagic fever: diagnosis, treatment, prevention and control, 2nd ed*. Geneva: WHO; 1997.

34. Gainor EM, Harris E, LaBeaud AD. Uncovering the burden of dengue in Africa: considerations on magnitude, misdiagnosis, and ancestry. *Viruses*. 2022;14(2):233. https://doi.org/10.3390/v14020233.

35. Lefeuvre C, Apaire-Marchais V. Dengue, chikungunya et Zika, des arboviroses émergentes en France métropolitaine. *Actual Pharm*. 2021;60(608):21-27. https://doi.org/10.1016/j.actpha.2021.06.008.

36. Kularatne SA, Dalugama C. Dengue infection: Global importance, immunopathology and management. *Clin Med*. 2022;22(1):9-13. https://doi.org/10.7861/clinmed.2021-0791.

37. Tayal A, Kabra SK, Lodha R. Management of dengue: an updated review. *Indian J Pediatr*. 2023;90(2):168. https://doi.org/10.1007/s12098-022-04394-8.

38. Chan CY, Ooi EE. Dengue: an update on treatment options. *Future Microbiol*. 2015;10(12):2017-2031. https://doi.org/10.2217/fmb.15.105.

39. Angelin M, Sjölin J, Kahn F, Ljunghill Hedberg A, Rosdahl A, Skorup P, *et al.* Qdenga® - A promising dengue fever vaccine; can it be recommended to non-immune travelers? *Travel Med Infect Dis*. 2023;54:102598. https://doi.org/10.1016/j.tmaid.2023.102598.

40. Guy B, Saville M, Lang J. Development of Sanofi Pasteur tetravalent dengue vaccine. *Hum Vaccin*. 2010;6(9).

41. Wong JM, Adams LE, Durbin AP, Muñoz-Jordán JL, Poehling KA, Sánchez-González LM, *et al.* Dengue: a growing problem with new interventions. *Pediatrics*. 2022;149(6):e2021055522. https://doi.org/10.1542/peds.2021-055522.

42. Kariyawasam R, Lachman M, Mansuri S, Chakrabarti S, Boggild AK. A dengue vaccine whirlwind update. *Ther Adv in Infect Dis*. 2023;10:20499361231167270. https://doi.org/10.1177/20499361231167274.

43. Patel SS, Rauscher M, Kudela M, Pang H. Clinical safety experience of TAK-003 for dengue fever: a new tetravalent live attenuated vaccine candidate. *Clin Infect Dis*. 2023;76(3):e1350-e1359. https://doi.org/10.1093/cid/ciac418.

44. Caragata EP, Dutra HLC, Sucupira PHF, Ferreira AGA, Moreira LA. Wolbachia as translational science: controlling mosquito-borne pathogens. *Trends Parasitol*. 2021;37(12):1050-1067. https://doi.org/10.1016/j.pt.2021.06.007.

45. Oliva CF, Benedict MQ, Collins CM, Baldet T, Bellini R, Bossin H, *et al.* Sterile insect technique (sit) against aedes species mosquitoes: a roadmap and good practice framework for designing, implementing and evaluating pilot field trials. *Insects*. 2021;12(3):191. https://doi.org/10.3390/insects12030191.

46. European Centre for Disease Prevention and Control. *Information for travellers to areas with chikungunya transmission*. Stockholm: ECDC. Available from: https://www.ecdc.europa.eu/en/chikungunya/threats-and-outbreaks/information-travellers; last visited 20 July 2023.

47. Schaffner F, Mathis A. Dengue and dengue vectors in the WHO European region: past, present, and scenarios for the future. *Lancet Infect Dis*. 2014;14(12):1271-1280. https://doi.org/10.1016/S1473-3099(14)70834-5.

48. Gjenero-Margan I, Aleraj B, Krajcar D, Lesnikar V, Klobučar A, Pem-Novosel I, *et al.* Autochthonous dengue fever in Croatia, August–September 2010. *Euro Surveill*. 2011;16(9):19805. https://doi.org/10.2807/ese.16.09.19805-en.

49. La Ruche G, Souarès Y, Armengaud A, Peloux-Petiot F, Delaunay P, Desprès P, *et al.* First two autochthonous dengue virus infections in metropolitan France, September 2010. *Euro Surveill*. 2010;15(39):19676.

50. Cassaniti I, Ferrari G, Senatore S, Rossetti E, Defilippo F, Maffeo M, *et al.* Preliminary results on an autochthonous dengue outbreak in Lombardy Region, Italy, August 2023. *Euro Surveill*. 2023;28(37). https://doi.org/10.2807/1560-7917.ES.2023.28.37.2300471.

51. Poinsignon A, Boulanger D, Binetruy F, Elguero E, Darriet F, Gallian P, *et al.* Risk factors of exposure to *Aedes albopictus* bites in mainland France using an immunological biomarker. *Epidemiol Infect*. 2019;147:e238. https://doi.org/10.1017/S0950268819001286.

52. Muller DA, Depelsenaire ACI, Young PR. Clinical and Laboratory diagnosis of dengue virus infection. *J Infect Dis*. 2017;215(suppl_2):S89-S95. https://doi.org/10.1093/infdis/jiw649.

53. Europe. Commission Implementing Decision (EU) 2018/945 of 22 June 2018 on the communicable diseases and related special health issues to be covered by epidemiological surveillance as well as relevant case definitions. *Official Journal of the European Union* L 170, 6/7/2018.

54. Kulkarni MA, Duguay C, Ost K. Charting the evidence for climate change impacts on the global spread of malaria and dengue and adaptive responses: a scoping review of reviews. *Global Health*. 2022;18(1):1. https://doi.org/10.1186/s12992-021-00793-2.

55. Semenza JC, Suk JE. Vector-borne diseases and climate change: a European perspective. *FEMS Microbiol Lett*. 2018;365(2):fnx244. https://doi.org/10.1093/femsle/fnx244.

56. Bouzid M, Colón-González FJ, Lung T, Lake IR, Hunter PR. Climate change and the emergence of vector-borne diseases in Europe: case study of dengue fever. *BMC public health*. 2014;14:781. https://doi.org/10.1186/1471-2458-14-781.

57. Tjaden NB, Suk JE, Fischer D, Thomas SM, Beierkuhnlein C, Semenza JC. Modelling the effects of global climate change on Chikungunya transmission in the 21st century. *Sci Rep*. 2017;7(1):3813. https://doi.org/10.1038/s41598-017-03566-3.

DISEASE X

Soushieta Jagadesh (a), Claudia Cataldo (b), Annapaola Rizzoli (c), Esther van Kleef (d), Wim Van Bortel (e), Luca Busani (b)
(a) International Society of Infectious Diseases, Boston (MA)
(b) Centro di Riferimento per la Medicina di Genere, Istituto Superiore di Sanità, Rome
(c) Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige, Trento
(d) Department of Public Health, Institute of Tropical Medicine, Antwerp
(e) Department of Biomedical Sciences, Outbreak Research Team, Institute of Tropical Medicine, Antwerp

Term definition

Disease X is a term coined by the World Health Organisation (WHO) in 2018 during the second annual review of the Blueprint list of priority diseases. The term refers to a disease caused by an unknown pathogen that could emerge as a future epidemic or pandemic (1). Disease X is caused by Pathogen X, an infectious agent that is not currently known to cause human disease, but it is an aetiologic agent of a future outbreak with epidemic or pandemic potential (2). Since the terms' introduction, COVID-19 caused by the SARS-CoV-2 virus strain was the first disease to meet the requirements of Disease X (3). Most emerging infectious diseases posing an international public health threat in the recent decades originated from wildlife reservoirs (4), as was seen with the emergence of the H1N1 influenza, the highly pathogenic H5N1 avian influenza (5), the Severe Acute Respiratory Syndrome Coronavirus (SARS) (6), Ebola Virus Disease (EVD) (7), COVID-19, and Mpox (previously known as Monkeypox) (8).

Various pathogens, including viruses, bacteria, fungi, parasites, and prions can potentially be causative agents of Disease X. Transmission characteristics, host requirements, and availability of antimicrobials have seen viruses as the predominant causative-agents of outbreaks (9-11). RNA viruses specifically make up about 94% of zoonotic viruses with their ability to spillover from non-human hosts to humans, evade host defences and reproduce across a variety of host species due to their great mutability (12-14). The high mutation rates in RNA viruses lead to their rapid evolution and environmental adaptability to reach adaptive equilibrium within their host species very quickly (15). Their ability to acquire significant resistance to antiviral treatment after a brief exposure as observed with HIV and antiretroviral treatment is also an additional cause of concern (16). Furthermore, RNA viruses are found to be the most common pathogens causing emerging infections in humans, with a rate of two to three novel viruses being discovered each year (14).

Therefore, in this report, we focus on viral families with pandemic potential characteristics rather than on a specific virus that may or may not present a future threat. Prevention and control countermeasures, including pharmacological measures and vaccines, against one member of the family could easily be adapted to another member in due course when the next threat emerges.

The pandemic potential characteristics defining the high-risk viral families are: 1) no pre-existing immunity in the global population; 2) transmittable by asymptomatic cases; 3) transmitted via airborne or direct contact; 4) zoonotic potential; and 5) no existing, effective therapeutics or vaccines (17-19). This criterion was developed following foundational assessments on viral families from Johns Hopkins University (17) and was subsequently used in the "Framework for U.S. Pandemic Preparedness Policy" (18). In Table 1, a list of high-risk viral families that could emerge to the next Disease X are presented.

Table 1. Description of the potential agents for Disease X

Viral family	Notable viruses	Mode of transmission	Zoonotic transmission	Genetic material	Existing interventions
Adenoviridae	Adenovirus 7, 14	Respiratory, fecal-oral	Confirmed	DNA	Vaccine available for Type 4 and 7
Arenaviridae	Lassa fever virus*, lymphocytic choriomeningitis	Direct contact with the blood or other body fluids of infected individuals or contaminated objects	Confirmed	RNA	None
Coronaviridae	SARS CoV 1 and 2*, MERS CoV*	Respiratory, fecal-oral, surface contact	Confirmed	RNA	Vaccines available SARS-CoV-2
Filoviridae	Ebola virus (EBOV)*, Marburg virus*	Direct contact with the blood or other body fluids of infected individuals or contaminated objects	Confirmed	RNA	None
Orthomyxoviridae	Influenza	Respiratory, water	Confirmed	RNA	Influenza (including seasonal H1N1, seasonal Influenza B, and H5N1)
Paramyxoviridae	Nipah virus*, Hendra virus*, Mumps virus, Rubeola virus	Respiratory, Direct contact with the blood or other body fluids of infected individuals or contaminated objects	Confirmed	RNA	Vaccines for Rubeola and mumps
Picornaviridae	Poliovirus, foot-and-mouth disease virus	Respiratory, surface contact	None confirmed	RNA	Vaccines for Poliovirus and Hepatitis A
Pneumoviridae	Respiratory Syncytial Virus (RSV)	Respiratory	None confirmed	RNA	Vaccine and monoclonal antibodies against RSV
Poxviridae	Variola virus, Varicella virus, Mpox virus*	Respiratory, surface contact	Confirmed	DNA	Vaccines against Variola and varicella

*Diseases included in the 2023 updated WHO Blueprint priority diseases (1)
adapted from "Viral Families and Disease X: A Framework for U.S. Pandemic Preparedness Policy", 2023 (18)

Apart from viruses, considering bacterial pathogens, the emergence of antibiotic multi-resistant bacteria poses a significant threat to global public health, prompting consideration of such strains as potential candidates for Disease X, representing an unknown and potentially catastrophic infectious disease. Treating antibiotic multi-resistant bacteria as Disease X has advantages and disadvantages. While it may enhance global preparedness and collaboration, there are potential negative consequences, such as stigmatization and resource diversion (20-22).

Emergence of newly multi-drug-resistant bacterial strains is often regarded as an existing, endemic condition that requires a different approach to address AntiMicrobial Resistance (AMR) including surveillance of AMR and antimicrobial consumption/use and ensuring universal access to quality diagnosis and appropriate treatment of infections (23, 24). Whereas current research towards Disease X is exclusively focused on research and development (R&D) for medical

countermeasures and health systems optimization against viral threats (25, 26). Therefore, in this report, while acknowledging the importance of AMR as global public health threat of high importance, we consider only viral threats for Disease X.

Description of the potential disease agents

In this report, we discuss the characteristics of the nine viral families mentioned above that the Pathogen X could belong to.

Adenoviridae

Adenoviruses are large (~950 Å) and complex non-enveloped virions with an icosahedral capsid (70 to 100 nm) is made up of 252 capsomeres with 240 hexons forming the faces and 12 pentons at the vertices (27). Each penton bears a trimetric fibre that aids in attachment to the host cell via the receptor on its surface (28). The double-stranded linear DNA, between 26K and 48K base pairs (bp) on length, is associated with two major core proteins and carries a 55-kDa protein covalently attached to its 5′ end (27).

Arenaviridae

Arenaviridae are spherical or pleomorphic in shape, 40-200 nm in diameter, with dense lipid envelopes containing bi-segmented negative strand uncapped RNA segments (29), L (ca. 7.3 kbp) and S (ca. 3.5 kbp), that are encapsidated independently (30–32). The virion surface layer is covered with club-shaped projections which consist of trimeric spike structures of two virus-encoded membrane glycoprotein subunits (GP1 and GP2) and, in some, a third component (stable signal peptide (SSP)) (33). Isolated ribonucleoprotein (RNP) complexes are organized into "beads-on-a-string"-like structures (34).

Coronaviridae

This family consists of large, enveloped particles decorated with 20nm long club- or petal-shaped surface projections (the "peplomers" or "spikes"), and single-stranded capped, polyadenylated, positive-strand RNA viruses with genomes ranging from 25 to 32 kbp and a roughly spherical virion of 118-136 nm in diameter (35,36). Within the envelope is a flexible (subfamily *Coronavirinae*) or a doughnut-shaped (subfamily *Torovirinae*) nucleocapsid.

Filoviridae

Filoviruses are filamentous, enveloped particles with a nucleocapsid containing non-segmented, negative-sense, single-stranded RNA genome, approximately 19 kb long and four viral structural proteins (37, 38).

Orthomyxoviridae

Influenza viruses are spherical or pleomorphic enveloped particles 80 to 120 nm in diameter (39, 40). The helically symmetric nucleocapsid consists of a nucleoprotein and a multipartite genome (10.0 to 14.6 kbp) of single-stranded negative sense RNA in seven or eight segments, 50-

150 nm in length. The envelope carries 500 distinct spike-like surface projections including the hemagglutinin attachment protein and a neuraminidase in a 10:1 ratio respectively (41).

Paramyxoviridae

All paramyxoviruses are enveloped virions 150 to 300 nm in diameter with a tubelike, helically symmetrical nucleocapsid containing a monopartite, single-stranded, negative-sense RNA genome (14 to 20 kbp in size) and an RNA-directed RNA polymerase (42, 43). The genome is uncapped, and the genome 3′-end is not polyadenylated. The nucleocapsid associates with the matrix protein (M) at the base of a double-layered lipid envelope.

Picornaviridae

Picornaviruses are small (22 to 30 nm) nonenveloped, single-stranded, uncapped positive sense (7 to 8.5 kbp) RNA viruses with cubic symmetry (44, 45). The virus capsid is composed of 60 identical subunits called protomers, each consisting of four polypeptides VP1–VP4 (46).

Pneumoviridae

This family consists of large filamentous enveloped negative-strand RNA viruses of 70-190 nm in diameter and up to 2 μm in length (47). Virions consist of a lipid envelope surrounding a nucleocapsid. The RNA length genome varies between genera, 13 to 15 kbp.

Poxviridae

Poxvirus virions are large and brick shaped, approximately 220-450 nm long, 140-260 nm wide and 140-260 nm thick with short surface tubules 10 nm wide in the lipoprotein surface membrane (48, 49). The genome consists of double-stranded DNA, from 128 to 375 kbp, and the core contains enzymes for virus entry and replication.

Priority level for EU

The COVID-19 pandemic highlighted limitations of outbreak response strategies and systems adopted within the European Union and its Member States, to respond quickly and effectively to a pandemic of this unprecedented scale, caused by an unknown pathogen (50, 51).

The pandemic demonstrated the ease with which a respiratory pathogen could spread via air travel and as a result of global connectivity (52). Coupled with densely populated cities with an immunologically naïve population, such an introduction of a novel pathogen led to an unprecedented public health crisis.

To prevent and ensure improved response to future threats, Health Emergency preparedness and Response Authority (HERA) was launched as a new European Commission Directorate-General on 16 September 2021 (53). HERA focuses on research on Pathogen X including threat assessment, horizon scanning for the identification of potential medical countermeasures and innovative technologies, and the development of standardized research protocols.

Drivers of the disease emergence and spread

Anthropogenic changes in the environment, such as human encroaching on forests and wild areas, biodiversity loss, and changes in host and vector population dynamics, have been associated with an increased risk of disease outbreaks and emerging diseases (54-58). Here we discuss the drivers of disease emergence and spread established for the high-risk viral families as summarised in Table 2.

Table 2. Drivers of Disease X emergence and spread for each high-risk viral family in an EU context

Viral family	Common drivers	Threat to the EU	Potential emergence and spread in the EU	Previous outbreaks in the EU
Adenoviridae	Land use changes and wildlife trade	Medium	Endemic with reports of antropozoonosis (57)	Endemic species in Europe
Arenaviridae	Land use changes, biodiversity loss, climate change	Low	Possibility of imported cases but currently no known reservoirs for arenaviruses in Europe	None
Coronaviridae	Land use changes, biodiversity loss, wildlife trade, monoculture, climate change and global travel	High	Although the risk of emergence is low in EU, coronaviruses being respiratory pathogens are easily transmitted	COVID-19 pandemic
Filoviridae	Land use changes, biodiversity loss, wildlife trade, global travel and conflict	Low	Possibility of imported cases but currently no known reservoirs for filoviruses in Europe	Laboratory outbreaks of Marburg virus in Germany in 1976 and EBOV in England in 1976. One imported case of Ebola in 2014
Orthomyxoviridae	Land use changes, monoculture, climate change, war/conflict, and global travel and trade	High	Avian and seasonal influenza are well established in Europe	Several outbreaks of different influenza strains have been recorded in Europe
Paramyxoviridae	Land use changes, biodiversity loss, wildlife trade, monoculture, and climate change	Low-medium	Rubeola and mumps are endemic vaccine preventable diseases in Europe, however the recent zoonotic diseases are currently geographically restricted to South and South-East Asia and Australia	Endemic species in Europe
Picornaviridae	No known environmental drivers.	Low-medium	Hepatitis A and Polio are vaccine preventable diseases	Hepatitis A endemic in Europe
Pneumoviridae	No known environmental drivers.	Low-medium	Human metapneumovirus and RSV are only known virus affecting humans in this family and they are endemic to Europe	Human outbreaks of known viruses endemic
Poxviridae	Biodiversity loss and wildlife trade	Medium-high	Imported cases from West Africa with sustained human to human	Mpox outbreak in May 2022 declared by WHO European region

Land-use changes

The extension of agricultural cultivation into forests through deforestation and habitat fragmentation may perturb the existing zoonotic transmission cycles (59, 60).

The emergence of bat-associated viruses: bats play a critical role as reservoirs for new infectious diseases due to their unique immune systems, extended lifespans, and ability to host diverse viruses. Their natural behaviours, such as roosting in large colonies and migrating over vast distances, facilitate viral transmission and spread (61). Zoonotic spillover events, where viruses jump from bats to other animals and eventually to humans, have been implicated in outbreaks like Ebola, SARS, and COVID-19. Outbreaks from the *Paramyxoviridae* family, Nipah and Hendra viruses were associated with loss of bat habitat due to deforestation and agricultural expansion (59, 62, 63).

The emergence of rodent-associated viruses: Rodents serve as reservoirs for various infectious diseases, harbouring pathogens with zoonotic potential. Their proximity to human habitats increases the risk of disease transmission. The adaptability and widespread distribution of rodents contribute to the emergence and spread of infectious diseases. Like bats, they provide examples of disease emergence due to agricultural encroachment into forests, like the outbreak of Argentine Haemorrhagic Fever (AHF), caused by the Junín virus, an arenavirus. As the pampas of Argentina was cleared for maize cultivation, the rodent *Calomys musculinus*, a natural host of Junín virus, increased in population (64,65). The infected *Calomys* species shed virus in their urine, which was aerosolised by agricultural machinery infecting agricultural workers. Similar outbreaks due agricultural expansion has documented with other arenavirus diseases such as Bolivian HF and Lassa fever, caused by the Machupo virus and Lassa fever virus, respectively, via their natural respective host rodents *Calomys callosus* and *Mastomys natalensis* (66, 67).

Biodiversity loss

Studies demonstrate that reforestation can increase biodiversity loss and disease transmission when land conversion of grasslands, savannas, and open-canopy woodlands in temperate regions are reforested (56, 68, 69). For example, the incidence of tick-borne encephalitis in humans in Italy was explained by the ratio of coppice to high stand forest in Italy with natural reforestation that favoured the abundance of the roe deers, reservoirs of tick-borne viruses (70). On the other hand, loss of biodiversity is found to play a major role with frequent emergence and transmission of zoonoses (71-73). Biodiversity loss in ecological communities is a consequence of loss of large-bodied species with slower life histories (74) and increasing abundance of smaller-bodied species with fast life histories (75), which are more likely to transmit zoonotic pathogens (76). Furthermore, land-use changes caused by humans has been found to increase in the abundance of a single species, often zoonotic host species and reduce the diversity of non-hosts due to habitat loss (60). Thus, biodiversity loss potentially leads to increase in of zoonotic reservoirs population and therefore, increased risk of spillover.

Wildlife trade

Wildlife trade for consumption and recreation plays a major role in zoonoses emergence. Hunting, preparing, and selling bushmeat has been linked with spillover due to frequent contact with infectious materials from wildlife (77, 78). For example, EBOV spillover events and subsequent outbreaks in the Congo Basin have been traced back to hunters who were exposed to Ebola contaminated ape carcasses (79, 80). Another example of spillover from bushmeat

consumption, is the SARS epidemic (2003-2004) that emerged from the Pearl River delta region in Guangdong, China (81). The first cases of the SARS, a disease caused by a coronavirus, were likely wild animal handlers in markets and restaurants serving bushmeat (82, 83). Wildlife trade such as importation of experimental mammals have led outbreak events as seen with Marburg virus in Marburg, Germany and EBOV in Virginia, US in 1969 (84) and 1989 (85), respectively.

Monoculture

Monocultures of livestock and poultry for the purpose of increasing productivity and improving management, promote susceptibility to infection (15) and facilitates disease transmission by increasing population size and density (86-88). Avian influenza which is of relatively low pathogenicity in wild water birds (89, 90) is highly pathogenic in domestic poultry (54) especially with some strains. Studies demonstrate that both extensive and intensive farming practices drive influenza virus spillover from wild birds to domestic birds and pigs and thereby, infecting humans (89, 91). Rice fields combined with free-grazing duck farming in wetlands bring wild water birds into proximity with domestic ducks (91) and can transmit the pathogen to domestic poultry (92). Low genetic diversity in the immunologically naïve domestic population encourages rapid dissemination and amplification of infection (71, 88). In regions where swine farming is in proximity to poultry farming, pigs serve as "mixing vessels" to generate reassortants which are potential candidates for new pandemic strains, are they are susceptible to both avian and human influenza viruses (93,94). Intensification of camel herding in the Arabian Peninsula was associated with Middle East Respiratory Syndrome (MERS) outbreak (95). MERS is caused was MERS-CoV, a member of *Coronaviridae.*

Climate change

Climate change such as increasing temperatures and precipitation changes causes shifts in the geographical range of various pathogen hosts, vectors, and reservoirs (96, 97). Climate change also has an indirect impact on vegetation and ecosystems which affects the geographical range and migration of pathogen hosts and reservoirs (98,99). Extreme climatic events such as drought, heatwaves, wildfires, storms, and floods cause habitat destruction and are linked to Nipah (100) and Ebola (101) spillovers from wildlife moving to safer areas foraging for limited food resources. Global warming was related to melting ice and thawing of the permafrost exposing once-frozen pathogens (102). Studies demonstrated a genetic analysis of an anthrax outbreak in the Arctic circle suggesting that the bacterial strain emerged from a once infected reindeer corpse as the frozen ground thawed (103). Climate also influenced human social gatherings and the transmissibility of viruses such as influenza (104) and COVID-19 (105). Some studies suggested that heavy rainfall could induce social isolation, thereby reducing COVID-19 cases (105). However, in Indonesia and Pakistan, increased rainfall and temperatures were associated with increased cases of COVID-19 reflecting different behavioural responses to weather (106). Destruction and damage to infrastructure such as sewage systems and portable drinking water due to climatic disasters influence disease outbreaks such as hepatitis A (107).

Global travel and conflict

Global travel enables asymptomatic viraemic travellers to transmit across borders, introducing a novel pathogen to a seeming naïve population. The effect of travel on global pandemics as in the case of the SARS outbreak 2003-2004 demonstrated that virus can be spread around the world

by international air travellers (108). Travelers from Singapore, Canada and Vietnam became infected following their visit to Hong Kong in March 2003, leading to SARS-CoV-1 infections in twenty countries in a month's time. A similar consequence of global connectivity was observed with SARS-CoV-2 transmission across 229 countries with a total of 698,446,176 confirmed cases of COVID-19 that originated from Wuhan, China (109). War and conflict lead to the displacement of large populations into temporary overcrowded settlements with inadequate safe water and sanitation, need for sustenance hunting of wildlife, and higher risk of exposure to disease vectors and reservoirs (110). Exacerbation and increased geographical spread of Lassa fever (110) and Ebola (111, 112) outbreaks, in West and Central Africa respectively, can be attributed to local unrest and conflict.

Diagnostic procedures and notification systems used at local, national, and European scale

The Zoonoses Directive 2003/99/EC obliges EU Member States to collect relevant and, when applicable, comparable data on zoonoses, zoonotic agents, antimicrobial resistance, and foodborne outbreaks (113). In 2004, the European Commission entrusted European Food Safety Authority (EFSA) with the task of setting up an electronic reporting system and database for monitoring zoonoses (EFSA Mandate No 2004-0178, continued by M-2015-0231) (114). However, this monitoring system was exclusively meant for assessing zoonoses and pathogens emerging within the European region, and not for detecting outside threats. To detect imported infectious disease threats, TropNet (European Tropical and Travel Medicine Network) was founded in 1999 to create a "European Network on Imported Infectious Disease Surveillance" (http://tropnetdev.netsysco.net/about-us/).

Epidemic intelligence systems rapidly detect and assess outbreak events of any origin to ensure the EU's health security by collating information from a variety of sources, which is then validated and analysed. Epipulse (restricted access) and Epitweeter (public access via a R package) are tools developed by the European Centre for Disease Prevention and Control (ECDC) for public health threat detection (115). Early Warning and Response System (EWRS) is another tool with restrictive access that monitors public health threats in the EU (116).

Surveillance of animal health and zoonotic threats in the EU is assessed by various disease intelligence and surveillance systems. Platform for Automated extraction of Disease Information from the web (PADI web) is a multilingual event-based surveillance system dedicated to the monitoring of online news sources for the detection of animal health infectious events (117). Nationally, France monitors animal diseases are a potential threat to public health, the economy and the environment via the epidemiological surveillance in animal health, ESA platform coordinated by ANSES (the French Agency for Food, Environmental and Occupational Health & Safety) and the French Ministry of Agriculture (Directorate General for Food, DGAL) (118).

The other EU agencies involved in outbreak preparedness for future threats include the Directorate-General for Health and Food Safety (DG-SANTE), The Directorate-General for Research and Innovation (DG-RTD), European Medicines Agency (EMA), EU Civil Protection Mechanism, and more recently, HERA (50).

Monitoring Outbreak events for Disease surveillance project (MOOD: https://mood-h2020.eu/about-mood/), a part of the European program Horizon 2020, aims to improve epidemic intelligence tools and services for the early detection, assessment, and monitoring of current and future infectious disease threats such as Disease X across Europe in the context of continuous global, environmental, and climatic change.

References

1. World Health Organization. Prioritizing diseases for research and development in emergency contexts. Geneva: WHO. Available from: https://www.who.int/activities/prioritizing-diseases-for-research-and-development-in-emergency-contexts; last visited 11 October 2023.
2. Simpson S, Kaufmann MC, Glozman V, Chakrabarti A. Disease X: accelerating the development of medical countermeasures for the next pandemic. *Lancet Infect Dis.* 2020;20(5):e108-e115. doi:10.1016/S1473-3099(20)30123-7.
3. Jiang S, Shi ZL. The first Disease X is caused by a highly transmissible acute respiratory syndrome Coronavirus. *Virol Sin* 2020;35 (3):263-265. https://doi.org/10.1007/s12250-020-00206-5.
4. Jones KE, Patel NG, Levy MA, *et al.* Global trends in emerging infectious diseases. Nature. 2008;451(7181):990-993. doi:10.1038/nature06536.
5. Joseph U, Su YCF, Vijaykrishna D, Smith GJD. The ecology and adaptive evolution of influenza A interspecies transmission. *Influenza Other Respir Viruses.* 2017;11(1):74-84. https://doi.org/10.1111/irv.12412.
6. LeDuc JW, Barry MA. SARS, the first pandemic of the 21st century. *Emerg Infect Dis.* 2004;10(11):e26. doi:10.3201/eid1011.040797_02
7. Kalra S, Kelkar D, Galwankar SC, *et al.* The emergence of ebola as a global health security threat: from 'lessons learned' to coordinated multilateral containment efforts. *J Glob Infect Dis.* 2014;6(4):164-177. doi:10.4103/0974-777X.145247.
8. Ren SY, Li J, Gao RD. 2022 Monkeypox outbreak: Why is it a public health emergency of international concern? What can we do to control it? *World J Clin Cases*. 2022;10(30):10873-10881. doi:10.12998/wjcc.v10.i30.10873.
9. Morse SS, Mazet JA, Woolhouse M, *et al.* Prediction and prevention of the next pandemic zoonosis. *Lancet*. 2012;380(9857):1956-1965. doi:10.1016/S0140-6736(12)61684-5.
10. Kelly TR, Karesh WB, Johnson CK, Gilardi KVK, Anthony SJ, Goldstein T, *et al.* One Health proof of concept: Bringing a transdisciplinary approach to surveillance for zoonotic viruses at the human-wild animal interface. *Prev Vet Med*. 2017;137:112-118. https://doi.org/10.1016/j.prevetmed.2016.11.023.
11. Institute of Medicine (US) Forum on Microbial Threats. *Microbial evolution and co-adaptation: a tribute to the life and scientific legacies of Joshua Lederberg: Workshop Summary*. Washington, DC: National Academies Press (US); 2009.
12. Kreuder Johnson C, Hitchens PL, Smiley Evans T, Goldstein T, Thomas K, Clements A, *et al.* Spillover and pandemic properties of zoonotic viruses with high host plasticity. *Sci Rep*. 2015;5:14830. https://doi.org/10.1038/srep14830.
13. Carrasco-Hernandez R, Jácome R, López Vidal Y, Ponce de León S. Are RNA viruses candidate agents for the next global pandemic? A review. *ILAR J*. 2017;58(3):343-358. https://doi.org/10.1093/ilar/ilx026.
14. Rosenberg R. Detecting the emergence of novel, zoonotic viruses pathogenic to humans. *Cell Mol life Sci: CMLS*. 2015;72(6):1115-1125. https://doi.org/10.1007/s00018-014-1785-y.
15. Hui E. Reasons for the increase in emerging and re-emerging viral infectious diseases. *Microbes Infect*. 2006;8(3):905-916. https://doi.org/10.1016/j.micinf.2005.06.032.
16. Lucas GM. Antiretroviral adherence, drug resistance, viral fitness and HIV disease progression: a tangled web is woven. *J Antimicrob Chemother*. 2005;55(4):413-416. https://doi.org/10.1093/jac/dki042.

17. Adalja AA, Watson M, Toner ES, Cicero A, Inglesby TV. Characteristics of microbes most likely to cause pandemics and global catastrophes. *Curr Top Microbiol Immunol.* 2019;424:1-20. doi:10.1007/82_2019_176

18. Schuerger C, Batalis S, Quinn K, Adalja A, Puglisi A. *Viral Families and Disease X: A Framework for U.S. Pandemic Preparedness Policy*. Washington, DC: Center for Security and Emerging Technology; 2023.

19. Coalition for Epidemic Preparedness Innovations. Using AI to speed up vaccine development against Disease X | *PreventionWeb*. 18 July 2023. Available from: https://www.preventionweb.net/news/using-ai-speed-vaccine-development-against-disease-x; last visited 27 November 2023.

20. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, *et al.* Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. 2012;18(3):268-281. https://doi.org/10.1111/j.1469-0691.2011.03570.x.

21. Salam MA, Al-Amin MY, Salam MT, Pawar JS, Akhter N, Rabaan AA, Alqumber MAA. Antimicrobial Resistance: A Growing Serious Threat for Global Public Health. *Healthcare*. 2023; 11(13):1946. https://doi.org/10.3390/healthcare11131946

22. Morens DM, Folkers GK, Fauci AS. The challenge of emerging and re-emerging infectious diseases. *Nature*. 2004;430(6996):242-249. https://doi.org/10.1038/nature02759.

23. MacIntyre CR, Bui CM. Pandemics, public health emergencies and antimicrobial resistance - putting the threat in an epidemiologic and risk analysis context. *Arch Public Health*. 2017;75:54. https://doi.org/10.1186/s13690-017-0223-7.

24. World Health Organization. *Antimicrobial resistance*. Geneva: WHO; 2023. Available from: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance; last visited 8 December 2023.

25. Radanliev P, De Roure D. Disease X vaccine production and supply chains: risk assessing healthcare systems operating with artificial intelligence and industry 4.0. *Health Technol (Berl)*. 2023;13(1):11-15. https://doi.org/10.1007/s12553-022-00722-2.

26. Coalition for Epidemic Preparedness Innovations. *CEPI and University of Oxford to accelerate vaccine development against Disease X. CEPI* 29th August 2023. Available: https://cepi.net/news_cepi/cepi-and-university-of-oxford-to-accelerate-vaccine-development-against-disease-x/; last visited 8 December 2023.

27. Doerfler W. Adenoviruses. In: Baron S (Ed.) *Medical microbiology*. 4th Ed. Galveston, TX: University of Texas Medical Branch at Galveston; 1996. Chapter 67.

28. Gallardo J, Pérez-Illana M, Martín-González N, San Martín C. Adenovirus structure: what is new? *Int J Mol Sci*. 2021;22(10):5240. https://doi.org/10.3390/ijms22105240.

29. Leung WC, Ghosh HP, Rawls WE. Strandedness of Pichinde virus RNA. *J Virol*. 1977;22 (1):235-237. https://doi.org/10.1128/JVI.22.1.235-237.1977.

30. Meyer BJ, de la Torre JC, Southern PJ. Arenaviruses: genomic RNAs, transcription, and replication. *Curr Top in Microbiol Immunol*. 2002;262:139-157. https://doi.org/10.1007/978-3-642-56029-3_6.

31. Fedeli C, Moreno H, Kunz S. Novel insights into cell entry of emerging human pathogenic Arenaviruses. *J Mol Biol*. 2018;430(13):1839-1852. https://doi.org/10.1016/j.jmb.2018.04.026.

32. Hetzel U, Sironen T, Laurinmäki P, Liljeroos L, Patjas A, Henttonen H, *et al.* Isolation, identification, and characterization of novel arenaviruses, the etiological agents of boid inclusion body disease. *J Virol*. 2013;87(20):10918-10935. https://doi.org/10.1128/JVI.01123-13.

33. Buchmeier MJ. Arenaviruses: protein structure and function. *Curr Top Microbiol Immunol*. 2002;262:159-173. https://doi.org/10.1007/978-3-642-56029-3_7.

34. Neuman BW, Adair BD, Burns JW, Milligan RA, Buchmeier MJ, Yeager M. Complementarity in the supramolecular design of arenaviruses and retroviruses revealed by electron cryomicroscopy and image analysis. *J Virol*. 2005;79 (6):3822-3830. https://doi.org/10.1128/JVI.79.6.3822-3830.2005.

35. Payne S. Family Coronaviridae. *Viruses*. 2017;149-158. https://doi.org/10.1016/B978-0-12-803109-4.00017-9.

36. Siddell SG, Anderson R, Cavanagh D, Fujiwara K, Klenk HD, Macnaughton MR, *et al.* Coronaviridae. *Intervirology*. 1983;20(4):181-189. https://doi.org/10.1159/000149390.

37. Feldmann H, Klenk HD. Filoviruses. In: Baron S (Ed.) *Medical microbiology*. 4th Ed. Galveston, TX: University of Texas Medical Branch at Galveston; 1996. Chapter 72.

38. Beer B, Kurth R, Bukreyev A. Characteristics of Filoviridae: Marburg and Ebola viruses. *Naturwissenschaften*. 1999;86(1):8-17. https://doi.org/10.1007/s001140050562.

39. Couch RB. Orthomyxoviruses. In: Baron S (Ed.) *Medical microbiology*. 4th Ed. Galveston, TX: University of Texas Medical Branch at Galveston; 1996. Chapter 58.

40. Noda T. Native morphology of influenza virions. *Front Microbiol*. 2011;2:269. https://doi.org/10.3389/fmicb.2011.00269.

41. Einav T, Gentles LE, Bloom JD. SnapShot: influenza by the numbers. *Cell*. 2020;182(2):532-532.e1. https://doi.org/10.1016/j.cell.2020.05.004.

42. Enders G. Paramyxoviruses. In: Couch RB. Orthomyxoviruses. In: Baron S (Ed.) *Medical microbiology*. 4th Ed. Galveston, TX: University of Texas Medical Branch at Galveston; 1996. Chapter 59.

43. Rima B, Balkema-Buschmann A, Dundon WG, Duprex P, Easton A, Fouchier R, *et al.* ICTV virus taxonomy profile: Paramyxoviridae. *J Gen Virol*. 2019;100(12):1593-1594. https://doi.org/10.1099/jgv.0.001328.

44. Tuthill TJ, Groppelli E, Hogle JM, Rowlands DJ. Picornaviruses. *Curr Top Microbiol Immunol*. 2010; 343:43-89. https://doi.org/10.1007/82_2010_37.

45. Yin-Murphy M, Almond JW. Picornaviruses. In: Baron S (Ed.) *Medical microbiology*. 4th Ed. Galveston, TX: University of Texas Medical Branch at Galveston; 1996. Chapter 53.

46. Zell R, Delwart E, Gorbalenya AE, Hovi T, King AMQ, Knowles NJ, *et al.* ICTV virus taxonomy profile: Picornaviridae. *J Gen Virol*. 2017;98(10):2421-2422. https://doi.org/10.1099/jgv.0.000911.

47. Rima B, Collins P, Easton A, Fouchier R, Kurath G, Lamb RA, *et al.* ICTV virus taxonomy profile: Pneumoviridae. *J Gen Virol*. 2017;98(12):2912-2913. https://doi.org/10.1099/jgv.0.000959.

48. Baxby D. Poxviruses. In: Baron S (Ed.) *Medical microbiology*. 4th Ed. Galveston, TX: University of Texas Medical Branch at Galveston; 1996. Chapter 69.

49. McInnes CJ, Damon IK, Smith GL, McFadden G, Isaacs SN, Roper RL, *et al.* ICTV Virus taxonomy profile: Poxviridae 2023. *J Gen Virol*. 2023;104(5). https://doi.org/10.1099/jgv.0.001849.

50. Anderson M, Mckee M, Mossialos E. Covid-19 exposes weaknesses in European response to outbreaks. *BMJ*. 2020;368:m1075. https://doi.org/10.1136/bmj.m1075.

51. Forman R, Atun R, McKee M, Mossialos E. 12 Lessons learned from the management of the coronavirus pandemic. *Health Policy*. 2020;124(6):577-580. https://doi.org/10.1016/j.healthpol.2020.05.008.

52. Arora P, Mrig S, Goldust Y, Kroumpouzos G, Karadağ AS, Rudnicka L, *et al.* New Coronavirus (SARS-CoV-2) crossing borders beyond cities, nations, and continents: impact of international travel. *Balkan Med J*. 2021;38(4):205. https://doi.org/10.5152/balkanmedj.2021.21074.

53. Villa S, van Leeuwen R, Gray CC, van der Sande M, Konradsen F, Fröschl G, *et al.* HERA: a new era for health emergency preparedness in Europe? *Lancet*. 2021;397(10290):2145-2147. https://doi.org/10.1016/S0140-6736 (21)01107-7.

54. Morse S. Factors in the emergence of infectious diseases. *Emerg infect Dis*. 1995;1(1):7-15. https://doi.org/10.3201/eid0101.950102.

55. Morse S. Factors and determinants of disease emergence. *Rev Sci Tech*. 2004;23(2):443-451. https://doi.org/10.20506/rst.23.2.1494.

56. Morand SS, Lajaunie C. Outbreaks of vector-borne and zoonotic diseases are associated with changes in forest cover and oil palm expansion at global scale. *Front Vet Sci*. 2021;8. https://doi.org/10.3389/fvets.2021.661063.

57. Borkenhagen LK, Fieldhouse JK, Seto D, Gray GC. Are adenoviruses zoonotic? A systematic review of the evidence. *Emerg Microb Infect*. 8(1):1679-1687. https://doi.org/10.1080/22221751.2019.1690953.

58. World Health Organization. *Mpox (monkeypox)*. Geneva: WHO. Available from: https://www.who.int/europe/emergencies/situations/monkeypox; last visited 7 December 2023.

59. Field HE. Bats and emerging zoonoses: henipaviruses and SARS. *Zoonoses Public Health*. 2009;56(6-7):278-284. https://doi.org/10.1111/j.1863-2378.2008.01218.x.

60. Gibb R, Redding DW, Chin KQ, Donnelly CA, Blackburn TM, Newbold T, Jones KE. Zoonotic host diversity increases in human-dominated ecosystems. *Nature*. 2020;584(7821):398-402. doi:10.1038/s41586-020-2562-8

61. Cowled C, Wang L-F (Ed.). *Bats and viruses: a new frontier of emerging infectious diseases*. Hoboken, NJ: Wiley-Blackwell; 2015.

62. Daszak P, Zambrana-Torrelio C, Bogich TL, Fernandez M, Epstein JH, Murray KA, *et al.* Interdisciplinary approaches to understanding disease emergence: The past, present, and future drivers of Nipah virus emergence. *Proceedings of the National Academy of Sciences*. 2013;110(supplement_1):3681-3688. https://doi.org/10.1073/pnas.1201243109.

63. Chua KB. Nipah virus outbreak in Malaysia. *J Clin Virol*. 2003;26(3):265-275. https://doi.org/10.1016/s1386-6532 (02)00268-8.

64. Maiztegui JI. Clinical and epidemiological patterns of Argentine haemorrhagic fever. *Bull World Health Organ*. 1975;52(4-6):567-575.

65. Enria DA, Briggiler AM, Feuillade MR. An overview of the epidemiological, ecological and preventive hallmarks of Argentine haemorrhagic fever (Junin virus). *Bull Inst Pasteur*. 1998;96(2):103-114. https://doi.org/10.1016/S0020-2452 (98)80004-3.

66. Vainrub B, Salas R. Latin American hemorrhagic fever. *Infect Dis Clin North Am*. 1994;8(1):47-59.

67. Richmond JK, Baglole DJ. Lassa fever: epidemiology, clinical features, and social consequences. *BMJ*. 2003;327(7426):1271-1275. https://doi.org/10.1136/bmj.327.7426.1271.

68. Bremer LL, Farley KA. Does plantation forestry restore biodiversity or create green deserts? A synthesis of the effects of land-use transitions on plant species richness. *Biodivers Conserv*. 2010;19(14):3893-3915. https://doi.org/10.1007/s10531-010-9936-4.

69. Veldman J, Overbeck G, Negreiros D, Mahy G, Le Stradic S, Fernandes G, *et al.* Where Tree Planting and Forest Expansion are Bad for Biodiversity and Ecosystem Services. *BIoScience*. 2015;65(10):1011-1018. https://doi.org/10.1093/biosci/biv118.

70. Rizzoli A, Hauffe HC, Tagliapietra V, Neteler M, Rosà R. Forest structure and roe deer abundance predict tick-borne encephalitis risk in Italy. *PloS One*. 2009;4(2):e4336. https://doi.org/10.1371/journal.pone.0004336.

71. Keesing F, Belden LK, Daszak P, Dobson A, Harvell CD, Holt RD, Hudson P, Jolles A, Jones KE, Mitchell CE, Myers SS, Bogich T, Ostfeld RS. Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature*. 2010 Dec 2;468(7324):647-52. doi: 10.1038/nature09575.

72. Schmeller DS, Courchamp F, Killeen G. Biodiversity loss, emerging pathogens and human health risks. *Biodivers Conserv*. 2020;29(11-12):3095-3102. doi: 10.1007/s10531-020-02021-6.

73. Keesing F, Ostfeld RS. Impacts of biodiversity and biodiversity loss on zoonotic diseases. *Proc Natl Acad Sci U S A.* 2021 Apr 27;118(17):e2023540118. doi: 10.1073/pnas.2023540118.

74. Hutchings JA, Myers RA, García VB, Lucifora LO, Kuparinen A. Life-history correlates of extinction risk and recovery potential. *Ecol Appl.* 2012;22(4):1061-1067. doi:10.1890/11-1313.1

75. Keesing FY, Truman P. Cascading consequences of the loss of large mammals in an African Savanna. *BioScience*. 2014;64(6):487-495. https://doi.org/10.1093/biosci/biu059.

76. Plourde BT, Burgess TL, Eskew EA, Roth TM, Stephenson N, Foley JE. Are disease reservoirs special? Taxonomic and life history characteristics. *PLoS One.* 2017;12(7):e0180716. doi:10.1371/journal.pone.0180716.

77. Milbank CV Bhaskar. Wildmeat consumption and zoonotic spillover: contextualising disease emergence and policy responses. *Lancet Planet Health*. 2022; 6 (5):e439-e448. https://doi.org/10.1016/s2542-5196 (22)00064-x.

78. Plowright RK; P Colin R; McCallum, Hamish; Hudson, Peter J; Ko, Albert I; Graham, Andrea L; Lloyd Smith, James O. Pathways to zoonotic spillover. *Nat Rev. Microbiol*. 2017;15(8):502-510. https://doi.org/10.1038/nrmicro.2017.45.

79. Georges-Courbot MC, Sanchez A, Lu CY, Baize S, Leroy E, Lansout-Soukate J, *et al.* Isolation and phylogenetic characterization of Ebola viruses causing different outbreaks in Gabon. *Emerg Infect Dis*. 1997;3(1):59-62. https://doi.org/10.3201/eid0301.970107.

80. Leroy EM, Rouquet P, Formenty P, *et al.* Multiple Ebola virus transmission events and rapid decline of central African wildlife [published correction appears in Science. 2004 Jan 20;303(5658):628]. Science. 2004;303(5656):387-390. doi:10.1126/science.1092528.

81. Wang M, Yan M, Xu H, Liang W, Kan B, Zheng B, *et al.* SARS-CoV Infection in a Restaurant from Palm Civet. *Emerg Infect Dis*. 2005;11(12):1860-1865. https://doi.org/10.3201/eid1112.041293.

82. Xu H fang, Wang M, Zhang Z bin, Zou X zhong, Gao Y, Liu X ning, *et al.* (An epidemiologic investigation on infection with severe acute respiratory syndrome coronavirus in wild animals traders in Guangzhou). *Zhonghua Liu Xing Bing Xue Za Zhi (Chinese Journal of Preventive Medicine)*. 2004; 38(2):81-83.

83. Xu RH, He JF, Evans MR, Peng GW, Field HE, Yu DW, *et al.* Epidemiologic clues to SARS origin in China. *Emerg Infect Dis*. 2004;10(6):1030-1037. https://doi.org/10.3201/eid1006.030852.

84. Slenczka WG. The Marburg virus outbreak of 1967 and subsequent episodes. *Curr Top Microbiol Immunol*. 1999; 235:49-75. https://doi.org/10.1007/978-3-642-59949-1_4.

85. Peters CJ, LeDuc JW. An introduction to Ebola: the virus and the disease. *The J Infect Dis*. 1999179 Suppl 1:ix-xvi. https://doi.org/10.1086/514322.

86. Cutler SJ; F Anthony R; van der Poel, Wim HM. Public health threat of new, reemerging, and neglected zoonoses in the industrialized world. *Emerg infect Dis*. 2010;16(1):1-7. https://doi.org/10.3201/eid1601.081467.

87. Graham J, Leibler J, Price L, Otte J, Pfeiffer D, Tiensin T, *et al.* The animal-human interface and infectious disease in industrial food animal production: Rethinking biosecurity and biocontainment. *Public Health Rep*. 2008; 123 (3):282-299. https://doi.org/10.1177/003335490812300309.

88. Drew TW. The emergence and evolution of swine viral diseases: to what extent have husbandry systems and global trade contributed to their distribution and diversity? *Rev Sci Tech*. 2011;30(1):95-106. https://doi.org/10.20506/rst.30.1.2020.

89. Artois M, Bicout D, Doctrinal D, Fouchier R, Gavier-Widen D, Globig A, Hagemeijer W, Mundkur T, Munster V, Olsen B. Outbreaks of highly pathogenic avian influenza in Europe: the risks associated with wild birds. *Rev Sci Tech.* 2009 Apr;28(1):69-92. doi: 10.20506/rst.28.1.1854.

90. Landolt GA, Olsen CW. Up to new tricks - a review of cross-species transmission of influenza A viruses. *Anim Health Res Rev*. 2007;8(1):1-21. https://doi.org/10.1017/S1466252307001272.

91. Gilbert M, Xiao X, Chaitaweesub P, Kalpravidh W, Premashthira S, Boles S, *et al.* Avian influenza, domestic ducks and rice agriculture in Thailand. *Agric Ecosyst Environ*. 2007;119(3-4):409-415. https://doi.org/10.1016/j.agee.2006.09.001.

92. Sims LD, Domenech J, Benigno C, Kahn S, Kamata A, Lubroth J, *et al.* Origin and evolution of highly pathogenic H5N1 avian influenza in Asia. *Vet Rec*. 2005; 157(6):159-164. https://doi.org/10.1136/vr.157.6.159.

93. Ito T, Couceiro JN, Kelm S, Baum LG, Krauss S, Castrucci MR, *et al.* Molecular basis for the generation in pigs of influenza A viruses with pandemic potential. *J Virol* 1998;72(9):7367-7373. https://doi.org/10.1128/JVI.72.9.7367-7373.1998.

94. Ninomiya A, Takada A, Okazaki K, Shortridge KF, Kida H. Seroepidemiological evidence of avian H4, H5, and H9 influenza A virus transmission to pigs in southeastern China. *Vet Microbiol*. 2002; 88 (2):107-114. https://doi.org/10.1016/s0378-1135 (02)00105-0.

95. Gossner C, Danielson N, Gervelmeyer A, Berthe F, Faye B, Aaslav K, *et al.* Human-Dromedary Camel Interactions and the Risk of Acquiring Zoonotic Middle East Respiratory Syndrome Coronavirus Infection. *Zoonoses Public Health*. 2016; 63(1):1-9. https://doi.org/10.1111/zph.12171.

96. Nava AS Juliana Suieko; Chmura, Aleksei A; Luz, Sérgio Luiz Bessa. The Impact of Global Environmental Changes on Infectious Disease Emergence with a Focus on Risks for Brazil. *ILAR J*. 2017;58(3):393-400. https://doi.org/10.1093/ilar/ilx034.

97. Mora C, McKenzie T, Gaw IM, *et al.* Over half of known human pathogenic diseases can be aggravated by climate change. *Nat Clim Chang.* 2022;12(9):869-875. doi:10.1038/s41558-022-01426-1.

98. Carlson CJ, Albery GF, Merow C, Trisos CH, Zipfel CM, Eskew EA, *et al.* Climate change increases cross-species viral transmission risk. *Nature*. 2022;607(7919):555-562. https://doi.org/10.1038/s41586-022-04788-w.

99. Altizer SB Rebecca A; Han, Barbara A. Animal migration and infectious disease risk. *Science*. 2011;331(6015):296-302. https://doi.org/10.1126/science.1194694.

100. Patz JA, Daszak P, Tabor GM, *et al.* Unhealthy landscapes: Policy recommendations on land use change and infectious disease emergence. *Environ Health Perspec*t. 2004;112(10):1092-1098. doi:10.1289/ehp.6877.

101. Kontra JM. Zombie Infections and other infectious disease complications of global warming. *The Journal of Lancaster General Hospital.* 2017;12(1):12-16.

102. Wu R, Trubl G, Tas N, Jansson J. Permafrost as a potential pathogen reservoir. *One Earth*. 2022;5(4):351-360. https://doi.org/10.1016/j.oneear.2022.03.010.

103. Gross M. Permafrost thaw releases problems. *Curr Biol*. 2019;29(2):R39-R41. https://doi.org/10.1016/j.cub.2018.12.045.

104. Liu Q, Tan Z, Sun J, Hou Y, Fu C, Wu Z. Changing rapid weather variability increases influenza epidemic risk in a warming climate. *Environ Res Lett.* 2020;15(4). https://doi.org/10.1088/1748-9326/ab70bc.

105. Shenoy A, Sharma B, Xu G, Kapoor R, Rho HA, Sangha K. God is in the rain: The impact of rainfall-induced early social distancing on COVID-19 outbreaks. *J Health Econ*. 2022;81:102575. https://doi.org/10.1016/j.jhealeco.2021.102575.

106. Raza A, Khan MTI, Ali Q, Hussain T, Narjis S. Association between meteorological indicators and COVID-19 pandemic in Pakistan. *Environ Sci Pollut Res Int*. 2021;28(30):40378-40393. https://doi.org/10.1007/s11356-020-11203-2.

107. Ligon BL. Infectious diseases that pose specific challenges after natural disasters: a review. *Semin Pediatr Infect Dis*. 2006;17(1):36-45. https://doi.org/10.1053/j.spid.2006.01.002.

108. Hung LS. The SARS epidemic in Hong Kong: what lessons have we learned? *J R Soc Med*. 2003;96 (8):374-378.

109. Worldometer. *COVID - Coronavirus Statistics*. Available from: https://www.worldometers.info/coronavirus/; last visited 30 November 2023.

110. Gayer M, Legros D, Formenty P, Connolly MA. Conflict and Emerging Infectious Diseases. *Emerg Infect Dis*. 2007;13(11):1625-1631. https://doi.org/10.3201/eid1311.061093.

111. Kraemer MUG, Pigott DM, Hill SC, Vanderslott S, Reiner RC, Stasse S, *et al.* Dynamics of conflict during the Ebola outbreak in the Democratic Republic of the Congo 2018-2019. *BMC Med*. 2020;18 (1):113. https://doi.org/10.1186/s12916-020-01574-1.

112. Chapwanya M, Lubuma J, Terefe Y, Tsanou B. Analysis of war and conflict effect on the transmission dynamics of the tenth Ebola outbreak in the Democratic Republic of Congo. *Bull Math Biol*. 2022;84(12):136. https://doi.org/10.1007/s11538-022-01094-4.

113. Europe. Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC. *Official Journal of the European Union* L 325, 12.12.2003.

114. EFSA. *Mandate Overview M-2015-0231*. Parma: European Food Safety Authority. *OpenEFSA* Available from: https://open.efsa.europa.eu/questions/EFSA-Q-2020-00787; last visited 28 November 2023.

115. European Centre for Disease Prevention and Control. *Information sources for epidemic intelligence and outbreak response*. Stockholm: ECDC; 2021. Available from: https://www.ecdc.europa.eu/en/training-and-tools/surveillance-and-outbreak-tools/information-sources-epidemic-intelligence-and; last visited 6 December 2023.

116. European Centre for Disease Prevention and Control. *Early Warning and Response System of the European Union (EWRS)*. Stockholm: ECDC; 2018. Available from: https://www.ecdc.europa.eu/en/publications-data/early-warning-and-response-system-european-union-ewrs; last visited 7 December 2023.

117. Valentin S, Arsevska E, Rabatel J, *et al.* PADI-web 3.0: A new framework for extracting and disseminating fine-grained information from the news for animal disease surveillance. *One Health.* 2021;13:100357. Published 2021 Dec 3. doi:10.1016/j.onehlt.2021.100357

118. Ministère de l'Agriculture et de la Souveraineté alimentaire. *The French platform for epidemiological surveillance in animal health by and for those involved in animal health*. *Info* +23 septembre 2015. Availble from: https://agriculture.gouv.fr/french-platform-epidemiological-surveillance-animal-health-and-those-involved-animal-health; last visited 7 December 2023.

INFLUENZA A

Claudia Cataldo (a), Maria Bellenghi (a), Francesca Dagostin (b), Marzia Facchini (c), Sara Piacentini (c), Simona Puzelli (c), Luca Busani (a)
(a) Centro di Riferimento per la Medicina di Genere, Istituto Superiore di Sanità, Rome
(b) Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige, Trento
(c) Dipartimento Malattie Infettive, Istituto Superiore di Sanità, Rome

Biological, ecological, and molecular features of the causative agent

Disease name

Influenza A (IA).

Disease agent

Common, scientific and Latin name

Influenza A viruses (IAVs) are the causative agents of one of the most critical viral respiratory diseases in humans, swine, poultry, and other species. Influenza is a descriptive term for a respiratory epidemic disease presenting with several symptoms: fever, cough, sore throat, runny nose, headache and muscle pain (1, 2). Common name of the disease is "influenza" in humans and other animal species (e.g., swine influenza in pigs, equine influenza in horses, and avian influenza in wild and domestic birds). In avian species, "fowl plague" is also used when the influenza virus subtypes responsible for the infection can cause systemic and highly lethal disease. Seasonal influenza occurs almost every winter in humans.

Taxonomy

IAVs belong to the family of *Orthomyxoviridae*. The multiple influenza A subtypes are defined by the two surface proteins, hemagglutinin (H) and neuraminidase (N). There are a total of 18 different hemagglutinin subtypes (H1 through H18) and 11 different neuraminidase subtypes (N1 through N11), including the bat-specific influenza A-like subtypes H17N10 and H18N11, unable to reassort with influenza A viruses. The remaining 16 HA and 9 NA could theoretically be found in any combination (3-5).

Disease agent characteristics

IAVs are enveloped viruses with a negative sense, single-stranded, segmented RNA genome, organized in 8 segments encoding for the 11 viral genes: hemagglutinin (HA), Viral Attachment Protein (VAP), neuraminidase (NA), matrix 1 (M1), matrix 2 (M2), nucleoprotein (NP), non-structural protein 1 (NSP1), non-structural protein 2 (NS2; also known as nuclear export protein, NEP), polymerase acidic protein (PA), polymerase basic protein 1 (PB1), polymerase basic protein 2 (PB2) and polymerase basic protein 1-F2 (PB1-F2). The lipid membrane of the virion harbours the HA and the NA, that project from the surface of the virion, and the M2 integral ion channel. The matrix M1 protein lies just beneath the envelope which, along with the viral proteins,

encloses and protects the virion core that contains the viral RiboNucleoProteins (vRNP) complexes and the Nuclear Export Protein (NEP). At one end of the vRNPs are the three polymerases (3P) proteins (PB1, PB2, and PA) that make up the viral RNA polymerase complex (3) (6). The influenza viruses are pleomorphic, mostly roughly spherical but filamentous particles have frequently been observed.

The influenza virus life cycle can be divided into the following stages: binding of the VAP at the N-acetylneuraminic (sialic) acid expressed on the host cell surface, internalization by receptor-mediated endocytosis; endosomal uptake and release; entry into the host cell; entry of viral ribonucleoproteins into the nucleus; transcription and replication of the viral genome; export of the viral ribonucleoproteins from the nucleus; assembly and budding of new virions at the host cell plasma membrane (2) (3).

IAVs are divided into subtypes based on the HA and NA proteins on the virus surface. All known subtypes of IAVs have been found among birds, except subtypes H17N10 and H18N11, which have only been found in bats, as mentioned before.

IAVs commonly circulating among other animal species, including humans, are fewer H and N subtypes than in birds.

The two most common IAVs in humans are H3N2 and H1N1(7). They are the causative agents of seasonal influenza characterized by a sudden rise in body temperature to > 38.5°C 1–3 days following infection. Other symptoms include headache, limb ache, tiredness, general faintness, and dry cough. The most severe outcomes are peracute death, primary influenza pneumonia, encephalitis, and myocarditis. In addition, severe and fatal consequences of primary viral and viral-bacterial pneumonia are known, particularly in older patients with underlying diseases (chronic heart or lung disease, metabolic disorders such as diabetes, immune disorders, etc.).

Avian Influenza Viruses (AIVs) found in wild aquatic birds worldwide present the most remarkable diversity of virus subtypes, from H1 to H16 and from N1 to N9. Avian IAVs can spill over from wild aquatic birds and can infect domestic poultry and other bird and animal species.

AIV of H5 and H7 subtypes exhibit two pathotypes in poultry: Low Pathogenic (LP) and Highly Pathogenic (HP).

LP strains result in mild or asymptomatic infections, whereas HP strains cause up to 100% morbidity and mortality. Therefore, any AIV that exhibits an Intravenous Pathogenicity Index (IVPI) in 6-week-old chickens greater than 1.2 or kills at least 75% of 4- to 8-week-old chickens during a 10-day-observation period is defined as a HP strain (8).

The LPAI of H5 and H7 subtypes can spontaneously mutate to the HP phenotype under natural conditions. The HA cleavage site mutation causes the HP phenotype, but additional mutations may play a role. Two different classes of proteases are responsible for cleavage-activation of the hemagglutinin of influenza viruses: the trypsin-like proteases that cleave LPAIV are present only in a limited number of cells or tissues, so that these viruses commonly cause localized infections in, for example, the respiratory tract of mammals or intestinal tract of birds. In contrast, furin and subtilisin-like proteases that activate HPAIV are ubiquitously expressed causing the systemic spread of the virus (9).

Three different subtypes of Influenza A Viruses of Swine (IAV-S) co-circulate worldwide: H1N1, H3N2, and H1N2. However, the origin, genetic background, and antigenic properties of those IAV-S vary considerably from region to region. For example, recently, the main IAV-S circulating in U.S. pigs have been swine triple reassortant (tr) H1N1, trH3N2 virus, and trH1N2 virus (10). Pigs could also be affected by avian influenza viruses and may play a role in the adaptation of avian IAVs to humans and other mammalian hosts, either as intermediate hosts in which avian influenza viruses may adapt to humans or as a “mixing vessel” in which influenza viruses from various origins may reassort, generating novel progeny viruses capable of replicating and spreading among humans.

For other species, Equine Influenza (EI) is mainly caused by two subtypes of IAVs, namely H7N7 (first isolated in the year 1956) and H3N8 (first isolated in the year 1963) (11), while H17-H18 and N10-N11subtypes have detected in bats only (4).

Physiochemical properties

These properties were mainly studied in avian viruses, which were most stable at slightly basic pH (7.4-8.2), low temperatures (<17°C), and mild to brackish salinities (0-20,000 parts per million (ppm)). Under acidic conditions (pH <6.6), warmer temperatures (>32°C), and high salinities (>25,000 ppm), AI viruses have much shorter stability (12).

Priority level for EU

IAVs cause one of the most important respiratory diseases in humans, avian species, and pigs. In addition, the IAVs constantly mutate to evade the host's immune systems, and new viruses could emerge and spread in naïve populations. In particular, influenza diversification occurs by two main mechanisms, known as "antigenic drift" and "antigenic shift". The first mechanism, which drives annual influenza epidemics, describes gradual antigenic changes in the HA or NA as a result of the accumulation of point mutations in the antigenic epitopes. The second process may occur when two different influenza viruses coinfect the same cells within an individual, causing the mixing and matching of viral genome segments; a change in HA and NA antigenic characteristics can occur due to this reassortment (2).

Interspecies transmission in animals can result in genetic reassortment of viral RNA segments during co-infections with different influenza A viruses and this is central to the emergence of novel influenza A viruses, typically through zoonotic transmission.

Repeated outbreaks and the rapid spread of genetically and antigenically distinct IAVs represent a considerable challenge for animal and public health. Spillover of IAVs from birds to animals and/or to humans could occur, and epidemiological and environmental processes influence the occurrence. Moreover, bidirectional transmission of IAV between pigs and people has altered the evolutionary dynamics of IAV, and a "One Health" approach is required to ameliorate morbidity and mortality in both hosts and improve control strategies.

Although only subtypes of H1N1, H1N2, and H3N2 are endemic in swine worldwide, considerable diversity can be found in their H, N, and the other six genes. Human and swine IAVs have demonstrated a particular propensity for interspecies transmission, leading to regular and sometimes sustained incursions from man to pig and vice versa. The diversity of IAVs in swine remains a critical challenge in diagnosing and controlling this important pathogen for swine health, contributing to a significant public health risk (13). From late 2016 to date, avian influenza continues to evolve in Europe and globally, with reports of new bird outbreaks and occasional infections in mammals. Sporadic human infections have been reported in countries outside the EU, while the risk to the public in the EU remains low. However, a surprising number of HPAI virus detections in sea birds were recently (2022-2023) observed, mainly in gull species and particularly in black-headed gulls (14).

The genetic analyses indicate that the virus persisted in Europe in residential wild birds during and after the summer months. Although the virus retained a preferential binding for avian-like receptors, several mutations associated with increased zoonotic potential were detected.

Distribution of the pathogen

IAVs have a broad host range and are among the most clinically and economically important pathogens for humans, food animals, companion animals, and wild birds. Influenza in humans occurs every year in the cold season, and the causative IAVs circulate in all parts of the world.

Avian Influenza occurs worldwide, but some subtypes can be restricted to specific geographical areas or periods. There is often a "wave" pattern of avian influenza viruses in both wild and domestic avian species, with periods of many outbreaks and others with few cases. In temperate climates, seasonal epidemics occur mainly during winter, while in tropical regions, influenza may occur throughout the year, causing outbreaks more irregularly.

In 2021/2022, the HPAI epidemic in the EU was the largest observed so far in terms of outbreaks, dead wild birds, and geographical spread. In Europe, between 2005 and 2022, all countries experienced at least one episode of HPAI (Figure 1). In recent years, the range of wild bird and mammal species affected by HPAI viruses has also expanded, with the detection of HPAI viruses showing genetic markers of adaptation to replication in mammals. In addition, animal-to-human transmission has occasionally occurred, while no transmissions between humans have been reported.

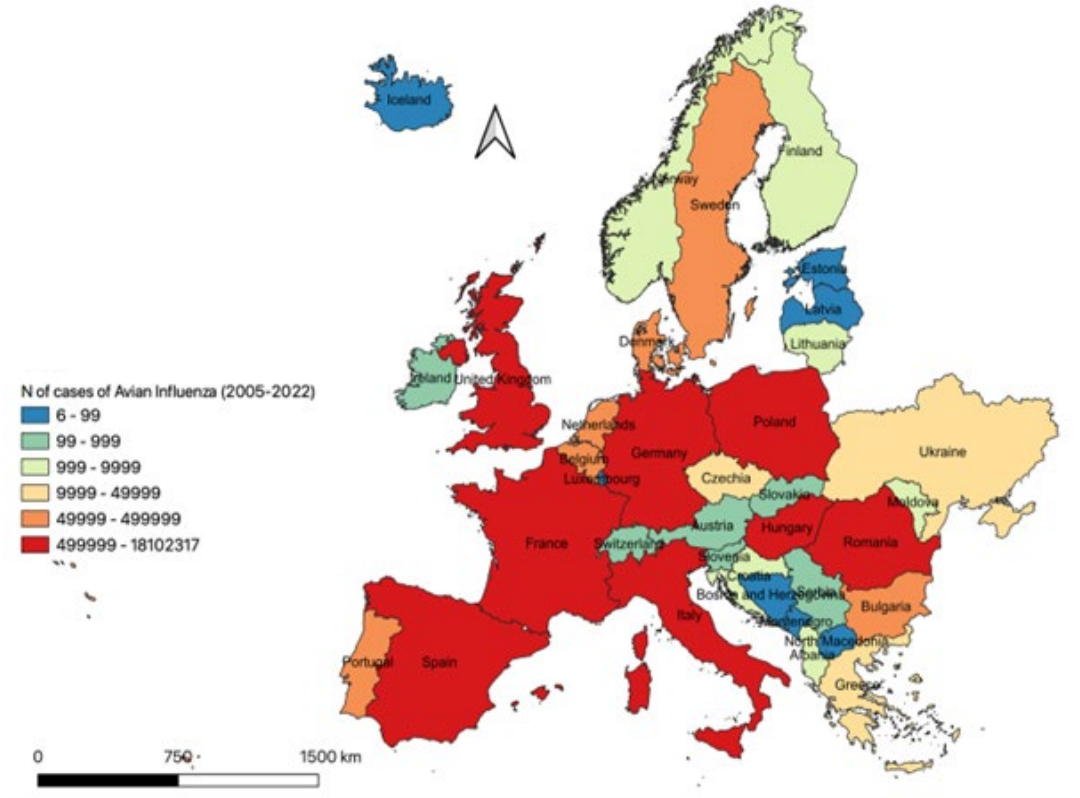

Figure 1. Cumulative cases of Avian influenza in wild and domestic birds in Europe and the Balkans from 2005 to 2022 (Data from WOAH-WAHIS)

Swine influenza in pigs regularly causes outbreaks worldwide, characterized by high levels of illness in pig herds. Still, since vaccination is standard in pigs, the disease's severity is usually

limited. Swine influenza viruses can circulate among swine throughout the year, but most outbreaks occur during the late fall and winter months, like human outbreaks.

Ecology and transmission routes

The ecology of IAVs is dynamic and complex, involving multiple host species (Table 1). Aquatic wild birds of the orders *Anseriformes* (ducks and geese) and *Charadriiformes* (shorebirds and gulls) are the primary reservoir of avian influenza viruses in nature, harbouring H1-H16 subtypes. In contrast, H17 and H18 subtypes were recently discovered in bats (5) (15). Almost all HA and NA subtypes have been detected in dabbling ducks (*Anas* spp.), suggesting that these species are the major reservoir of IAVs (16).

Table 1. List of influenza A animal species included in the selected studies in decreasing order according to the number of references

Genera	Type	Species	n. of papers[#](n=15)	% of impact[*](n=29)
Avian	Domestic	Poultry	4	13.8%
		Domestic birds	3	10.3%
		Turkeys	1	3.4%
		Guinea fowl	1	3.4%
		Quail	1	3.4%
		Chicken	1	3.4%
		Goose	1	3.4%
		Pigeon	1	3.4%
	Wild	Anatidae	5	17.2%
		Wild bird	2	6.9%
		Ducks	2	6.9%
		Aquatic bird	1	3.4%
	ND	Other avian	1	3.4%
Mammals	Domestic	Swine	2	6.9%
		Equine	1	3.4%
ND	ND	Host	2	6.9%

ND=not defined, # number of papers extracted, the number of references per covariates is higher because more covariates may have been extracted from a document; *% calculated on the total number of references

From an ecological point of view, IAVs are natural components of wetland ecosystems in which they occupy trophic niches represented by susceptible hosts while interacting with other biotic and environmental elements. However, ecosystem interactions underlie possible bidirectional viral flows between natural and anthropogenic habitats. Domestic pigs and poultry are two other critical reservoirs of IAVs (17).

Natural avian reservoirs, mainly aquatic wild birds, maintain the environment's low-pathogenic avian influenza viruses (LPAIVs). In these animals, virus prevalence can be >20% in the autumn migration season. The IAV can occasionally move from aquatic bird reservoirs and jumps to poultry or various mammalian species, including humans, resulting in sporadic infections, disease epidemics, or pandemics. If IAVs are transmitted to poultry, they can occasionally evolve into highly pathogenic (HP) strains. In recent years, a new scenario was observed with the increasing potential involvement of wild birds in the long-distance spread by migratory populations of HP avian influenza viruses, particularly H5 subtypes.

From an evolutionary perspective, sequence data generated about influenza viruses have provided a general understanding of the extent and structure of virus genetic diversity, the evolutionary processes that gave rise to it, from where influenza viruses originate, and the mutations that underpin host adaptation, antigenic drift, and antiviral resistance (18).

Human seasonal influenza waves display a complex spatiotemporal pattern resulting from biological, sociodemographic, and environmental factors. Wave-like spread on a small scale (country or area) and long-range seeding events synchronizing distant populations are the two main observed patterns, mainly due to short-distance commuting and air travel flows that facilitate inter-human virus transmission (19).

Animal IAVs follow different transmission patterns. Commercial poultry farms, "wet markets" (where live birds and other animals are sold), backyard poultry farms, commercial and family poultry slaughtering facilities, swine farms, human dietary habits, and the global trade in exotic animals have all been implicated in the spread of IAVs (20).

Direct transmission of IAV from wild birds to humans is rare; most IAV spillover events have occurred through close contact with infected domestic poultry. An example of a persistent transmission from domestic poultry to humans of an avian influenza virus is the outbreak of H7N9 in China, which has become one of the more severe zoonotic infections from avian IAV, with about 1600 human cases from 2013 to 2022, with high morbidity and 40% of case fatality. Most H7N9 human infections have resulted from close exposure to live poultry markets, whereas human-to-human virus transmission is limited (15).

Live poultry markets act as a major source of reassortment of IAV, causing new HA and neuraminidase (NA) subtype combinations and gene constellations, with the H9N2 subtype acting as a significant donor of internal gene segments to other subtypes (15).

Transmission of IAV in wild bird populations depends on several factors, including virus shedding, virus stability in the environment, and the degree of close contact/mixing with other hosts. The maintenance of this diverse pool of viruses globally is in part due to the migratory nature of the bird species, a mechanism in which the viruses are shed in bird faeces and later acquired by other birds that share the same habitat along migratory flyways (15). It has also been suggested that switching transmission dynamics might be a critical strategy for pathogens, such as IAVs, associated with mobile hosts, such as wild water birds, and that both intraspecies and interspecies transmission is essential to maintaining gene flow across seasons (21). In backyard poly-culture farming, domestic poultry often comes into contact with wild birds. The movement and mixing of domestic poultry to live poultry markets enhance the spread and mixing of IAVs (15). AIVs continue to cause both morbidity and mortality in poultry worldwide. Increased mortality is strongly related to infection with highly pathogenic IAVs (HPAIVs) (21).

The IAV hemagglutinin (HA) binds to sialic acid (SIA) receptors, widely represented in mammals and birds. Most avian and human influenza viruses preferentially bind to specific receptor types having SAα2,3Gal (avian receptor)- or SAα2,6Gal (mammalian receptor)-terminated saccharides, respectively. Thus, pigs are widely recognized as a "mixing vessel" of IAV with the presence of both α- 2,3-SA and α- 2,6-SA residues distributed throughout their respiratory tracts, where avian, swine, and human IAV strains reassort following co-infection and give rise to new genetic variants, potentially leading to epidemics and/or epizootics (20).

The transmission of Equine influenza viruses (EIVs) occurs by inhalation through aerosol that can spread effectively through the air up to 1-2 km of distance. Droplet infection plays a significant role in the transmission as nasal discharge/fomites aid in animal-to-animal transfer (22). Horse-to-horse spread is relatively rapid and faster than other respiratory infections in the equine species (23). International trade and traffic also lead to the spread of disease to disease-free zones of the world. The virus can persist for three days in the environment leading to the spread in other animals through fomites. The incubation period is 1-3 days, and the infected horses

can shed the virus for up to 10 days via nasal discharge (24). EIV is a self-limiting sterile disease in horses since the virus does not persist in recovered animals (25).

Drivers of the disease emergence and spread

Ecological drivers

Patterns of influenza outbreaks are different in the tropics than in temperate regions. Although considerable experimental progress has been made in identifying climate-related drivers of influenza, the apparent latitudinal differences in outbreak patterns raise fundamental questions about how potential environmental variables combine and act across the globe.

Some studies clarify that absolute humidity drives influenza outbreaks across latitudes, find that the effect of absolute humidity on influenza is U-shaped, and show that this U-shaped pattern is mediated by temperature (26). A study on seasonal influenza in children showed socio-demographic drivers regarding the role of children in influenza transmission to their elderly contacts. It was demonstrated that Influenza-Like Illness (ILI) was 3.4 times lower in the elderly contacts of immunized children than in contacts of the control group (27).

Different environmental drivers of Highly Pathogenic Avian Influenza H5N1 outbreaks in poultry and wild birds were described. In wild birds, outbreaks were strongly associated with an increased Normalized Difference Vegetation Index (NDVI) and lower elevation, though they were similarly affected by climatic conditions as poultry outbreaks. Outbreaks in poultry mainly occurred in areas where the location of farms or trade areas overlapped with habitats for wild birds. In contrast, outbreaks in wild birds were primarily found in areas where food and shelters are available. The different environmental drivers suggest that other spread mechanisms might be involved: HPAI H5N1 spread to poultry via poultry and wild birds, whereas contact with wild birds alone seems to drive the outbreaks in wild birds (28, 29). Table 2 reports leading influenza A environmental drivers.

Table 2. List of influenza A environmental covariates included in the selected studies in decreasing order according to the number of references

Environmental drivers	n. of papers# (n=26)	% of impact* (n=54)
Temperature (average daily, monthly or seasonal temperature)	19	35.2%
Humidity (absolute and relative)	8	14.8%
Time (month/season of the year)	6	11.1%
Precipitation (monthly/total seasonal rainfall)	5	9.2%
Chemical Characteristic (salinity and pH of the water)	3	5.5%
Water (if stagnant or flowing)	3	5.5%
Wind (speed and direction)	3	5.5%
Distance (to the nearest wetland)	2	3.7%
Normalized Difference Vegetation Index (NDVI)	2	3.7%
Altitude	1	1.8%
Land use (CORINE)	1	1.8%
Light (daylight time length)	1	1.8%

number of papers extracted, the number of references per covariates is higher because more covariates may have been extracted from a document; *% calculated on the total number of references

Natural history of disease in humans and animals, including symptoms, morbidity, and mortality

Brief history of the pathogen and disease

Records show that the flu has existed for at least 1,500 years. The history of influenza begins with Hippocrates (5th century BC), who first reported that an influenza-like illness spread from northern Greece to the islands south and elsewhere. In the 1300s, a flu epidemic hit Florence, Italy, which they called "influenza da freddo" ("cold flu"). History records various large flu epidemics or pandemics, from one in 1580 that spread from Asia to Europe and Africa to others that came over the centuries both on the continent of Europe and to Britain. Influenza pandemic episodes have been described since the end of the 19th century, and virus characteristics and associated host responses vary from one pandemic to another.

Different "pandemic events" have been documented, such as the 1889 "Russian flu" (H3N8) followed by the 1918 "Spanish flu" (H1N1), the "Asian flu" in 1957 (H2N2), the "Hong Kong flu" in 1968 (H3N2), the re-emergence of H1N1 viruses in 1977 - "Russian flu" (H1N1) and most recently the 2009 "swine flu" (H1N1pdm09).

Interestingly, the most well-known influenza pandemic, the 1918 Spanish flu, and the most recent pandemic, the swine influenza from 2009, were caused by an H1N1 virus. Also called "the mother of all pandemics", the 1918 virus infected around one-third of the world population and was responsible for the death of at least 50 million people within a year, appearing in three successive waves. Although the 1918 pandemic has been extensively studied, the virus characteristics responsible for its fast spread, associated with a high mortality rate, especially in the population aged 20 to 40, largely remain obscure. The 2009 "swine flu," since then renamed as "novel influenza A (H1N1)" or "pandemic 2009 H1N1 flu", resulted in 18,500 reported laboratory cases, and a modelling study estimates that, in total, the 2009 H1N1 virus caused more than 200,000 influenza-associated deaths due to respiratory and 80,000 deaths due to cardiovascular failure. Notably, more than 80% of these fatal cases affected a young population (<65 years) (30).

The first record of Avian Influenza (AI) dates back to 1878 in northern Italy when it was described as a contagious disease of poultry associated with high mortality called "fowl plague". It was not until 1955 that the classical fowl plague virus was shown to be a type A influenza virus based on the presence of type A influenza virus type-specific ribonucleoprotein. The term fowl plague was substituted by the more appropriate term, Highly Pathogenic Avian Influenza (HPAI), at the First International Symposium on Avian Influenza in 1981 (30).

Swine influenza was first proposed to be a disease related to human flu during the H1N1 pandemic in 1918 when pigs showed similar symptoms simultaneously as humans. The first identification of an influenza virus as a cause of disease in pigs occurred about ten years later, in 1930. For the following 60 years, swine influenza strains were almost exclusively H1N1. Then, between 1997 and 2002; H3N2 strains followed by H1N2, a reassortant between H1N1 and H3N2, were observed first in North America, and in the following years, they circulated in the pig population worldwide. Unlike human influenza viruses, swine viruses have different epizootiological patterns according to the area of the world with enzootic and geographic dependence. Currently, three predominant subtypes of influenza virus are prevalent in pig populations worldwide: H1N1, H3N2, and H1N2, and these include classical swine H1N1, avian-like H1N1, human-like H3N2, reassortant H3N2, and various genotype H1N2 viruses. In Europe, North America, and China, IAVs circulating in pigs are distinct in their genetic characteristics and genetic sources.

Disease in humans

The IAVs currently responsible for seasonal influenza in humans are H1N1 pdm09 and H3N2. Clinically, human influenza is characterized by an acute onset of symptoms after 24 to 48 hours from infection (incubation time). These symptoms constitute influenza-like illness (ILI) and include headache, cough, myalgias, malaise, chills, and fever that can persist for 2 to 8 days. The severity of the disease varies from mild to severe, and the elderly and immunocompromised are at higher risk of complications and coinfections. Pandemic and, to a lesser extent, seasonal IAV have been described to cause gastrointestinal illness with vomiting or diarrhoea, especially in children. Frequently, coinfection with colonizing bacteria aggravates the course of the disease. Coinfections with *Streptococcus pneumoniae* and *Staphylococcus aureus* are the most frequent and have been observed at a high rate during a pandemic, leading to an increase in pneumonia-associated death (31). Coinfection with other respiratory viruses, such as respiratory syncytial virus, aggravates the severity of the disease (32), especially in immunocompromised patients (33). Avian influenza viruses infecting humans are many; the most important are H5N1 and H7N9, confirmed to induce severe disease in humans (34, 35). Avian, swine, and other zoonotic influenza virus infections in humans may cause diseases ranging from mild upper respiratory tract infection (fever and cough) to severe pneumonia, sepsis with shock, acute respiratory distress syndrome, and even death. Conjunctivitis, gastrointestinal symptoms, encephalitis, and encephalopathy have also been reported to varying degrees depending on subtype.

Disease in animals

In domestic poultry, AIV can cause LPAI with asymptomatic or mild disease, with HA subtypes H1, H3, H5, H6, H7, and H9 most commonly isolated. In contrast, specific AIV lineages in subtypes H5 and H7 can cause severe disease and rapid mortality and are referred to as HPAI (15). Low pathogenicity avian influenza viruses typically produce respiratory signs such as sneezing, coughing, ocular and nasal discharge, and swollen infraorbital sinuses in poultry. Sinusitis is common in domestic ducks, quail, and turkeys.

Lesions in the respiratory tract typically include congestion and inflammation of the trachea and lungs. In layers and breeders, there may be decreased egg production or infertility, ova rupture or involution, or mucosal oedema and inflammatory exudates in the lumen of the oviduct. A few layer and breeder chickens may have acute renal failure and visceral urate deposition (visceral gout). The morbidity and mortality are usually low unless accompanied by secondary bacterial or viral infections or aggravated by environmental stressors. In wild water birds, LPAIV infection probably does not affect movements during the stopover, resulting in the potential for the virus to spread along the migration route (21). HPAI viruses cause severe, systemic disease with high mortality in chickens, turkeys, and other gallinaceous poultry; mortality can be as high as 100% in a few days. Clinical signs or gross lesions may be lacking in peracute cases before death. However, in acute cases, lesions may include cyanosis and oedema of the head, comb, wattle, and snood (turkey); ischemic necrosis of comb, wattles, or snood; oedema and red discoloration of the shanks and feet due to subcutaneous ecchymotic haemorrhages; petechial haemorrhages on visceral organs and in muscles; and blood-tinged oral and nasal discharges.

Since the mid-2000s, spillover of highly pathogenic H5Nx viruses has occurred on multiple occasions, from poultry to wild birds. It has resulted in subsequent inter and trans-continental spread of H5Nx viruses via wild bird movements across Eurasia, Africa, and North America.

Birds that survive the peracute infection may develop CNS involvement as torticollis, opisthotonos, incoordination, paralysis, and drooping wings. The location and severity of microscopic lesions are highly variable and may include oedema, haemorrhage, and necrosis in

parenchymal cells of multiple visceral organs, skin, and CNS. In recent years, evidence of HPAI infections in wild birds without signs of illness was provided by several studies (8, 36). However, when migrating, they can carry the disease to new areas, potentially exposing domestic poultry to the virus. On the other hand, in wild birds, HPAI viruses can cause mass mortality, frequently observed along migratory routes.

Swine influenza virus infection causes acute respiratory distress in pigs. The incubation period for the swine influenza virus ranges from 1 to 3 days. The virus is inhaled and deposited on the surface of the lower respiratory tract. It has been documented that these pigs can lose from 5 to 12 pounds of body weight over a 3 to 4-week period. Often the bronchial and mediastinal lymph nodes are enlarged. Severe cases may result in fibrinous pleuritis (37).

About EIV, the three most common signs of equine influenza are pyrexia (peak 42°C), a serous and subsequently mucopurulent nasal discharge, and a persistent, harsh, dry cough. Other clinical signs include depression, anorexia, myalgia, limb oedema and enlarged mandibular lymph nodes. Haematological changes are non-specific but may consist of anaemia, leukopenia and lymphopenia. Secondary bacterial infection may occur with persistent pyrexia, coughing, purulent nasal discharge, pneumonia, or pleuritis (25).

Availability of preventive, therapeutic, and control measures, including licensed or pipelines vaccines

Therapy in humans

Treatment for most patients with influenza is symptomatic; it includes rest, hydration, and antipyretics as needed. Complicating bacterial infections require appropriate antibiotics. Antiviral drugs given within 1 to 2 days from the symptom onset decrease the duration of fever, severity of symptoms, and time to return to normal activity. Treatment with antiviral drugs is recommended for high-risk patients (including all hospitalized patients) who develop influenza-like symptoms; this recommendation is based on data suggesting that early treatment may prevent complications in these patients.

The H7N9 and H5N1 viruses are resistant to the earlier antiviral drugs amantadine and rimantadine; resistance or reduced susceptibility to oseltamivir has also been reported. The antiviral agent, baloxavir, has also shown *in vitro* activity against various avian influenza viruses, but clinical data still need to be provided.

Therapy in animals

There is no effective treatment, although antimicrobials may reduce secondary bacterial infections, and antipyretics may provide symptomatic relief. Expectorants also may help relieve signs in severely affected pig herds. Vaccination and strict import controls are the only specific preventive measures to control influenza in pigs and horses. Sow vaccination, pre-farrowing or the entire herd at once (mass vaccination), are the most common vaccination protocols. Sow vaccination attempts to maximize the transfer of maternal immunity to the progeny. Piglet vaccination is possible, but the reduced efficacy due to maternal antibodies is an issue. Good management practices, such as strict all-in/all-out procedures, limiting movement of pigs and sows within farrowing rooms and between pens, rooms, and barns, and stress reduction, mainly due to crowding and dust, help reduce virus transmission and losses.

Licensed or pipelined vaccines

Specific vaccines against the most important virus subtypes are widely used in pigs and horses. In poultry, some vaccines are available, but due to the many immunologically distinct viral subtypes that cause influenza in poultry and the virus's ability to rapidly evolve new strains, the preparation of effective vaccines is complicated. While avian flu vaccines are currently available, they are not used on a large scale on poultry farms. This hinders the ability to conduct surveillance testing, which helps detect the virus in unvaccinated flocks and limits the spread of the disease. Thus, the most effective control of outbreaks in poultry remains rapid culling of infected farm populations and decontaminating farms and equipment. This measure also reduces the chances of human exposure to the virus. However, vaccines in poultry against some H5 and H7 avian influenza viruses have been used in several countries. In 2007 the U.S. Food and Drug Administration approved a vaccine to protect humans against one subtype of the H5N1 virus. It was the first vaccine approved for use against bird flu in humans. No vaccines are currently available for the other avian influenza viruses rarely associated with human disease (H7N7, H9N2, H7N3, and H7N9).

Other prevention measures

In addition to vaccination, people may take several personal measures to reduce their risk of acquiring influenza. Influenza spreads from person to person principally when people cough or sneeze or by direct or indirect contact with respiratory secretions from infectious people on their hands or surfaces. Avoid close contact with sick people, washing hands frequently, and increase ventilation in all settings are general measures that, applied consistently, can help reduce the spread of the infection.

Prevention is indicated for all patients but is especially important for high-risk patients and health care practitioners.

At the farm level, appropriate biosecurity and management measures to prevent the direct or indirect contact of poultry with wild birds and preventive hygiene measures such as cleaning and disinfection are crucial. Biosecurity is a set of practices you can use to avoid exposing animals to the disease, and can be summarized in:

- restrict traffic onto and off of the farms;
- disinfect shoes, clothes, hands, egg trays or flats, crates, vehicles, and tires;
- avoid contact with other poultry farms or bird owners.

Disease awareness among farmers and cooperation by all persons in the poultry sector must ensure that the strictest bio-security measures are applied to prevent the introduction of the HPAI virus in the establishments and the (further) spread of the disease.

Similar measures are also implemented in pig farms.

Disease specific recommendations

The competent authorities deliver specific recommendations for preventing and controlling human influenza to the population and are also in charge of Influenza pandemic preparedness. Pandemics require a multisectoral response over several months or even years, and operational plans at national and subnational levels support the national strategies for responding to a pandemic. A pandemic plan is a living document reviewed at intervals and revised if there is a change in global guidance or evidence-based; lessons learned from a pandemic, an exercise, or other relevant outbreak; or changes to national or international legislation related to

communicable disease prevention and control. The Directive 2005/94/EC on Community measures for the control of AI sets out a list of specific provisions concerning preventive biosecurity measures, surveillance, and mass poultry vaccination. In addition, it establishes the measures to be applied in case of outbreaks, like the epidemiological investigation, tracking back and forward, and the restriction to animal movement in the area around the outbreaks to prevent further spread. The restricted zone consists of a protection zone and a surveillance zone with a radius of at least 3 and 10 km around the outbreak.

Epidemiological situation at different spatial scales: past and current trends

Seasonal influenza is a vaccine-preventable disease that each year infects approximately ten to thirty percent of Europe's population and causes hundreds of thousands of hospitalizations across Europe. Older people, younger children, and those with chronic conditions suffer the most. Still, everyone is at risk of developing severe complications, including pneumonia, myocarditis, and encephalitis, that may result in death. The European Centre for Disease Prevention and Control (ECDC) coordinates European influenza surveillance through the European Influenza Surveillance Network (EISN), which combines epidemiological and virological surveillance. Epidemiological and virological surveillance data are regularly sent by all the EU Member States, Iceland, and Norway to the European Surveillance System (TESSy) database hosted by ECDC (info at https://www.ecdc.europa.eu/en/about-us/partnerships-and-networks/disease-and-laboratory-networks/eisn). Analysing the data of the influenza seasons 2018-2021, there are differences among countries. France and Norway reported the highest positive specimens in Europe between 2019 and 2021 (Figure 2).

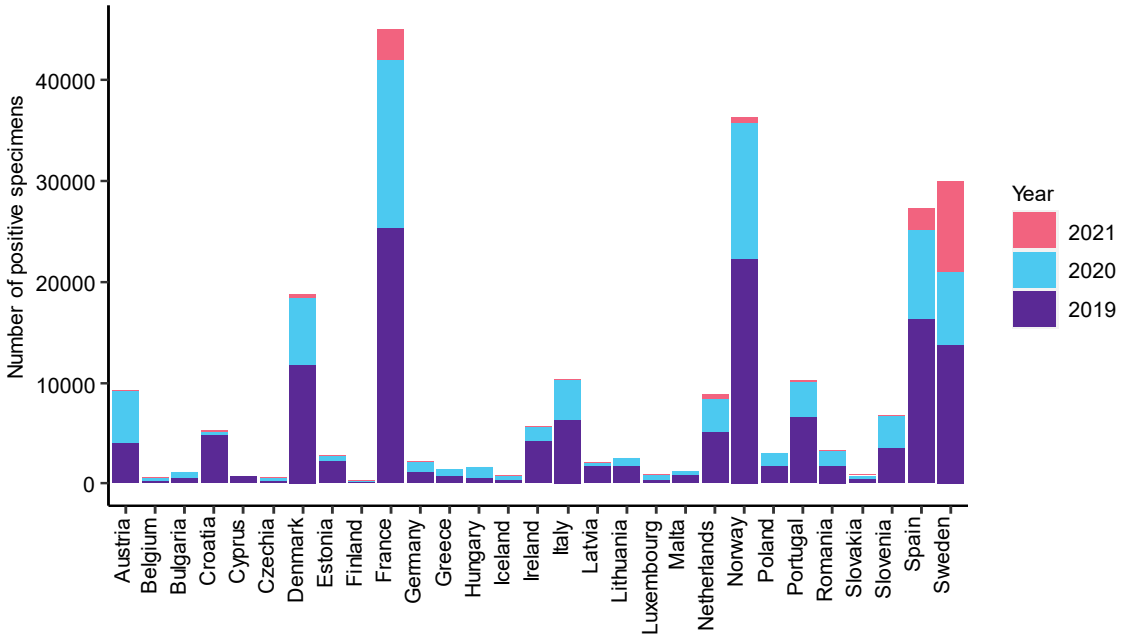

Figure 2. Number of positive specimens of influenza A reported in 29 countries of the European Union and European Economic Area, 2019-2021 (data from http://atlas.ecdc.europa.eu/public/index.aspx)

The weekly number of positive specimens reported over the years (Figures 3-5) showed a remarkable decrease in 2021 due to the restrictive measures implemented during the COVID-19 pandemic (38). Trends were more apparent in individual countries: Sweden showed an increasing number of cases while decreasing case numbers were apparent in Portugal and Slovakia (Figure 4).

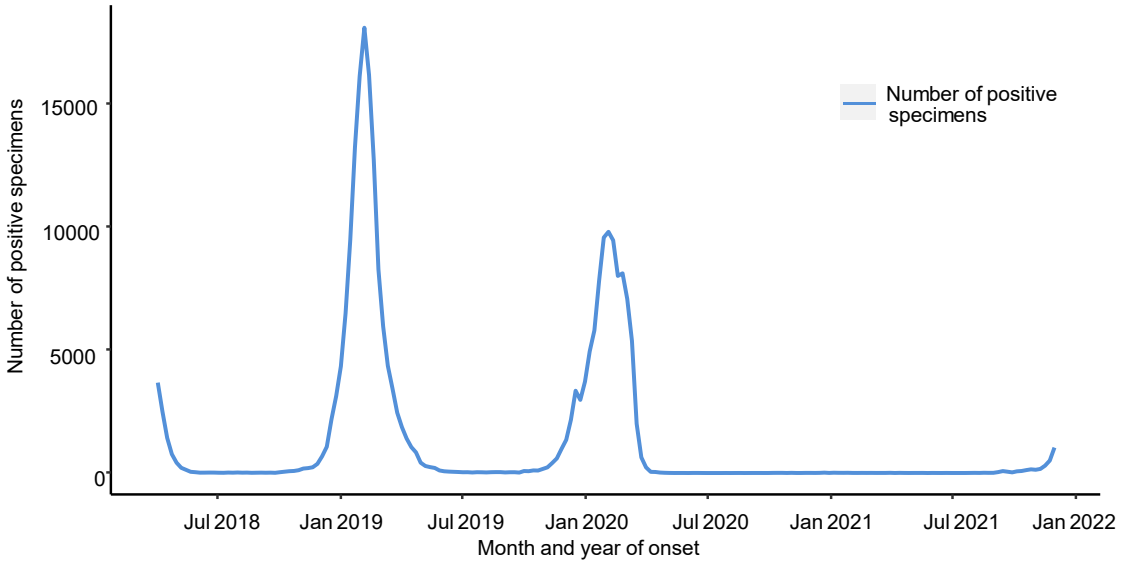

Figure 3. Number of weekly positive specimens by month and year of onset of influenza A in the European Union and European Economic Area countries, 2019-2021 (data from TESSy-ECDC)

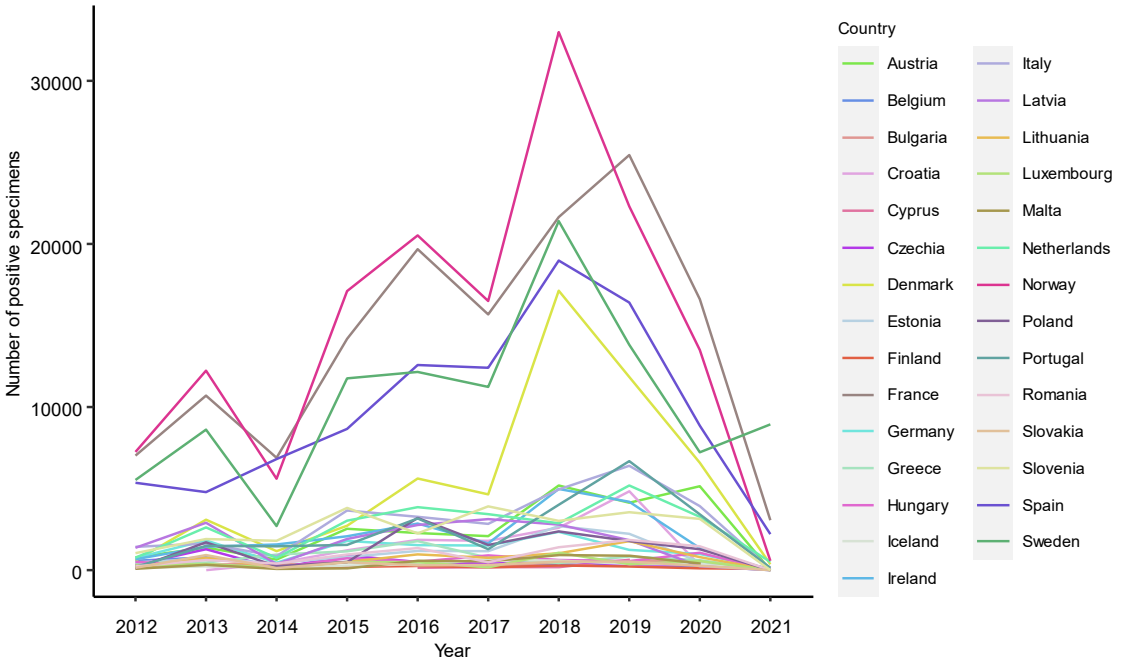

Figure 4. Number of positive specimens of influenza A in the European Union and European Economic Area countries, 2012-2021 (data from http://atlas.ecdc.europa.eu/public/index.aspx)

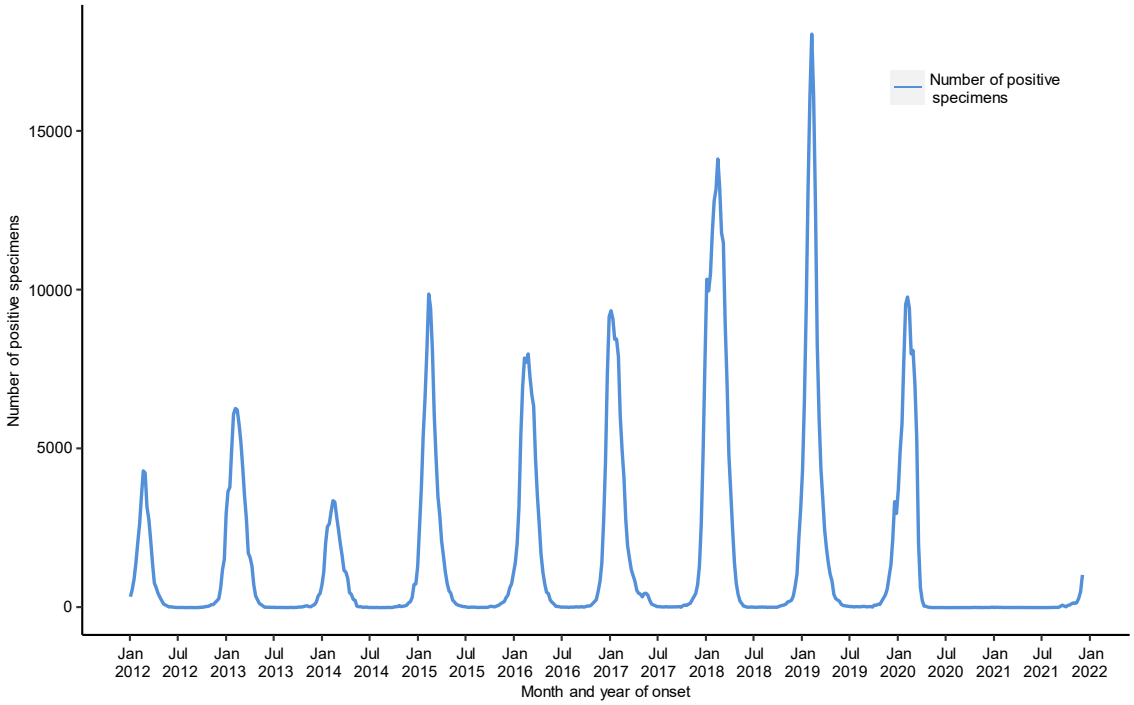

Figure 5. Number of weekly positive specimens by month and year of onset of influenza A in the European Union and European Economic Area countries, 2012-2021 (data from TESSy-ECDC)

Sociological and demographical dimensions affecting susceptibility and exposure, including gender

At-risk groups include infants, older adults, farmers, veterinarians, live animal sellers, swine workers, and poultry workers. Serological screenings in rural populations in China found different proportions of positivity to IAVs of animal origin, including H1, H3, H5, H7, H9, and others. The risk of infection is also related to contact with wild birds, as serological evidence of H5N1 infection among Alaskan hunters who handled dead wild avian species demonstrated (15).

The severity of the consequences is related to additional risk factors or comorbidities that comprise age, chronic respiratory, renal, hematologic, neurologic or cardiovascular disease, diabetes, obesity, immunosuppression/-deficiency, and pregnancy/postpartum period, as many clinical studies on hospitalized 2009 influenza have demonstrated (39-41). The impact of sex differences on morbidity and mortality after multiple behavioural, environmental, and social factors influence IAV infection.

Studies comparing male and female susceptibilities and disease outcomes after IAV infection reported different results, with some suggesting that young males are more susceptible. In contrast, others indicate that females are at an increased risk for severe and fatal outcomes (42).

Table 3 shows the most important influenza A human covariates. Among them, the infection rate and the human density are the most critical.

Table 3. List of influenza A human covariates included in the selected studies in decreasing order according to the number of references

Human drivers	n. of papers #(n=7)	% of impact*(n=31)
Infections (rate of infection)	11	35.5%
Population (human density)	10	32.2%
Economic condition (high/low-income countries)	3	9.7%
Outcomes (ICI admission, chronic disease, mortality)	3	9.7%
Migration (average length of weekly movements)	2	6.45%
Vaccination	2	6.45%

number of papers extracted, the number of references per covariates is higher because more covariates may have been extracted from a document; *% calculated on the total number of references

Diagnostic procedures and notification systems used at local, national and European scale

Diagnosis

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) assay applied to organic material taken using a nasal or throat swab is the routine method to confirm IA infection in humans and animals. Patients with lower respiratory tract illness can have samples taken from sputum, endotracheal aspirate, or bronchoalveolar lavage fluid. The culture of the organism should not be attempted because special precautions are required for these highly pathogenic viruses.

In pigs, IAV is primarily diagnosed by RT-PCR, virus isolation, and occasionally by detecting antibodies against IAV in non-vaccinated animals. Viruses can be isolated from nasal and oral secretions in the febrile phase, affected lung tissue in the early acute stage, or udder wipes collected from sows with infected suckling piglets. Sequencing and characterization of the influenza viral isolates may be needed to select epidemiologically relevant strains for evaluating custom vaccine production. A clinical diagnosis (presumptive diagnosis) can be made by observing the sudden onset of coughing, fever, and nasal secretions in many pigs. However, subclinical and chronic influenza infections are common; cough and nasal secretions may be sporadic in those cases. A retrospective diagnosis can be made by demonstrating a rise in virus-specific antibody titres in acute and convalescent serum samples using the hemagglutination inhibition test. Both H3 and H1 subtype antigens should be included. This test is also used for herd surveys, and an ELISA (Enzyme-Linked Immunosorbent Assay) against the nucleoprotein (not subtype specific) is also available.

All suspected cases of HPAI in poultry or captive birds must be investigated. Appropriate measures must be taken according to the Regulation (EU) 2016/429 ("Animal Health Law"), and the rules for the prevention and control of certain diseases laid down in Commission Delegated Regulation (EU) 2020/687 have to be taken in case of confirmation. Since 2003 EU Member States must carry out surveillance programs for avian influenza aimed at the early detection of highly pathogenic avian influenza viruses and at detecting infections with low pathogenic avian influenza viruses of the H5 and H7 subtypes in poultry which have the potential to mutate to the highly pathogenic form of the virus. The surveillance for avian influenza is compulsory and, by Implementing Regulation (EU) 2020/690, highly pathogenic avian influenza and infection with

low pathogenic avian influenza viruses are subject to European Union surveillance programs. Surveillance of avian influenza in poultry and wild birds must be implemented on the entire territory of all EU Member States and by the provisions laid down in Annex II to Delegated Regulation (EU) 2020/689. By Implementing Regulation (EU) 2020/2002, Member States shall submit to the Commission every year data on the results of the implementation of the Union surveillance programs. The data shall be submitted electronically via the Animal Disease Information System (ADIS).

Infrastructure capacity to identify pathogens for each Member State

In Europe, the Istituto Zooprofilattico Sperimentale delle Venezie, a veterinary public health regional laboratory in Italy, is the designated European Reference Laboratory (EURL) for avian influenza and Newcastle disease, according to the Decision (EU) 2018/662. A European reference laboratory for IA in humans is still not available, but the activities are carried out at the national level, according to national infrastructures and regulations.

Estimated influence of environmental change on the disease's future trends

It is thought that the most significant change in AI epidemiology resulting from climate change will be brought about by changes in the distribution, composition, and migration behaviour of wild bird populations that harbor the genetic pool of AI viruses and in which natural AI transmission cycles occur. In contrast, HPAI, primarily confined to domestic poultry, has spread worldwide successfully in various climatic conditions. Although the effect of the environment on HPAI transmission and persistence is poorly understood, these observations support the idea that climate change will have minimal impact on HPAI epidemiology. However, the indirect effects are mainly those occurring due to the influence of climate change on agroecosystems associated with duck and crop production and changes in the distribution of domestic–wild waterfowl contact points (43). In addition, including abiotic factors such as temperature, UV index, and other meteorological parameters in IV surveillance systems could further our understanding of virus stability and transmissibility and help develop accurate predictive models of influenza epidemics. Moreover, combining epidemiological, meteorological, and genetic studies could unravel the evolution of influenza viruses and improve early intervention and long-term control strategies for future influenza outbreaks (44).

References

1. Fleming DM. Influenza diagnosis and treatment: a view from clinical practice. *Philos Trans R Soc Lond B Biol Sci.* 2001;356:1933-43.
2. Krammer F, Smith GJD, Fouchier RAM, *et al.* Influenza. *Nat Rev Dis Primer.* 2018;4:3.
3. Samji T. Influenza A: understanding the viral life cycle. *Yale J Biol Med.* 2009;82:153-9.
4. Tong S, Li Y, Rivailler P, *et al.* A distinct lineage of influenza A virus from bats. *Proc Natl Acad Sci U S A.* 2012;109:4269-74.

5. Lipsitch M, Barclay W, Raman R, *et al.* Viral factors in influenza pandemic risk assessment. *eLife* 2016;5:e18491.
6. Bouvier NM, Palese P. The biology of influenza viruses. *Vaccine.* 2008;26:D49-D53.
7. Allen JD, Ross TM. H3N2 influenza viruses in humans: Viral mechanisms, evolution, and evaluation. *Hum Vaccines Immunother.* 2018;14:1840-7.
8. Swayne DE. *Avian Influenza*. John Wiley & Sons, 2009.
9. Beerens N, Heutink R, Harders F, *et al.* Emergence and selection of a highly pathogenic avian influenza H7N3 virus. *J Virol.* 2020; 94: e01818-19.
10. Loubet P, Enouf V, Launay O. The risk of a swine influenza pandemic: still a concern? *Expert Rev Respir Med.* 2019;13:803-5.
11. Singh RK, Dhama K, Karthik K, *et al.* A Comprehensive review on equine influenza virus: etiology, epidemiology, pathobiology, advances in developing diagnostics, vaccines, and control strategies. *Front Microbiol*. 2018;9:1941. doi:10.3389/fmicb.2018.01941
12. Brown JD, Goekjian G, Poulson R, *et al.* Avian influenza virus in water: Infectivity is dependent on pH, salinity and temperature. *Vet Microbiol* .2009;136:20-6.
13. Vincent AL, Anderson TK, Lager KM. A Brief introduction to influenza A virus in swine. *Methods Mol Biol.* 2020;2123:249-271. doi:10.1007/978-1-0716-0346-8_18.
14. European Food Safety Authority, European Centre for Disease Prevention and Control, European Union Reference Laboratory for Avian Influenza, Adlhoch C, Fusaro A, *et al.* Avian influenza overview March - April 2023. *EFSA J.* 2023;21:e08039.
15. Joseph U, Su YCF, Vijaykrishna D, *et al.* The ecology and adaptive evolution of influenza A interspecies transmission. *Influenza Other Respir Viruses.* 2017;11:74-84.
16. Howley PM, Knipe DM. *Emerging viruses*. 7th ed. Philadelphia: Wolters Kluwer, 2021.
17. Borkenhagen LK, Salman MD, Ma M-J, *et al.* Animal influenza virus infections in humans: A commentary. *Int J Infect Dis.* 2019;88:113-9.
18. Wille M, Holmes EC. The ecology and evolution of influenza viruses. *Cold Spring Harb Perspect Med* 2020; 10: a038489.
19. Coletti P, Poletto C, Turbelin C, *et al.* Shifting patterns of seasonal influenza epidemics. *Sci Rep.* 2018; 8: 12786.
20. Ma W, Kahn RE, Richt JA. The pig as a mixing vessel for influenza viruses: Human and veterinary implications. *J Mol Genet Med Int J Biomed Re.s* 2008;3:158-66.
21. Venkatesh D, Poen MJ, Bestebroer TM, *et al.* Avian influenza viruses in wild birds: virus evolution in a multihost ecosystem. *J Virol*. 2018;92:e00433-18.
22. Timoney PJ. Equine influenza. *Comp Immunol Microbiol Infect Dis.* 1996;19:205-11.
23. Chambers TM. Equine influenza. *Cold Spring Harb Perspect Med.* 2022;12:a038331.
24. Daly JM, Newton JR, Mumford JA. Current perspectives on control of equine influenza. *Vet Res.* 2004;35:411-23.
25. Cullinane A, Newton JR. Equine influenza—A global perspective. *Vet Microbiol.* 2013;167:205-14.
26. Deyle ER, Maher MC, Hernandez RD, *et al.* Global environmental drivers of influenza. *Proc Natl Acad Sci U S A.* 2016;113:13081-6.
27. Villani L, D'Ambrosio F, Ricciardi R, *et al.* Seasonal influenza in children: Costs for the health system and society in Europe. *Influenza Other Respir Viruses.* 2022;16:820-31.
28. Si Y, De Boer WF, Gong P. Different environmental drivers of highly pathogenic avian influenza H5N1 outbreaks in poultry and wild birds. *PLoS ONE.* 2013;8:e53362.

29. Wang Y, Li P, Wu Y, *et al.* The risk factors for avian influenza on poultry farms: A meta-analysis. *Prev Vet Med* 2014;117:1-6.
30. Lupiani B, Reddy SM. The history of avian influenza. *Comp Immunol Microbiol Infect Dis* 2009;32:311-323.
31. Rynda-Apple A, Robinson KM, Alcorn JF. Influenza and Bacterial Superinfection: Illuminating the Immunologic Mechanisms of Disease. *Infect Immun* 2015;83:3764-3770.
32. Goka EA, Vallely PJ, Mutton KJ, *et al.* Single and multiple respiratory virus infections and severity of respiratory disease: a systematic review. *Paediatr Respir Rev* 2014;15:363-370.
33. Stefanska I, Romanowska M, Donevski S, *et al.* Co-infections with influenza and other respiratory viruses. *Adv Exp Med Biol* 2013;756:291-301.
34. European Centre for Disease Prevention and Control. Zoonotic influenza. In: *ECDC. Annual epidemiological report for 2022*. Stockholm: ECDC; 2023.
35. Simon PF, McCorrister S, Hu P, *et al.* Highly Pathogenic H5N1 and Novel H7N9 Influenza A Viruses Induce More Profound Proteomic Host Responses than Seasonal and Pandemic H1N1 Strains. *J Proteome Res* 2015;14:4511-4523.
36. Kwon J-H, Lee D-H, Swayne DE, *et al.* Experimental infection of H5N1 and H5N8 highly pathogenic avian influenza viruses in Northern Pintail (Anas acuta). *Transbound Emerg Dis* 2018;65:1367-1371.
37. Kothalawala H, Toussaint MJM, Gruys E. An overview of swine influenza. *Vet Q* 2006;28:45-53.
38. Binns E, Koenraads M, Hristeva L, *et al.* Influenza and respiratory syncytial virus during the COVID-19 pandemic: Time for a new paradigm? *Pediatr Pulmonol* 2022;57:38-42.
39. Van Kerkhove MD, Mumford E, Mounts AW, *et al.* Highly pathogenic avian influenza (H5N1): pathways of exposure at the animal-human interface, a systematic review. *PloS One* 2011;6:e14582.
40. Centers for Disease Control and Prevention. *People at high risk of flu*. Available from: https://www.cdc.gov/flu/highrisk/index.htm (2021, accessed 19 April 2022).
41. Rodríguez-Rieiro C, Carrasco-Garrido P, Hernández-Barrera V, *et al.* Pandemic influenza hospitalization in Spain (2009): incidence, in-hospital mortality, comorbidities and costs. *Hum Vaccines Immunother* 2012;8:443-447.
42. Gounder AP, Boon ACM. Influenza pathogenesis: the effect of host factors on severity of disease. *J Immunol* 2019;202: 341-350.
43. Gilbert M, Slingenbergh J, Xiao X. Climate change and avian influenza. *Rev Sci Tech Int Off Epizoot* 2008;27:459-466.
44. Ianevski A, Zusinaite E, Shtaida N, *et al.* Low temperature and low UV indexes correlated with peaks of influenza virus activity in Northern Europe during 2010-2018. *Viruses* 2019;11:207.

LEPTOSPIROSIS

Claudia Cataldo (a), Maria Bellenghi (a), Alessandra Ciervo (b), Francesca Dagostin (c), Luca Busani (a)
(a) Centro di Riferimento per la Medicina di Genere, Istituto Superiore di Sanità, Rome
(b) Dipartimento Malattie Infettive, Istituto Superiore di Sanità, Rome
(c) Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige, Trento

Biological, ecological and molecular features of the causative agent

Disease name

Leptospirosis.

Disease agent

Common, scientific and Latin name

Leptospira species are the causative microbial agents of leptospirosis, an emerging zoonotic disease of worldwide and ubiquitous distribution.

Taxonomy

From 1989, the genus *Leptospira* was separated into *L. interrogans*, including pathogenic strains, and *L. biflexa*, including saprophytic strains (1). Due to the biological intricacy, leptospires were classified by their serological and genomic characteristics.

Serological classification

Leptospira species are separated in different serovars through the agglutination test with homologous antigens. Over 200 and 60 serovars are recorded for *L. interrogans* and *L. biflexa*, respectively (Table 1).

Genomic classification

The genetic heterogeneity of *Leptospira* does not match with the previous two species differentiation, and pathogenic and non-pathogenic serovars occur within the same species (Table 2).

The genotypic classification of *Leptospira* is taxonomically correct, but it must be taken into consideration that the molecular typing is problematic for clinical microbiologists for its incompatibility with the serogroup system identification.

From 1998, phylogenetic studies on 16S rRNA *Leptospira* strains identified three clades based on the pathogenicity status: pathogenic, saprophytic, and intermediate. The last group includes strains of uncertain pathogenicity in humans (2).

Table 1. *L. interrogans* serogroups and some serovars

Serogroup	Serovar(s)
Icterohaemorrhagiae	Icterohaemorrhagiae, copenhageni, lai, zimbabwe
Hebdomadis	hebdomadis, jules, kremastos
Autumnalis	autumnalis, fortbragg, bim, weerasinghe
Pyrogenes	pyrogenes
Bataviae	bataviae
Grippotyphosa	grippotyphosa, canalzonae, ratnapura
Canicola	canicola
Australis	australis, bratislava, lora
Pomona	pomona
Javanica	javanica
Sejroe	sejroe, saxkoebing, hardjo
Panama	panama, mangus
Cynopteri	cynopteri
Djasiman	djasiman
Sarmin	sarmin
Mini	mini, georgia
Tarassovi	tarassovi
Ballum	ballum, aroborea
Celledoni	celledoni
Louisiana	louisiana, lanka
Ranarum	ranarum
Manhao	manhao
Shermani	shermani
Hurstbridge	hurstbridge

Table 2. *Leptospira* serogroups associated with genomospecies

Serogroup	Genomospecies
Andamana	*L. biflexa*
Australis	*L. interrogans, L. noguchii, L. borgpetersenii, L. kirschneri*
Autumnalis	*L. interrogans, L. noguchii, L. santarosai, L. borgpetersenii, L. kirschneri*
Ballum	L. borgpetersenii
Bataviae	*L. interrogans, L. noguchii, L. santarosai, L. borgpetersenii, L. kirschneri*
Canicola	*L. interrogans, L. inadai, L. kirschneri*
Celledoni	*L. weilii, L. borgpetersenii*
Codice	*L. wolbachii*
Cynopteri	*L. santarosai, L. kirschneri*
Djasiman	*L. interrogans, L. noguchii, L. kirschneri*
Grippotyphosa	*L. interrogans, L. santarosai, L. kirschneri*
Hebdomadis	*L. interrogans, L. weilii, L. santarosai, L. borgpetersenii, L. kirschneri, L. alexanderi*
Hurstbridge	*L. fainei*
Icterohaemorrhagiae	*L. interrogans, L. weilii, L. inadai, L. kirschneri*
Javanica	*L. weilii, L. santarosai, L. borgpetersenii, L. meyeri, L. inadai, L. alexanderi*
Louisiana	*L. interrogans, L. noguchii*
Lyme	*L. inadai*
Manhao	*L. weilii, L. inadai, L. alexanderi*
Mini	*L. interrogans, L. weilii, L. santarosai, L. borgpetersenii, L. meyeri, L. alexanderi*
Panama	*L. noguchii, L. inadai*
Pomona	*L. interrogans, L. noguchii, L. santarosai, L. kirschneri*
Pyrogenes	*L. interrogans, L. noguchii, L. weilii, L. santarosai, L. borgpetersenii*
Ranarum	*L. interrogans, L. meyeri*
Sarmin	*L. interrogans, L. weilii, L. santarosai*
Sejroe	*L. interrogans, L. weilii, L. santarosai, L. borgpetersenii, L. meyeri*
Semaranga	*L. meyeri, L. biflexa*
Shermani	*L. noguchii, L. santarosai, L. inadai*
Tarassovi	*L. noguchii, L. weilli, L. santarosai, L. borgpetersenii, L. inadai*

The number of available *Leptospira* genome sequences increased rapidly in the genomic era and with the whole-genome sequencing (WGS) tools. It forced a revisiting of the taxonomy of the genus in two major clades and four subclades. The first clade includes pathogen species for humans and animals and is divided into two subclades, P1 pathogens group and P2 intermediate group. The second clade contains saprophyte species, which is also subdivided into two subclades, S1 and S2 (Table 3) (3).

Table 3. *Leptospira* species belonging to subclades: pathogenic (P1, P2) and saprophytic (S1, S2)

Species	Subclade	Species	Subclade
L. adleri	P1	*L. saintgironsiae*	P2
L. ainazelensis	P1	*L. sarikeiensis*	P2
L. ainlahdjerensis	*P1*	*L. selangorensis*	P2
L. alexanderi	P1	*L. semungkikensis*	P2
L. alstonii	P1	*L. venezuelensis*	P2
L. barantonii	P1	*L. wolffii*	P2
L. borgpetersenii	P1	*L. abararensis*	S1
L. ellisii	P1	*L. bandrabouensis*	S1
L. gomenensis	P1	*L. biflexas.s.*	S1
L. interroganss.s.	P1	*L. bourretii*	S1
L. kirschneri	P1	*L. bouyouniensis*	S1
L. kmetyi	P1	*L. brenneri*	S1
L. mayottensis	P1	*L. chreensis*	S1
L. noguchii	P1	*L. congkakensis*	S1
L. santarosai	P1	*L. ellinghauseni*	S1
L. stimsonii	P1	*L. harrisiae*	S1
L. tipperaryensis	P1	*L. jelokensis*	S1
L. weilii	P1	*L. kanakyensis*	S1
L. yasudae	P1	*L. kemamanensis*	S1
L. andrefontaineae	P2	*L. levettii*	S1
L. broomii	P2	*L. meyeri*	S1
L. dzoumogneensis	P2	*L. montravelensis*	S1
L. fainei	P2	*L. mtsangambouensis*	S1
L. fletcheri	P2	*L. noumeaensis*	S1
L. fluminis	P2	*L. perdikensis*	S1
L. haakeii	P2	*L. terpstrae*	S1
L. hartskeerlii	P2	*L. vanthielii*	S1
L. inadai	P2	*L. wolbachii*	S1
L. johnsonii	P2	*L. yanagawae*	S1
L. koniamboensis	P2	*L. idonii*	S2
L. langatensis	P2	*L. ilyithenensis*	S2
L. licerasiae	P2	*L. kobayashii*	S2
L. neocaledonica	P2	*L. ognonensis*	S2
L. perolatii	P2	*L. ryugenii*	S2

Disease agent characteristics

Leptospires are Gram-negative coiled spirochetes with typical hook-ends and thickness of about 0.1 to 0.15 µm and 6 to 20 µm in length. Commonly with other spirochetes, leptospires have a double membrane structure consisting of the cytoplasmic membrane and peptidoglycan cell wall. This structure is associated with an outer membrane, other functional proteins, and a periplasmic flagellum that allow the bacteria to be motile. All species are obligate aerobes with an optimum growth temperature of 28 to 30°C.

Physiochemical properties

Leptospiral survival studies have thus far only been performed for environmental matrices such as water, soil and mud. Once they are excreted into the environment, many factors such as temperature, pH value, moisture and humidity, UV light, salt and mineral concentrations, and other microorganisms, affect survival. In soil, the reported survival times span from a few hours to 193 days. In tap water, distilled water, sea- and river water, different *Leptospira* species survived between a few hours and 20 months. Survival is temperature-dependent and increases with increasing incubation temperatures. Increasing the temperature survival time also increases, from 130 days at 4˚C to 263 days at 20˚C and to 316 days at 30˚C in fresh water (4). In Table 4 *Leptospira* serovars are grouped based on their capability to cause disease (5).

Table 4. Relationship between *Leptospira* serovars and biological and physiochemical properties in relation to their capability to cause disease

Leptospira serovars	Biological and physiochemical properties
L. illina *L. biflexa* *L. meyeri* *L. wolbachii* *L. yanagawae* *L. vanthielii* *L. terpstrae*	Saprophytic Do not cause disease 1°C>T<35°C
L. inadai *L. parva* *L. broomi* *L. inadai* *L. licerasiae* *L. wolffii* *L. fainei*	Biochemical intermediate Do live as saprophytic or pathogen 1°C>T<37°C
L. kirschnerii *L. interrogans* *L. weilii* *L. noguchii* *L. borgpetersenii* *L. santarosai*	Pathogenic Do cause disease in humans and rodents 20°C>T<37°C

Priority level for EU

Leptospirosis is among the communicable diseases that, according to the Commission Implementing Decision (EU) 2018/945 are covered by epidemiological surveillance. In Europe, the number of human cases of leptospirosis is about 800 per year. France is the country that reports the highest number of cases, followed by Germany. There are several European countries where leptospirosis has just been reported in recent years (Luxemburg, Cyprus, Iceland). In Europe, leptospirosis occurs mainly in the Mediterranean and East European regions. About 160 mammalian species have been identified as natural carriers of pathogenic leptospires. These include feral, semi-domestic and farm and pet animals as important infection sources. The

infectious period of natural hosts can be lifelong. Accidental hosts can act as intermediate infection sources and may shed leptospires for days or months.

Distribution of the pathogen

Since warm and humid conditions facilitate the transmission of leptospirosis (6), outbreaks typically occur in tropical areas (7) and sometimes during summer or fall in temperate regions. Table 5 lists the maintenance and incidental host linked with different *Leptospira* serovars (8).

Table 5. Maintenance and Incidental Hosts for the most important Serovars of *Leptospira interrogans*

Serovar	Maintenance host	Incidental host
L. bratislava	Pig	Horse, Dog
L. canicola	Dog	Pig, Cattle
L. grippotyphosa	Rodent	Cattle, Pig, Horse, Dog
L. hardio	Cattle	Human
L. Icterohaemorrhagiae	Brown rat	Domestic animals and Human
L. pomona	Pig, Cattle	Sheep, Horse, Dog

Ecology and transmission routes

The main modes of transmission of pathogenic *Leptospira* spp. to humans are through direct contact or by contact with contaminated water and soil, as well as infected animals (9). Contact with contaminated water due to floods or recreational activities (such as swimming, fishing, kayaking, surfing, canoeing, rafting, and triathlons) in lakes, rivers, and ponds was associated with an increased risk of leptospirosis (10). The risk is also increased for occupations, especially in developing countries, that may have direct or indirect contact with rodent urine, such as sewage workers, garbage collectors, and agricultural workers (10). Manual labourers tend to be more prone to skin abrasions, which may further increase the risk of infection (11). Increased shedding of the pathogen into the environment results in a heightened risk of occupational and nonoccupational exposure to *Leptospira* spp. in both rural and urban settings (12). *L. borgpetersenii* (mainly host-to-host transmission) and *L. interrogans* (mainly via contaminated water) are the two most common pathogenic species causing leptospirosis in animals (13). Generally, all animal pathogenic strains can be transmitted and be pathogenic to humans. Rats and other rodents are known to be maintenance hosts. Studies have shown that these animals are chronic carriers of *Leptospira* spp., commonly not manifesting any signs of infection when examined (14). Small mammals, particularly rats, are the main reservoir hosts for *L. interrogans*, with large herbivores as significant additional sources of infection. The leptospiral life cycle involves shedding in the urine by an infected animal, persistence in the environment, acquisition of a new host, and hematogenous dissemination to the kidneys. Once leptospires gain access to the renal tubular lumen of the kidney, they colonize the brush border of the proximal renal tubular epithelium, from which urinary shedding can persist for a long time without significant pathogenic effects on the reservoir host (12). Leptospirosis is primarily a zoonosis, with humans

serving as accidental hosts. However, it is worth noting that transient leptospiral shedding occurs during human infection and human-to-human infection, although extremely rare, through sexual intercourse and during lactation (12). Portals of entry include cuts and abrasions on the skin or mucous membranes such as the conjunctival, oral, or genital surfaces. Exposure may occur through either direct contact with an infected animal or through indirect contact via soil or water contaminated with urine from infected animals. Individuals with occupations at risk for direct contact with potentially infected animals include veterinarians, abattoir workers, farm workers (particularly in dairy milking situations), hunters and trappers, animal shelter workers, personnel in laboratories or during fieldwork.

Drivers of the disease emergence and spread

Ecological drivers

According to the Leptospirosis Burden Epidemiology Reference Group (LERG), leptospirosis increases due to rainfall, flooding, open sewers, crowding, animal contacts, and poor hygiene (15). Recent studies have demonstrated that the trend of leptospirosis is spreading worldwide as several countries are prone to become seriously affected after the effects of global warming and severe floods. Besides that, the prevalence of outbreaks is highly associated with various outdoor activities, such as recreational wildlife programs, adventure travels, and army expeditions or training (16,17). In Table 6 are reported the environmental drivers impacting the most on *Leptospira* spreading, while in Table 7 are reported the animal drivers.

Table 6. List of *Leptospira* environmental covariates included in the selected studies in decreasing order of importance according to the number of references

Environmental drivers	n. of papers# (n. 54)	% of impact* (n. 63)
Time (seasonal rate of infection or seasonal incidence in a period)	15	23.8%
Temperature (average daily/monthly)	10	15.8%
Location (area where cases or outbreaks occurred)	9	14.3%
Precipitation (total, monthly or seasonal rainfall)	9	14.2%
Water (eventually contaminated water)	8	12.69%
Altitude	3	4.7%
Land Use (Corine)	3	4.7%
Distance (Rho/spatial correlation or mean distance between infected colonies)	2	3.17%
Soil	2	3.17%
Chemical Characteristics (soil pH)	1	1.58%
Food (contact with animal excreta-contaminated water, soil or food)	1	1.58%

number of papers extracted, the number of references per covariates is higher because more covariates may have been extracted from a document; *% calculated on the total number of references

Table 7. List of *Leptospira* animal covariates identified from the selected studies in decreasing order according to the number of references

Animal drivers	n. of papers# (n. 54)	% of impact* (n. 115)
Rodent	28	24.3%
Dog	15	13.0%
Wild boar	8	6.9%
Cattle	7	6.1%
Fox	7	6.1%
Horse	7	6.1%
Shrew	5	4.3%
Wolf	3	2.6%
Bears	3	2.6%
Goat	3	2.6%
Lynx	3	2.6%
Pig	3	2.6%
Hare	2	1.7%
Jackal	2	1.7%
Mustelidae	2	1.7%
Porcupine	2	1.7%
Sheep	2	1.7%
Badger	1	0.9%
Bat	1	0.9%
Cat	1	0.9%
Cow	1	0.9%
Deer	1	0.9%
Erinaceomorphae	1	0.9%
Foina	1	0.9%
Herpestedae	1	0.9%
Lagomorph	1	0.9%
Otter	1	0.9%
Swine	1	0.9%
Wild bird	1	0.9%
Wild cat	1	0.9%

number of papers extracted, the number of reference per species is higher because from one paper more species could have been extracted) *% calculated on the total number of references

Natural history of disease in humans and animals, including symptoms, morbidity and mortality

Brief history of the pathogen and disease

The modern history of leptospirosis began in 1886 when Adolph Weil described a particular type of jaundice accompanied by splenomegaly, renal dysfunction, conjunctivitis, and skin rashes. It was subsequently named Weil's disease. Although the aetiology of the disease is unknown, it appeared to be infectious in nature and often associated with outdoor occupations in which people came into contact with water (sewer workers, rice-field workers, and coal miners). The first demonstration of leptospires was made by Stimson (18), who used the recently described Levaditi

silver deposition staining technique to observe spirochetes in kidney tissue sections of a patient described as having died of yellow fever. Stimson called the organism *Spirocheta interrogans*. The first isolation of *Leptospira* followed just a few years later. In Japan, where Weil's disease was common in coal miners (19). The organism was named *Spirochaeta icterohaemorrhagiae*. One of the first isolates survives to these days, and Ictero No. 1 was accepted by the Subcommittee on the Taxonomy of *Leptospira* in 1990 as the Type Strain of *L. interrogans* (20). The Japanese group Campo reported the key finding that rats were renal carriers of *Leptospira* (21). Ido and colleagues observed and cultured spirochetes from the kidneys and urine of a range of house and wild rat species and identified them as *S. icterohaemorrhagiae* based on specific reactivity with immune sera. They also observed that leptospires were restricted to the kidneys and that the rats appeared healthy, the first observation of the asymptomatic carrier state. The following decades saw significant advances in the understanding of leptospirosis: one of the more important was the recognition of leptospirosis as an infectious disease of almost all mammalian species, especially in an increasing range of rodent species, and the importance of domestic animals as a source of human infection. The genus name *Leptospira* was first proposed by Noguchi (22) to differentiate the Weil's disease spirochete from others known at the time, especially *Treponema pallidum*, *Spirochaeta and Spironema* (later *Borrelia*) *recurrentis*; the differentiation was based almost entirely on morphological characteristics. As new serovars were isolated, they were given species status, e.g. *L. pomona, L. canicola, L. hardjo*, *L. copenhageni*, and so on. Species (serovars) with related antigens were grouped together in serogroups. Even with the limited taxonomic tools available for *Leptospira* at the time, it was apparent that there were not >200 species, and so in 1982 the Subcommittee on the Taxonomy of *Leptospira* adopted the notion of two species of *Leptospira*, with *L. interrogans* containing the pathogenic serovars and *L. biflexa* containing the saprophytic serovars (23).

Disease in humans

Clinical symptoms of leptospirosis are highly variable and nonspecific. Most cases remain subclinical or asymptomatic, while symptoms typically manifest 2-30 days after the initial exposure (7). The infection is responsible for various clinical features ranging from subclinical symptoms to fatal pulmonary haemorrhage and Weil's syndrome (a combination of jaundice, renal failure, and haemorrhage). Most symptomatic cases (up to 90%) follow a biphasic pattern, consisting of an initial symptomatic leptospiremic phase lasting 5 to 7 days and an immune phase during which symptoms can gradually improve as the host mounts an antibody response (7). Muscle pain is often focused in the calves and lower back, gastrointestinal symptoms (anorexia, nausea, vomiting, and diarrhoea) are common, and nonproductive cough occurs in approximately half of the cases (8). Septic meningitis is also relatively frequent (up to 80% of cases) and usually manifests approximately seven days from the onset of the illness, as the immune phase begins. In a minority of cases, leptospirosis can progress to severe, fulminant disease with a mortality rate of 5-40% (7). Kidney involvement is expected because of the organism's predilection for renal tubules in their natural hosts, and renal failure occurs in 16-40% of cases (9).

Renal dysfunction in leptospirosis is typically non-oliguric and associated with hypokalaemia. Pulmonary manifestations of severe leptospirosis include alveolar haemorrhage (termed Severe Pulmonary Haemorrhagic Syndrome or SPHS) and pulmonary oedema, both of which can result in Acute Respiratory Distress Syndrome (ARDS) (24). Pulmonary involvement is associated with significantly higher mortality from leptospirosis, with case fatality rates estimated at 50-70% (25). *Leptospira* infection can also involve the heart, most commonly causing nonspecific echocardiogram abnormalities (even in mild disease). Myocarditis, pericarditis, heart block, and arrhythmias may occur, and repolarization abnormalities are a poor prognostic sign (26). Even

after recovery, patients may have continued late sequelae, including neuropsychiatric and ocular symptoms.

Disease in animals

Infection most frequently occurs through the mucous membranes of the eye, mouth, nose, or genital tract. The oral infection has also been shown in predators. Vertical transmission can also occur. A period of bacteraemia, which may last for a week, begins 1 or 2 days after infection. During this period, leptospires can be isolated from blood, most body organs, and cerebrospinal fluid. This primary bacteremic phase ends with the appearance of circulating antibodies, which are usually detectable after 10–14 days. A secondary bacteremic period (after 15–26 days) has rarely been reported (27). Acute clinical disease coincides with the bacteremic phase of the disease. It is seen mainly in young animals. It is usually associated with incidental infections, particularly hemolysin-producing strains such as *pomona* or *icterohaemorrhagiae* serogroup strains, which cause haemolytic disease, haemoglobinuria, jaundice, and death. Renal damage can be essential, particularly in *canicola* infection in dogs. Antileptospiral agglutinins appear detectable in the blood at approximately 10-14 days after infection and reach maximum levels at around 3-6 weeks. Peak titres vary considerably (1,000 to 100,000 in the Microscopic Agglutination Test, MAT). Depending on the species, these may be maintained for up to 6 weeks, after which a subsequent gradual decline occurs.

Low titres may be detectable for several years in many animals. The duration and intensity of urinary shedding vary from species to species, animal to animal, and with the infecting serovar. In the case of *pomona* infection in pigs, the intensity of excretion is highest during the first month of shedding (28). Leptospires may also localize in the uterus of pregnant females; abortion, abortion, stillbirth, and neonatal disease may result from intrauterine infections in late gestation. An additional feature seen in host-maintained infection is the persistence of leptospires in the oviduct and uterus of non-pregnant females and in the genital tracts of males (29).

Availability of preventive, therapeutic, and control measures, including licensed or pipelines vaccines

Therapy in humans

Most cases of leptospirosis are mild and self-limiting, and patients often do not present for care, while for milder cases, oral doxycycline, azithromycin, ampicillin or amoxicillin are indicated (30). Azithromycin or doxycycline are the drugs of choice as per standardized guidelines in geographical locations where also rickettsial diseases are endemic (31). Among pregnant and young children, doxycycline is contraindicated (32). In severe cases, intravenous penicillin G has proven to be equally effective as cefotaxime and ceftriaxone (32). Administration of fluids is recommended to correct hypovolemia, hypotension, or bleeding; transfusion with saline/blood is mainly suggested. In patients suffering from complications like acute kidney injury, treatment with fluids or diuretics is initiated in mild cases, and dialysis can be performed in severe stages of the disease. Ventilator support is often needed for patients who encounter complications like ARDS and pneumonia.

Therapy in animals

The treatment of acute leptospirosis in individual animals or herds depends on antibiotics plus supportive symptomatic treatment. Antibiotics used may vary according to their safety in a particular species, their availability for a specific country, the cost, and the route of administration. A combination of penicillin and streptomycin has been the antibiotic therapy of choice for the treatment of acute leptospirosis, but ampicillin, amoxycillin, tetracyclines, tulathromycin and third generation cephalosporins have also been used (33).

Licensed or pipelined vaccines

Vaccines composed of inactivated whole cells (bacterins) are the only vaccines currently licensed for the control of leptospirosis (34). They are mainly for animal use, but in countries such as France, Cuba, China and Japan, bacterins are approved for use in at-risk human populations (35). Although there are some negative aspects to the use of bacterins, e.g., short-term protection and the lack of cross-protection, they have significantly reduced the incidence of the disease and remain one of the most viable strategies to control the infection in humans (36).

The most extensively studied vaccine candidates to date are the Leptospiral immunoglobulin-like (Lig) proteins (37), which are highly conserved and only found in the pathogenic *Leptospira* spp. Vaccines using the LigA or LigB recombinant proteins have demonstrated a wide range of protection, even if LigA is not present in all pathogenic species, potentially limiting its role in a universal vaccine against leptospirosis. Also, several Outer Membrane Proteins (OMP: Lp11, Lp21, Lp22, Lp25, Lsa30, and Lp35), identified using a bioinformatics approach, induced a partially protective immune response when pooled in a single vaccine preparation (38).

Other prevention measures

Prevention of leptospirosis in humans starts by reducing exposure risk by avoiding contact with water contaminated with animal urine, either by not wading or swimming in contaminated water or wearing protective clothing for those with an occupational risk of exposure to contaminated water.

Measures to decrease the risk of infection should include: a) surveillance for both human and animal populations; b) control of rodents to reduce the risk of infection; c) covering skin lesions with impermeable dressings to prevent contact with contaminated water c) use of protective clothing, such as gloves and galoshes, for those working in contact with animals, sewage or during heavy rains; d) post exposure prophylactic treatment with antibiotics may be advised in situations of accidental exposure to rodent, or contaminated water or soil (39).

Disease specific recommendations

Contaminated urine is highly infectious for people and for susceptible animal species; therefore, contact with urine on mucous membranes or skin abrasions should be avoided. Handling infected animals or working in contaminated areas should be done with protective gloves, eye protection, and face masks (8).

Epidemiological situation at different spatial scales: past and current trends

Leptospirosis is the most widespread zoonotic disease. The disease is (re-) emerging globally and numerous outbreaks have occurred worldwide during the past decade (Factsheets about leptospirosis by the European Centre for Disease Prevention and Control, ECDC). France has consistent annual numbers of infections, and France and Germany reported the highest number of cases notified in Europe between 2018 and 2020 (Figure 1).

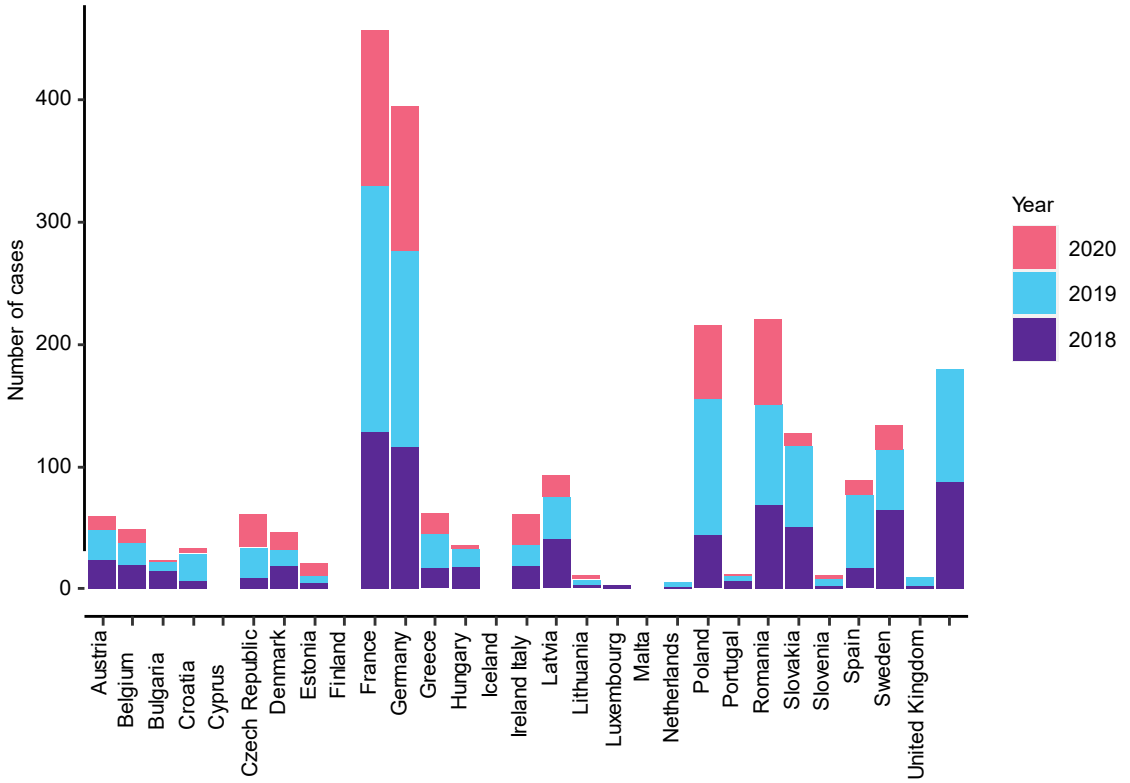

Figure 1. Number of reported cases of leptospirosis in 29 countries of the European Union and European Economic Area, 2018–2020 (data from http://atlas.ecdc.europa.eu/public/index.aspx)

On the other hand, in Europe, countries like Luxemburg, Finland, Cyprus, and Iceland did not notify cases in the last years. Other European countries that consistently notified human cases were Netherlands, Portugal, Romania, Slovenia, and Spain, almost all with an increasing trend, probably due in part to better surveillance and increased public awareness. Based on the reporting of human cases the significant hotspots for leptospirosis are in central and Mediterranean areas and East Europe. The data reported over the 2012-2020 period (Figure 2) has been relatively consistent over the years, and considering all EU/EEA (European Union/European Economic Area) countries, a slight upward trend can be observed. Trends were more apparent in individual countries: Czech Republic and Ireland showed an increasing number of cases, while decreasing case numbers was apparent in Italy, Greece and Austria.

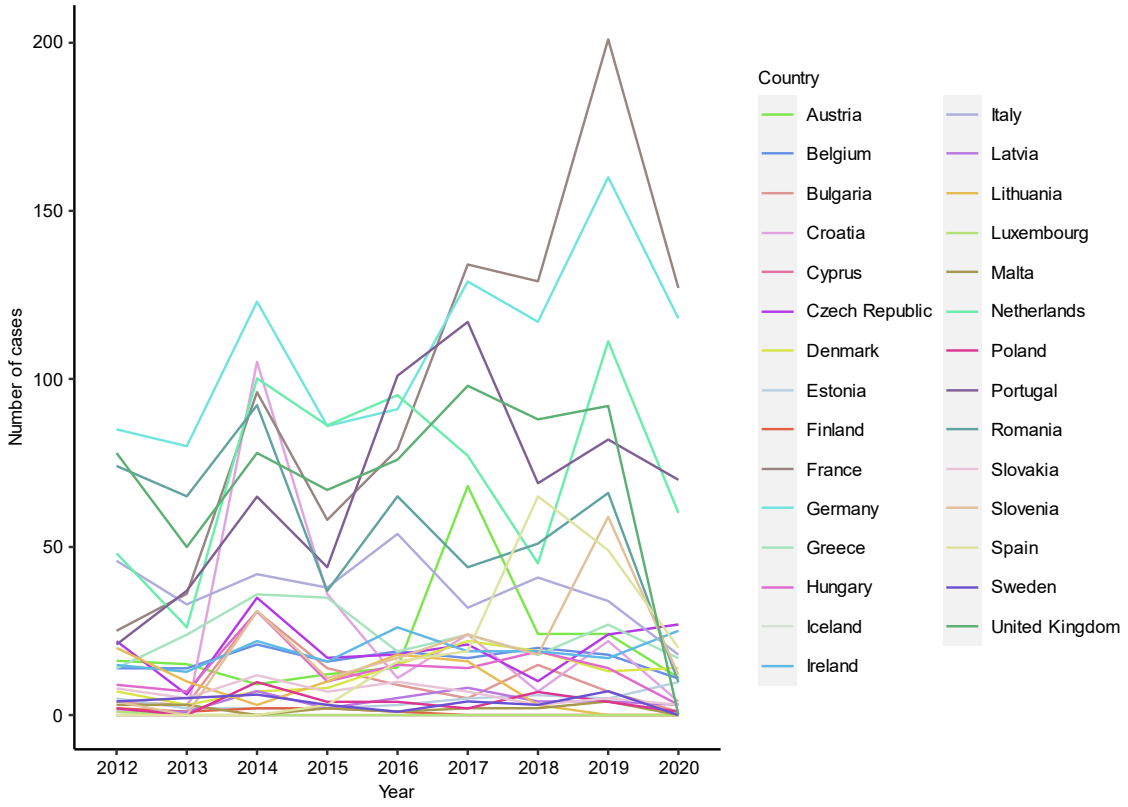

Figure 2. Number of reported cases of *Leptospira* in 29 countries of the European Union and European Economic Area, 2012-2020 (data from http://atlas.ecdc.europa.eu/public/index.aspx)

Sociological and demographical dimensions affecting susceptibility and exposure, including gender

Occupation, migratory behaviour, gender and age are all significant risk factors of leptospirosis. In the past, leptospirosis was first considered an occupational disease, whereby coal miners were the first occupational risk groups to be documented (40). In addition, various mammals including feral, farm, and pet animals can harbour leptospires. This extends the occupational risk to other professions, including farmers, miners, slaughterhouse labourers, pet traders, veterinarians, rodent catchers, sewer workers, garbage collectors and livestock ranchers (41). A report submitted from the second leptospirosis Burden Epidemiology Reference Group (LERG) meeting showed that the median case-fatality percentage was higher in women than men. However, that does not mean that women are more likely to be infected with the disease. The same report shows men are more likely to be infected with leptospirosis as they are more prone to occupational exposure in outdoor settings. The median incidence of the disease was the highest in men older than 59 years, followed by those between 20 to 29 years. For women, approximately 37% of leptospirosis cases were reported in the 40 to 49 age category (42). Table 8 reported the most important human covariates linked with leptospirosis spreading, while Figure 3 reported leptospirosis distribution by sex and age.

Table 8. List of *Leptospira* human covariates identified from the selected studies in decreasing order according to the number references

Human drivers	n. of papers# (n. 30)	% of impact* (n. 119)
Infection (incidence rates or presence of antibodies)	65	54.6%
Age (prevalence or titre of seropositive response by age)	19	15.9%
Gender (correlation of incidence with gender)	16	13.4%
Socio economic (professional soldiers or civilians)	7	5.9%
Clinical outcomes (hospitalization, mortality)	5	4.2%
Population (human density)	4	3.3%
Mortality	2	1.7%

number of papers extracted-the number of references per covariates is higher because from one paper more covariates could have been extracted) *% calculated on the total number of references

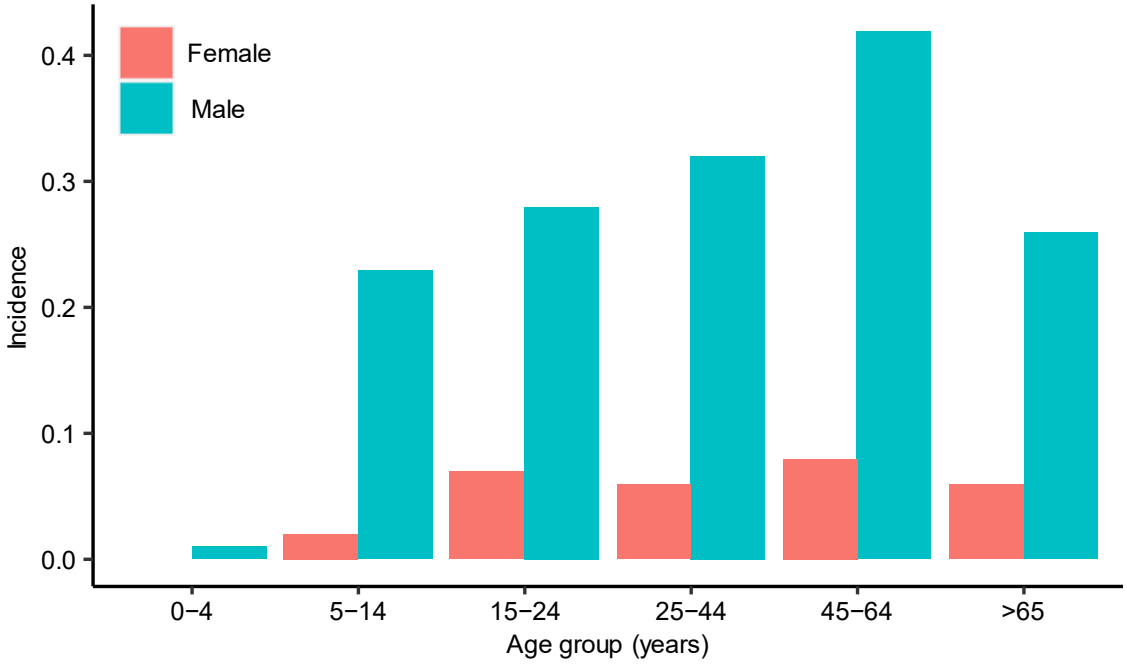

Figure 3. Distribution of *Leptospira* incidence by gender and age group (data from TESSy-ECDC)

Diagnostic procedures and notification systems used at local, national and European scale

Diagnosis

Because the clinical manifestations of leptospirosis are nonspecific and common many other febrile diseases, the combination of exposure history and symptoms should prompt confirmatory testing. In general, a definitive diagnosis of leptospirosis can be made via either traditional

microbiological methods (direct detection, culture) or serology. Culture of *Leptospira* from patient samples is challenging: the organisms typically take 1-2 weeks to grow but may take over a month, and specific growth media is required; blood and CSF cultures are most useful during the first ten days of illness (leptospiremic phase), when organisms are spreading hematogenously (43). After the second week of the disease, urine cultures for *Leptospira* are more likely to be positive due to the organism's tendency for renal tubule deposition. They may remain positive for up to 30 days after the resolution of symptoms. Serological methods are the most commonly used for confirming a diagnosis of leptospirosis. The "gold standard" is the microscopic agglutination test (MAT), in which acute and convalescent sera from a suspected case are mixed with a panel of live antigens from different serogroups of *Leptospira* organisms and examined for agglutination (12). Though test characteristics are overall superior to culture and microscopy (90% sensitivity, > 90% specificity), this method has several limitations: i) the test requires a panel of live organisms specific to the area the patient is suspected of having acquired the infection, ii) specialized lab expertise, iii) there is significant cross-reactivity both between different serogroups of *Leptospira*, as well as with other spirochetes (Treponema and Borrelia species) and iv) antibody response required for MAT testing is often insufficient for detection until the second week of disease (when the immune phase begins). As both culture and serological methods are limited in early detection, newer molecular methods have been developed to facilitate early detection. Both conventional and real-time PCR techniques are highly sensitive, even at early stage of the disease, before the development of antibody response (44). Because this period correlates with the leptospiremic phase, blood is the best sample for detecting leptospiral nucleic acid. However, urine, CSF, or tissue may also have detectable levels later in the disease. Of note, because PCR detects nucleic acid and is not dependent on the presence of live organisms, this technique can be used even after empiric therapy with antibiotics.

Infrastructure capacity to identify pathogens for each Member State

According to the Commission Implementing Decision (EU) 2018/945, leptospirosis is among the communicable diseases that are covered by epidemiological surveillance. It means that EU Member States must establish the national capacity to detect and report human cases.

The decision provides a case definition and laboratory criteria for case confirmation, which are at least one of the following four: 1) isolation of *Leptospira interrogans* or any other pathogenic *Leptospira* spp. from a clinical specimen; 2) detection of *L. interrogans* or any other pathogenic *Leptospira* spp. nucleic acid in a clinical specimen; 3) demonstration of *L. interrogans* or any other pathogenic *Leptospira* spp. by immunofluorescence in a clinical specimen; 4) *Leptospira interrogans* or any other pathogenic *Leptospira* spp. specific antibody response. Clinical microbiology laboratories routinely make the diagnosis.

However, most EU countries do not have a national reference laboratory, and there is not a European network of laboratories. In animals, the disease is not reportable at the EU level – Regulation (EU) 2016/429 and Commission Implementing Regulation (EU) 2020/2002. Yet, it is reportable internationally to the World Organization for Animal Health (WOAH). As for human surveillance, veterinary microbiology laboratories routinely make the diagnosis, and there is no European-wide reference laboratory network or national laboratories in most EU countries.

Estimated influence of environmental change on the disease future trends

The potential for leptospirosis to spread to new territories, either through the reappearance of sylvatic transmission in rural territories or through urbanization, is highlighted. Furthermore, some risk scenarios highlight that the leptospirosis burden in Europe might increase in the coming years due to several factors: (i) alterations in climate (current global warming and/or heavy rainfalls with flooding), (ii) the increasing population of urban rodents in European cities in close contact with human beings and with associated high leptospirosis carriage rates, (iii) human population growth and subsequent urbanization of affected rural territories, and (iv) the increase in intercontinental travels. Based on these aspects, it can be assumed that leptospirosis will be a growing public health problem in Europe, particularly in urban settings (45).

References

1. Russell JC, Faine S. *Bergey's manual of systematic bacteriology*. Baltimore, MD: Williams & Wilkins, 1984.
2. Brenner DJ, Kaufmann AF, Sulzer KR, *et al.* Further determination of DNA relatedness between serogroups and serovars in the family Leptospiraceae with a proposal for *Leptospira alexanderi* sp. nov. and four new Leptospira genomospecies. *Int J Syst Bacteriol.* 1999;49Pt2:839-858.
3. Vincent AT, Schiettekatte O, Goarant C, *et al.* Revisiting the taxonomy and evolution of pathogenicity of the genus Leptospira through the prism of genomics. *PLoS Negl Trop Dis.* 2019;13:e0007270.
4. Tekemen D, Franz M, Bier NS, *et al.* Survival time of Leptospira kirschneri on strawberries. *PloS One* 2020; 15: e0237466.
5. Samrot AV, Sean TC, Bhavya KS, *et al.* Leptospiral infection, pathogenesis and its diagnosis – A review. *Pathogens.* 2021;10:145.
6. Jorge S, Schuch RA, de Oliveira NR, *et al.* Human and animal leptospirosis in Southern Brazil: A five-year retrospective study. *Travel Med Infect Dis.* 2017;18:46-52.
7. Kurilung A, Chanchaithong P, Lugsomya K, *et al.* Molecular detection and isolation of pathogenic Leptospira from asymptomatic humans, domestic animals and water sources in Nan province, a rural area of Thailand. *Res Vet Sci.* 2017;115:146-154.
8. Ame MM. Review on leptospirosis and its public health significance. *Vet Med Open J.* 2021; 6(2): 51-59. doi: 10.17140/VMOJ-6-159.
9. Evangelista KV, Coburn J. Leptospira as an emerging pathogen: a review of its biology, pathogenesis and host immune responses. *Future Microbiol.* 2010;5:1413-1425.
10. Mwachui MA, Crump L, Hartskeerl R, *et al.* Environmental and behavioural determinants of leptospirosis transmission: a systematic review. *PLoS Negl Trop Dis.* 2015;9:e0003843.
11. Barragan V, Olivas S, Keim P, *et al.* Critical knowledge gaps in our understanding of environmental cycling and transmission of *Leptospira* spp. *Appl Environ Microbiol.* 2017;83:e01190-17.
12. Haake DA, Levett PN. Leptospirosis in humans. In: Adler B (Ed.) *Leptospira and Leptospirosis*. Berlin, Heidelberg: Springer Berlin Heidelberg; 2015. p. 65-97.
13. Bulach DM, Zuerner RL, Wilson P, *et al.* Genome reduction in *Leptospira borgpetersenii* reflects limited transmission potential. *Proc Natl Acad Sci U S A.* 2006;103:14560-14565.

14. Sunbul M, Esen S, Leblebicioglu H, *et al.* Rattus norvegicus acting as reservoir of *Leptospira interrogans* in the Middle Black Sea region of Turkey, as evidenced by PCR and presence of serum antibodies to *Leptospira* strain. *Scand J Infect Dis.* 2001;33:896-898.

15. World Health Organization. *Report of the first meeting of the leptospirosis burden epidemiology reference group*. Geneva: WHO; 2010.

16. Dierks J, Servies T, Do T. A study on the leptospirosis outbreak among US Marine Trainees in Okinawa, Japan. *Mil Med*. 2018;183(3-4):e208-e212. doi:10.1093/milmed/usx013.

17. Pappas G, Papadimitriou P, Siozopoulou V, *et al.* The globalization of leptospirosis: worldwide incidence trends. *Int J Infect Dis.* 2008;12:351-357.

18. Stimson AM. Note on an organism found in yellow-fever tissue. *Public Health Rep.* 1907;22:541.

19. Inada R, Ido Y, Hoki R, *et al.* The etiology, mode of infection, and specific therapy of Weil's disease (spirochaetosis icterohaemorrhagica). *J Exp Med.* 1916;23:377-402.

20. International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of Leptospira: Minutes of the Meetings, 13 and 15 September 1990, Osaka, Japan. *Int J Syst Bacteriol* 1992;42:330-334.

21. Ido Y, Hoki R, Ito H, *et al.* The rat as a carrier of spirochaeta icterohaemorrhagiae, the causative agent of weil's disease (spirochaetosis icterohaemorrhagica). *J Exp Med.* 1917;26:341-353.

22. Noguchi H. morphological characteristics and nomenclature of leptospira (spirochaeta) icterohaemorrhagiae (inada and ido). *J Exp Med.* 1918;27:575-592.

23. Faine S, Stallman ND. Amended descriptions of the genus Leptospira Noguchi 1917 and the species L. interrogans (Stimson 1907) Wenyon 1926 and L. biflexa (Wolbach and Binger 1914) Noguchi 1918. *Int J Syst Bacteriol.* 1982;32:461-463.

24. Helmerhorst HJF, van Tol EN, Tuinman PR, *et al.* Severe pulmonary manifestation of leptospirosis. *Neth J Med.* 2012;70:215-221.

25. Segura ER, Ganoza CA, Campos K, *et al.* Clinical spectrum of pulmonary involvement in leptospirosis in a region of endemicity, with quantification of leptospiral burden. *Clin Infect Dis.* 2005;40:343-351.

26. Navinan MR, Rajapakse S. Cardiac involvement in leptospirosis. *Trans R Soc Trop Med Hyg.* 2012;106:515-520.

27. Hathaway SC, Ellis WA, Little TW, *et al.* Leptospira interrogans serovar hardjo in pigs: a new host-parasite relationship in the United Kingdom. *Vet Rec.* 1983;113:153-154.

28. Bolt I, Marshall RB. The epidemiology of Leptospira interrogans serovar pomona in grower pig herds. *N Z Vet J.* 1995;43:10-15.

29. Oliveira SJ de, Bortolanza F, Passos DT, *et al.* Diagnóstico molecular de Leptospira spp em matrizes suínas descartadas. *Braz J Vet Res Anim Sci.* 2007;44:18.

30. Griffith ME, Hospenthal DR, Murray CK. Antimicrobial therapy of leptospirosis: *Curr Opin Infect Dis.* 2006;19:533-537.

31. Lane AB, Dore MM. Leptospirosis: a clinical review of evidence based diagnosis, treatment and prevention. *World J Clin Infect Dis.* 2016;6:61.

32. Khattak MB, Khan SJ, Khan A. Leptospirosis: a disease with global prevalence. *J Microbiol Exp.* 2018;6(5):219–221. doi:10.15406/jmen.2018.06.00218.

33. Cortese VS, Behan S, Galvin JE, *et al.* Evaluation of two antimicrobial therapies in the treatment of Leptospira borgpetersenii serovar hardjo infection in experimentally infected cattle. *Vet Ther Res Appl Vet Med.* 2007;8:201-208.

34. Vernel-Pauillac F, Werts C. Recent findings related to immune responses against leptospirosis and novel strategies to prevent infection. *Microbes Infect* .2018;20:578-588.

35. Adler B, de la Peña Moctezuma A. Leptospira and leptospirosis. *Vet Microbiol.* 2010;140:287-296.
36. Zuerner RL. Host response to leptospira infection. In: Adler B (Ed.). *Leptospira and leptospirosis.* Berlin, Heidelberg: Springer Berlin Heidelberg; 2015. p. 223-250.
37. Matsunaga J, Barocchi MA, Croda J, *et al.* Pathogenic Leptospira species express surface-exposed proteins belonging to the bacterial immunoglobulin superfamily: Leptospiral Ig-like protein family. *Mol Microbiol.* 2003;49:929-946.
38. Monaris D, Sbrogio-Almeida ME, Dib CC, *et al.* Protective immunity and reduced renal colonization induced by vaccines containing recombinant Leptospira interrogans outer membrane proteins and flagellin adjuvant. *Clin Vaccine Immunol.* 2015;22:965-973.
39. Desai S, van Treeck U, Lierz M, *et al.* Resurgence of field fever in a temperate country: an epidemic of leptospirosis among seasonal strawberry harvesters in Germany in 2007. *Clin Infect Dis.* 2009;48(6):691-697. doi:10.1086/597036.
40. Spirochaetal jaundice. *BMJ* 1927;1:844-844.
41. Hartskeerl RA, Collares-Pereira M, Ellis WA. Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. *Clin Microbiol Infect.* 2011;17:494-501.
42. World Health Organization. *Report of the second meeting of the leptospirosis burden epidemiology reference group*. Geneva: WHO; 2011.
43. Schreier S, Doungchawee G, Chadsuthi S, *et al.* Leptospirosis: current situation and trends of specific laboratory tests. *Expert Rev Clin Immunol.* 2013;9:263-280.
44. Ahmed A, Anthony RM, Hartskeerl RA. A simple and rapid molecular method for Leptospira species identification. *Infect Genet Evol.* 2010;10:955-962.
45. Dupouey J, Faucher B, Edouard S, *et al.* Human leptospirosis: an emerging risk in Europe? *Comp Immunol Microbiol Infect Dis.* 2014;37:77-83.

LYME BORRELIOSIS

Isabelle Lebert (a, b), Francesca Dagostin (c), Luca Busani (d), Valentina Tagliapietra (c), Annapaola Rizzoli (c), Luciano Toma (e), Xavier Bailly (a, b), Magalie René-Martellet (a, b)
(a) Université de Lyon, INRAE, VetAgro Sup, UMR EPIA, Marcy l'Etoile
(b) Université Clermont Auvergne, INRAE, VetAgro Sup, UMR EPIA, Saint-Genès-Champanelle
(c) Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige, Trento
(d) Centro di Riferimento per la Medicina di Genere, Istituto Superiore di Sanità, Rome
(e) Dipartimento Malattie Infettive, Istituto Superiore di Sanità, Rome

Biological, ecological and molecular features of the causative agent

Disease name

Lyme Disease, Lyme Borreliosis (LB).

Disease agent

Common, scientific and Latin name

Bacteria of the *Borrelia burgdorferi* sensu lato (Bbsl) species complex.

Taxonomy

Phylum: Spirochaetes; Order: Spirochaetales; Family: *Borreliaceae;* Genus: *Borrelia/Borreliella*

The etiologic agents of human Lyme Borreliosis (LB) belong to the phylum of Spirochaetes, the order of Spirochaetales and the family of *Borreliaceae*. Within this family, DNA sequences analyses illustrated that diversity is clustered in two major clades of related species, i.e. species complexes, that respectively include: i) the etiologic agents of human relapsing fever; and ii) the agents of human LB. The classification of these two groups in different genus is the subject of a lively scientific controversy (1-4). Depending on defended positions, the species complex including Lyme disease agents is assigned to a specific genus called *Borreliella*, or remains in its historical genus *Borrelia*. We will hereafter use *Borrelia burgdorferi* sensu lato (Bbsl) to describe the species complex that contains all agents responsible for human LB as in the latest version of the Bergey's manual of systematic bacteriology (5), the reference text book on the subject (6), and the latest publication in the *International Journal of Systematic and Evolutionary Microbiology* (4) corresponding to the official publication of the International Committee on Systematics of Prokaryotes.

The Bbsl complex of bacteria comprises at least 21 genospecies worldwide (7). Among the nine genospecies present in Europe, five have confirmed human pathogenicity (*Borrelia afzelii, B. garinii, B. burgdorferi* sensu stricto*, B. bavariensis, B. spielmanii*) – with *B. afzelii* and *B. garinii* considered as the major cause of human illness – and four are considered to have potentially or unknown human pathogenicity (*B. bissettiae*, *B. lusitaniae*, *B. valaisiana* and *B. turdi*) (7, 8).

Disease agent characteristics

Bbsl bacteria are helical in shape, comprising several non-cohesive coils. They range in size from 3 to 30 µm long and from 0.2 to 0.5 µm wide. They are surrounded by a surface layer, an outer membrane, periplasmic flagella, and a protoplasmic cylinder and are motile by rotation and translational movement. The protoplasmic cylinder consists of a peptidoglycan layer and an inner membrane which encloses the internal components of the cells.

Because of its clinical relevance, a reference genome of the Bbsl complex was obtained before the emergence of high-throughput sequencing, based on *B. burgdorferi* sensu strictu strain B31 (9). This reference genome illustrated the genome structure of the species complex, characterized by a linear chromosome and a large set of linear and circular plasmids, called lp and cp respectively. The genomic diversity of the species complex has then been explored, benefiting from the progress of sequencing techniques, either based on the sequencing of isolated strains (10, 11) or the sequence capture of genome components from infected material (12). Completed assemblies of Bbsl result in chromosomes from 850 kb to 910 kb long. The organization of the chromosome is similar within the whole species complex. Homologous recombination of chromosomal fragments occurs both within and among the chromosomes of species of the complex Bbsl. However, association measures among polymorphisms suggest that homologous recombination has a limited impact on the distribution of variability within species and modelling suggests that chromosomal homologous recombination rate is lower between species than within species (11).

Most of the 30 plasmid types of the species complex are distributed in different species, but show an extensive presence/absence polymorphism, with rearrangement occurring especially among linear plasmids (13). The most frequent plasmids are lp17, lp28–4, lp36, lp54 and cp26, followed by lp28–3, lp38 and cp32–5. The critical role of some of these plasmids in the process of vertebrate host infection has been documented (14). Most of the acquisition of new genes occurs on plasmids, at a regular rate, mostly through duplication of lipoprotein genes rather than by the import of heterologous material (15). The dynamics of plasmid evolution and exchange result in non-systematic but significant associations between chromosomal and plasmid diversity patterns (16).

Due to the relative consistency of polymorphism patterns, multilocus sequence typing (17) and eventually single gene barcoding still represent common methods to characterize the diversity of the Bbsl complex from either bacterial isolates or infected material (18). This is particularly convenient as Bbsl isolation is difficult. In recent years, the use of high-throughput sequencing, either through amplicon sequencing (19, 20) or sequence capture (21), proved useful to resolve and study co-infections by different Bbsl genotypes, which occur both in ticks and vertebrate hosts.

Physiochemical properties

Bbsl isolates are usually grown at an optimal temperature between 30 and 37°C. Isolates that have been grown *in vitro* are microaerophilic and have complex nutritional requirements usually satisfied by the Barbour-Stoenner-Kelly (BSK II) medium (22) and later derivatives (23). They grow slowly, typically dividing every 8-12 h during the exponential growth phase *in vitro*. Culture-adapted isolates can usually reach cell densities of 10^7 to 10^8 per mL after *in vitro* cultivation for 5-7 days.

Priority level for EU

In 2018, the European Centre for Disease Control (ECDC) has added the neurological form of LB called Lyme NeuroBorreliosis (LNB) to the list of communicable diseases (24). This is a first step for uniformization of data retrieval from EU countries surveillance systems and will allow comparison of incidence data at EU level.

In 2021, notification of LB human cases was mandatory in 19 European countries: Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Norway, Poland, Portugal, Romania, Slovakia and Slovenia (25). However, case definitions, reporting and surveillance systems differ from one country to the other.

Distribution of the pathogen

The pathogen is widely distributed in Europe (Figure 1), where it varies spatially, but also temporally (26-28). The distribution of the pathogen is highly dependent on the distribution of infected *Ixodes* spp. ticks (Figure 2a and 3) and hosts (Figure 2b).

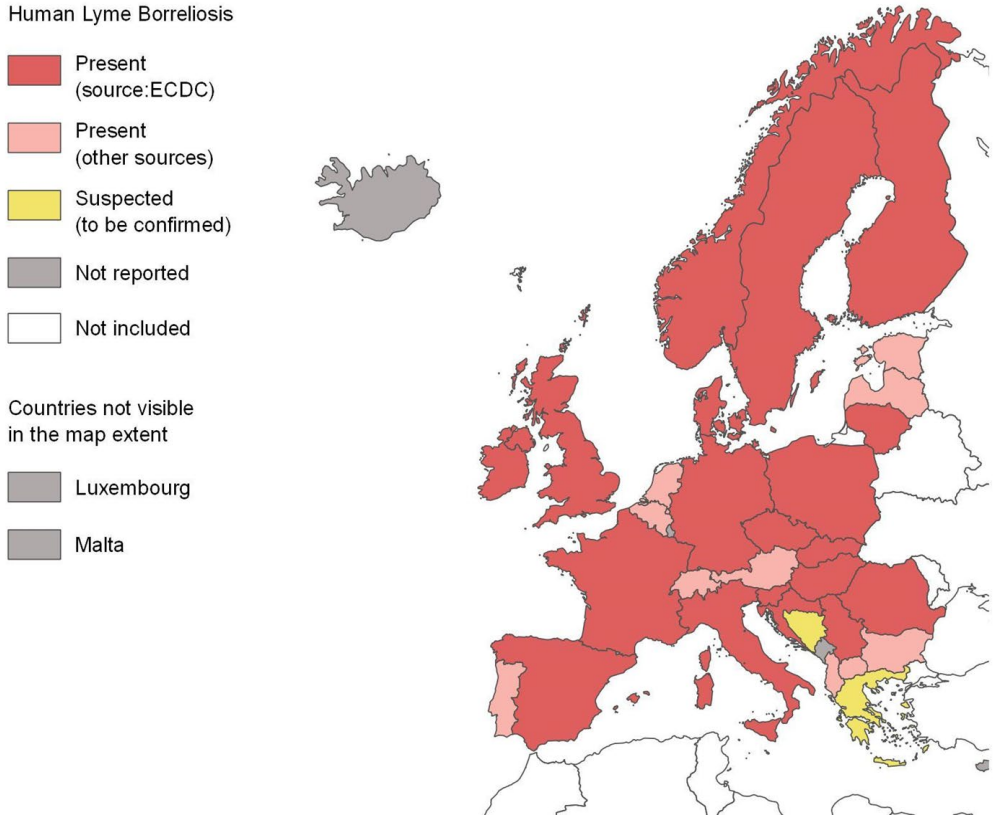

Figure 1. European Union and European Economic Area countries that reported at least one locally-acquired human Lyme borreliosis (hLB) case (including all clinical forms of the disease and all Bbsl pathogenic strains): hLB information was extracted from the TESSy-ECDC; Lyme neuroborreliosis (cases from 2017 to 2021) and from other sources (all clinical forms of hLB), such as national surveillance systems and published literature

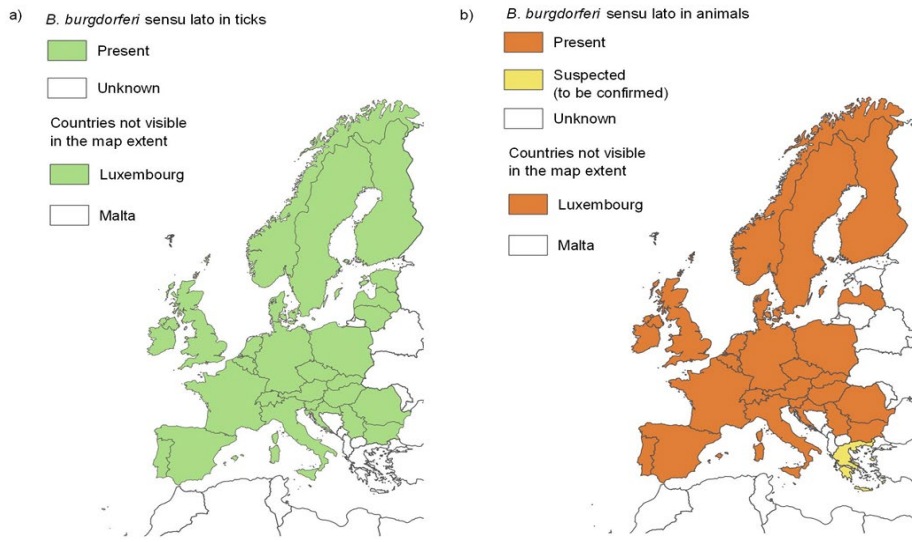

Figure 2. Distribution of Bbsl in questing *Ixodes* spp. ticks (a) and animal hosts (b) (derived from a literature review updated to 2023)

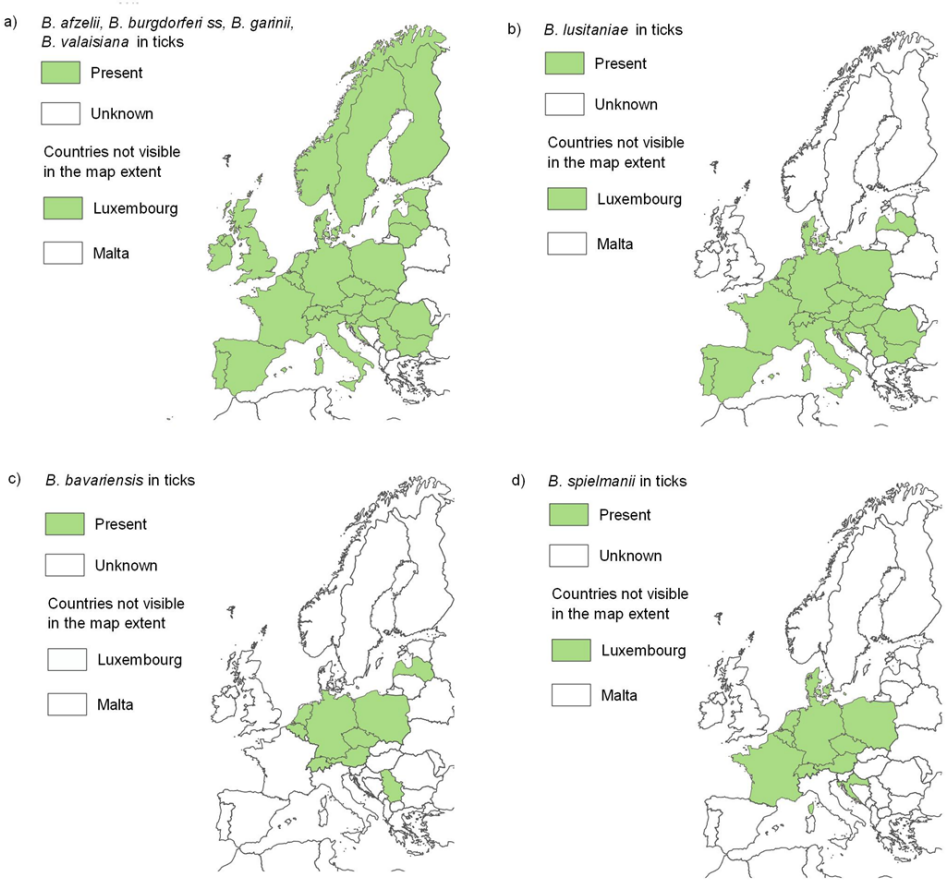

Figure 3. Distribution of: *B. afzelii*, *B. burgdorferi* s.s., *B. garinii*, *B. valaisiana* present in each country (a); *B. lusitaniae* (b); *B. bavariensis* (c); and *B. spielmanii* (d) in questing *Ixodes* spp. ticks (derived from a literature review updated to 2023)

Ecology and transmission routes

Bbsl transmission occurs mainly during a tick bite, either from an infected tick to its host, or from the infected host to the tick. Transmission can also take place by cofeeding (29). Trans-ovarian transmission (vertical transmission from adult female to larvae) seems absent or negligible in *Ixodes* tick (30). The occasional detection of bacteria in larvae can result either from an infection during a first blood meal attempt on an infected host or by cofeeding. In fact, bacteria can be detected in the larvae as early as 24 hours after the bite on an infected host (31, 32).

Vectors: Ticks of the *Ixodes* genus are the only confirmed vectors of Bbsl spirochetes. Among them, three species were experimentally confirmed to transmit Bbsl spirochetes in Europe (Table 1). However, several other species of this genus are suspected vectors of these bacteria based on epidemiological evidence (7, 8, 33). The main vector of the bacteria of the Bbsl complex in Europe is the hard tick *I. ricinus* (Figure 4).

Table 1. Tick species that are experimentally confirmed vectors of *Borrelia burgdorferi* s.l.

Genus	Species	Distribution	Bbsl strains	References
Ixodes	*ricinus*	Widely distributed in Europe except under Mediterranean climate	*B. burgdorferi s.s; B. afzelii; B. garinii*	34-41
Ixodes	*persulcatus*	Widely distributed from Poland to Japan	*B. garinii*	42,43
Ixodes	*hexagonus*	Reported from most European countries	*B. burgdorferi s.s.*	44

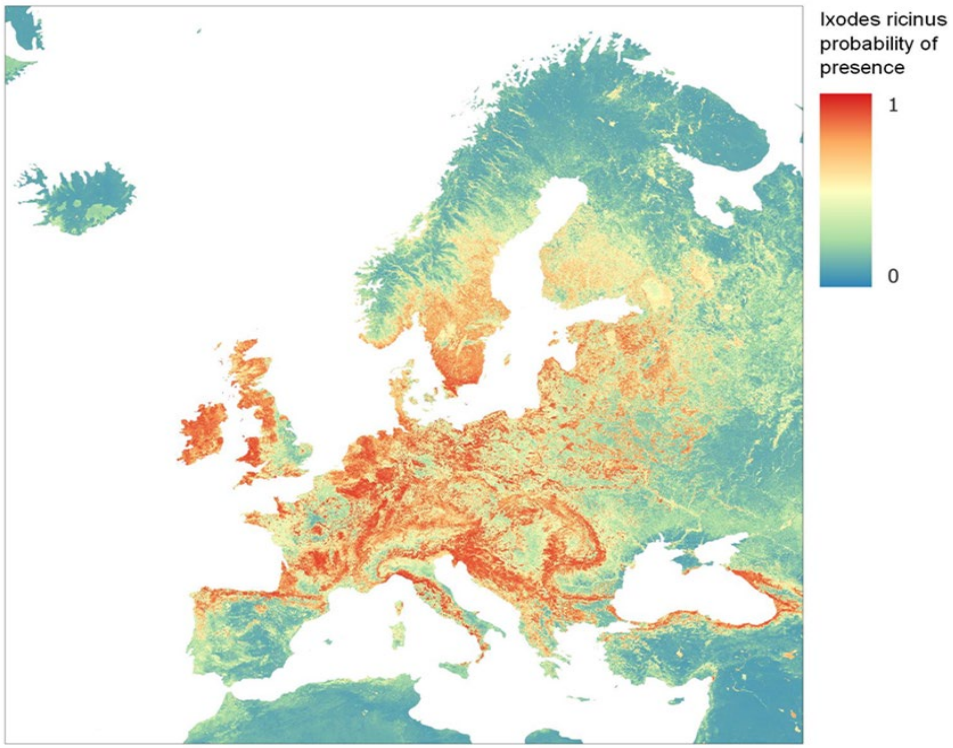

Figure 4. Current 1-km probability of presence of *Ixodes ricinus* across Europe, produced using random forest and boosted regression trees based spatial modelling techniques (source ERGO group)

Ixodes ricinus is the most common and most widely distributed tick species in Europe. It is an exophilic tick capable of feeding on a wide number of vertebrate species including the main reservoirs of Bbsl spirochetes (birds and rodents) and humans.

Ixodes ricinus is vulnerable to desiccation at all life stages and needs to return regularly to the moist litter layer to rehydrate (45). Thus, tick development and questing depend strongly on local environmental conditions related to climate, meteorology, seasonality, abundance of hosts. These features highly affect the duration of tick life cycle but also the timing of its activity and distribution (46).

Ixodes ricinus, like all other Ixodidae, goes through three life stages: larva, nymph, and adult (Figure 5). Each stage feeds on vertebrate hosts for an average duration of seven days. During this long engorgement period, some hosts might be able to move over large distances, thus enabling the dissemination of ticks. After feeding, a tick falls from the host and moults to the next stage. Larvae and nymphs mainly feed on small hosts such as birds, small mammals, and rodents, while adult ticks generally feed on large mammals such as wild ungulates (47,48). Mating generally occurs on the host. The engorged female subsequently falls to the ground and lays between 2000 and 3000 eggs, leading to local multiplication of ticks. The life cycle of the tick thus alternates between several steps: i) resting and developing on the ground; ii) exophilic questing; and iii) blood feeding on a host. All steps are essential for the maintenance of the life cycle.

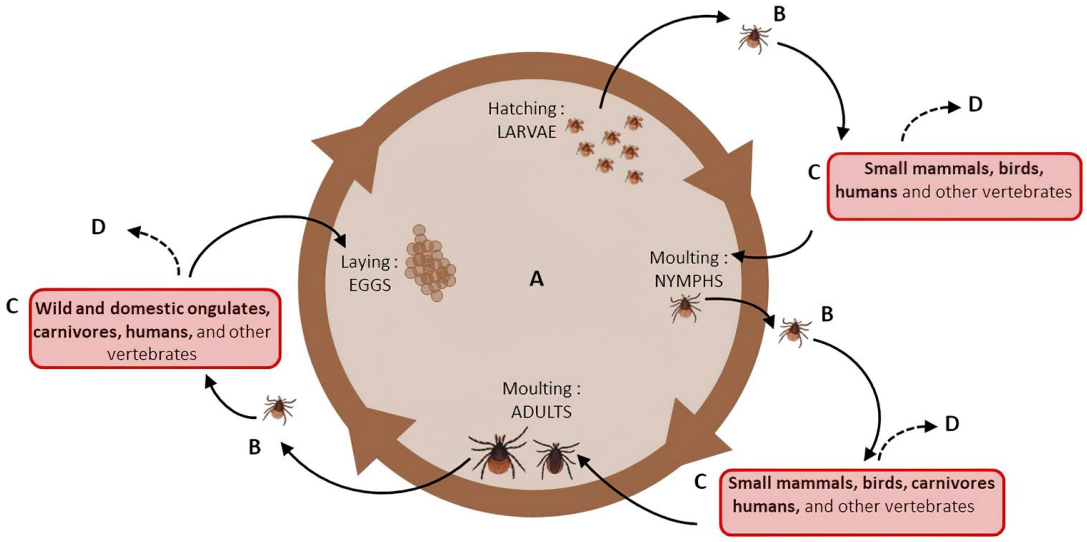

Figure 5. The *Ixodes ricinus* life cycle: A. development on the ground, B. questing activity, C. feeding on a vertebrate host, D. host-mediated dispersal

Ixodes persulcatus is another confirmed vector of Bbsl spirochetes in Eastern Europe. The species is present in at least 14 countries from Poland to Japan and, as *I. ricinus,* is an exophilic species capable of engorging on a high number of different hosts increasing its capacity of pathogen transmission (49).

The vector competence of *I. hexagonus* for Bbsl spirochetes was also confirmed in the laboratory (44). *I. hexagonus* is an endophilic species reported from most European countries. Their main hosts are hedgehogs and carnivorous mammals (50).

Nevertheless, other species of ticks of the genus *Ixodes* are suspected vectors of Bbsl. Even if these tick species rarely bite humans, they can contribute to maintaining the zoonotic cycle of bacteria and therefore influence their prevalence and spatial distribution. These species are *I. acuminatus* (51), *I. arboricola* (52, 53), *I. frontalis* (52, 54), *I. trianguliceps* (55) and *I. uriae* (56). Hosts: The main reservoir hosts of *B. afzelii*, *B. bavariensis* and *B. spielmanii* are small mammals (57-61), those of *B. garinii* and *B. valaisiana* are birds (57, 58, 62), those of *B. lusitaniae* are lizards (62-64) and those of *B. burgdorferi* s.s. are small mammals and birds (58, 65, 66). Other vertebrate species can be reservoirs of one or more *Borrelia* species (33). For example, *B. afzelii* and *B. valaisiana* have been detected in the badger, *Meles meles* (67), *B. spielmanii* seems mainly restricted to glirid rodents, namely the garden dormouse, *Eliomys quercinus* and the hazel dormouse, *Muscardinus avellanarius*, but not the European edible dormouse, *Glis glis* (60). Similarly, *B. afzelii* and *B. burgdorferi* s.s. can be found in the red squirrel, *Sciurus vulgaris* and the Siberian chipmunk, *Tamias sibiricus barberi* (65).

Deer are not proved to be reservoirs and are considered incompetent hosts for *Borrelia* genospecies (68, 69), even if positive serologies were observed (70, 71).

Drivers of disease emergence and spread

Ecological drivers

Drivers responsible for tick multiplication and questing activity

Disease presence, emergence and spread are highly related to *Ixodes* spp. tick distribution and questing activity. Many papers presented results of longitudinal studies performed in order to model spatial and temporal variations in tick abundance (27, 28, 46, 72-77). They provided observational data useful for the comprehension of the main ecological drivers that might contribute to disease emergence and spread:

- Climate: *Ixodes ricinus* ticks are able to develop in a wide range of climates if local conditions, in particular humidity and temperature, are suitable (78). To date, the species has been reported from Northern Africa to Northern Russia (79) and was recently detected in Iceland (80). A wide range of territories is thus suitable for their development in Europe. An exception is in the area around the Mediterranean basin, where the hot and dry conditions are generally considered unfavourable for the species (81, 82).
- Seasons: Due to their high sensitivity to desiccation tick questing activity highly depend on meteorological conditions and seasons. In most of European countries, density of questing ticks shows a high seasonality pattern with a peak of activity between April and August depending on the latitudes of the countries (83-85).
- Altitude: Despite many studies have shown that tick densities decrease with increasing altitude (with a sharper decrease above 1,000 m asl) (77, 86-89) since 2003, expansions in the distribution of ticks have been observed up to 1,100 m asl in the Czech Republic and Switzerland (90, 91), up to 1,250 m asl in Slovakia and the Czech Republic (77, 87), and up to 1,700-1,800 m asl in the Northern Alps (92) and the French Pyrenees (93).
- Land cover: *Ixodes ricinus* can be encountered in many types of vegetation, but forests and, in general, wooded areas offer better conditions for its development (94–96). In European countries, tick abundance was found higher in deciduous such as oaks and beech forests than in coniferous ones (92, 94, 97-99). The species has also been detected in suburban forests and public parks, as well as in private gardens (97, 100-102).

– Hosts: The development and local abundance of *I. ricinus* ticks is linked with the density and the wide variety of vertebrate hosts on which they feed. Local densities of rodents and other small mammals play an important role in sustaining populations of larvae and nymphs (103-105). Abundance of rodents is modulated by food availability, such as acorn density that can be used as a proxy for rodent density (76). The density of wild ungulates has been found to affect the local densities of *I. ricinus* and the risk of tick-borne disease transmission (97,106-108). Deer specifically, play an important role in the developmental cycle of ticks, with several studies demonstrating a significant correlation between deer population density and tick abundance (109-112). Birds are thought to play a relatively small role in maintaining tick populations, but an important role in tick infections and tick dissemination, through short or long-distance flights and migratory behaviour (113).

Many other key drivers may influence the local abundance of *I. ricinus*, in particular local weather conditions, soil composition, thickness of the litter layer, vegetation period, habitat structure and human management of wildlife and forests (78, 97, 111, 114, 115).

Drivers of tick infections: reservoirs of Bbsl

As competent reservoirs of Bbsl, rodents and birds highly contribute to tick infections. However, it was shown that the influence of local rodent populations on tick abundance and local infection is difficult to estimate due to their rapid development and pronounced seasonal variations (116). Introduced chipmunks and lizards were also proved to be competent reservoirs of Bbsl pathogens (28, 117).

On the contrary, deer are considered incompetent reservoirs of Bbsl therefore playing a complex role in its circulation (118, 119).

The dilution effect of biodiversity on Bbsl prevalence in ticks and reservoirs, defined as "the effect that occurs when the diversity of an ecological community reduces the transmission of a pathogen" is discussed in many articles (120-124). The concept is debated and continues to be questioned (120-122, 125). In North America, where the only species of *Borrelia* infecting humans is *B. burgdorferi* s.s., the tick vector *I. scapularis* and the main hosts are the white-tailed deer and the white-footed mice, potential dilution effects have been observed (121, 123, 126). In Europe, due to the presence of several *Borrelia* genospecies with different reservoir hosts, the dilution effect remains uncertain and more difficult to evaluate (118, 124).

Other drivers

Studies on demographic and geographic drivers of LB emergence in Europe are lacking. In a study performed in Wales and England, people with Lyme disease were from areas with higher socioeconomic status (127). New studies should be performed to better understand the consequences of such drivers on LB incidence in humans.

Natural history of disease in humans and animals, including symptoms, morbidity and mortality

Brief history of the pathogen and disease

The first descriptions of clinical manifestations of Lyme disease were performed between 1883 and 1909 by Buchwald and Afzelius (128-131). Afzelius described the cutaneous lesion now known as *erythema migrans* (EM) and suggested the role of the tick in the disease. In 1922, Garin

and Bujadoux reported the first case of neuroborreliosis in France confirming the role of the tick and the infectious nature of the disease (132).

Finally, in the mid-1970s, several patients, mainly children presented oligoarthritis with, for approximately ¼ of them, an expanding, annular skin lesion suggesting EM, before the onset of arthritis. All cases clustered around the town of Lyme in Southeastern Connecticut, USA, giving the name of Lyme disease (133). The aetiological agent was then identified first in the North American deer tick, *I. scapularis* (previously named *I. dammini)*, by Willy Burgdorfer in 1982 (134). Molecular studies revealed the isolate to be a new species within the genus *Borrelia*, and the species was subsequently named *B. burgdorferi* (108).

Disease in humans

In human health, the risk of infection is linked to the risk of exposure, defined as the product of the abundance of infected questing ticks and the human exposure to ticks. Transmission to humans is incidental and generally occurs during outdoor activities in a suitable habitat for ticks. The probability of pathogen transmission is considered low when the tick is removed within 24 hours (135).

However, the earliest the infected tick is removed, the lower the probability of *Borrelia* transmission is (136, 137). Humans are more commonly bitten by *I. ricinus* nymphs (70%) (138). Due to their small size, nymphs are sometimes only discovered late, typically few hours or days after infestation. Some patients may not remember any tick bite despite confirmed Bbsl infection (139). Thus, it is considered that the nymphal stage is the riskier for *Borrelia* transmission. In large surveys performed in humans, the seroconversion rate after a Bbsl-infected tick bite varied between 1.8% and 4.2% and the rate of clinical LB ranged between 0.7% to 4.0% (137,140,141).

Bbsl bacteria is mainly responsible for cutaneous, neurological and musculo-skeletal symptoms. Erythema migrans is the earliest and most common manifestation of LB and is also defined as the early localized stage of LB. It occurs in the first weeks after a tick bite. Systemic flu-like symptoms may accompany EM. If left untreated, the spirochetes can disseminate and cause early disseminated LB (weeks to months after tick bite) or late disseminated LB (from months to several years after tick bite) (142,143).

Three types of cutaneous lesions are usually observed:

- Erythema migrans (reported in 70 to 95% of cases in Europe depending on countries and studies). The classical form of erythema migrans corresponds to a pinkish to reddish colour cutaneous lesion, oval-shaped, with a central clearing, a regular growth, a centrifugal extension that appear in most cases at the site of the tick bite (144).
- Acrodermatitis Chronica Atrophicans (reported up to 10% of cases in Europe) corresponds to a macule or a plaque on a limb segment, of varying colour, more visible at bone tips, with progression from an initial oedematous stage to atrophy of the skin (145,146).
- Borrelial lymphocytoma is the rarest cutaneous manifestation of Lyme disease. It generally corresponds to a single lesion, very slow-growing, with varying colours, rarely pruritic with unusual localizations (ear lobe in children, breast in adults, exceptionally on the face, thorax or limbs) (133, 147).

Neurological manifestations can appear either in the early stages of the disease (more than 90% of cases) (148) or in the later stages (approximately 10%) and follow skin manifestations. Prevalence varies from 6 to 23% in European patients (133, 147) and affects mainly children/adolescents and adults over 50 years. LNB is generally due to infections by *B. garinii*

(the most frequent, ⅔ of cases) and *B. afzelii* and mainly manifests as meningoradiculitis and cranial nerve palsy (facial nerve, mainly in children). Other neurological manifestations such as clinical meningitis, acute myelitis, and encephalitis are less frequent. Cognitive disorders and dementia are rarely linked with LB infections but should be investigated in the absence of other aetiology (133, 147).

Arthralgia monoarthritis is a common sign of LB at the early stages and generally appears in an average time of 6 months (few weeks to two years) after an infected tick bite (133, 147). Without antibiotic therapy the affection can progress towards a chronic presentation. In Europe, the proportion of patients with Lyme arthritis with no EM or no tick bite recall seems higher than in the USA. Chronic myalgia has been associated with DNA detection of Bbsl in muscle samples (149). Many other atypical musculo-skeletal manifestations have been reported with low levels of scientific evidence.

Cardiac manifestations are reported in up to 4% of cases of LB. Outcome is favourable if correctly treated, but conduction disorders may require temporary cardiac pacing (147). Myocarditis, left ventricular dysfunction or cardiac failure are very rarely associated with LB (150, 151).

Several cases of patients with ophthalmological disorders and a positive serology for LB were reported. However, the link between symptoms and infection with Bbsl remains uncertain (147).

Other clinical manifestations are attributed to infection with the agents of LB with sometimes poor scientific evidence of their implication. Cases of death are rarely reported in patients with LB. Obel *et al.* did not show any significant differences in mortality rates between patients with LNB and the global population (152).

Disease in animals

As for humans, the risk of transmission to animals is proportional to their probability to encounter infected ticks in a suitable habitat (153-157).

Lyme disease in dogs and cats

Knowledge on consequences of Bbsl infection in domestic carnivores is incomplete. Most Bbsl-seropositive dogs and cats show no clinical signs of illness, neither after experimental nor after field infections. Koch's postulates have only been fulfilled experimentally for a clinical picture of transient fever, anorexia and clinical arthritis with lameness as well as reluctance to move, which was detected in puppies but not in dogs > 6 months. In contrast to humans, no EM has been observed in dogs (158), but synovial lesions were significant in infected dogs in all canine experimental studies (158-160).

When clinical signs compatible with Lyme disease are observed in dogs in the field, confirmation of the role of Lyme disease agents in the clinical outcome is difficult because diagnosis is based mainly on serology and no DNA of viable agents is detected (159). A small subset of infected dogs (<5%) presents transient signs of Lyme arthritis – corresponding to acute monoarticular or polyarticular lameness with joint swelling, fever, lethargy, and mild local lymphadenopathy – that generally responds well to an adapted antibiotic treatment. Nephritis (immune-complex glomerulonephritis) associated with Lyme infection is another supposed consequence of Bbsl infection described in dogs, less frequent but with the most serious impact on health. Other forms of infections described in humans are rare or not well documented in dogs (159). A predisposition of infection of Bernese Mountain Dogs was suggested due to a higher rate of seropositivity in this species in Central Europe but no clear consequences on health was found (no more risk of getting sick than other dog species) (161).

Vertical transmission and other non-vector borne modes of transmission (via semen, urine or blood) of *Borrelia* spp. are considered unlikely in dogs under natural conditions (160).

Seropositivity for Bbsl was detected in cats in Europe. However, no clear evidence of the consequence of infection with Bbsl on cats' health was demonstrated (159).

Lyme disease in horses

Horses are often exposed to *I. ricinus* ticks in Europe and seroprevalence of Bbsl infections in this group was evaluated from 12.4% to 48.4% depending on the studies and the countries (162, 163). Contrary to the high seroprevalence observed in the field, the paucity of documented cases of Lyme disease has made Bbsl infection and Lyme disease a controversial topic in equine practice (164). In horses, a broad spectrum of clinical manifestations, including arthritis, lameness, anterior uveitis, encephalitis and abortion, has been attributed to Bbsl infections (163). However, several other frequently described tick-borne pathogens – in particular *A. phagocytophilum*, *Babesia caballi* and *Theileria equi* – infect horses in Europe with sometimes similar clinical manifestations (162). Experimental inoculations in ponies led to systemic infection but did not induce any clinical signs nor histopathological alterations, except for skin lesions.

The association of Bbsl infection with other clinical signs is not well documented (164). It remains unclear if European Bbsl isolates are capable of causing such clinical manifestations in horses.

Lyme disease in cattle

As grazing vertebrates, cattle are often exposed to Bbsl (as well as *A. phagocytophilum* and *Babesia*) infected ticks in Europe (165). However, clinical relevance of Bbsl for ruminants is questionable. Active *B. burgdorferi* s.s., and *B. afzelii* infections with associated symptoms (skin erythema, fever, acute lameness due to arthritis) have been described in cattle in rare cases. Other studies suggested a link between serological evidence of Bbsl infection and clinical signs but causality remain uncertain. Experimental infections of cattle with *B. burgdorferi* s.s., *B. garinii*, and *B. afzelii* produced no clinical signs in cattle and in sheep. Neither clinical cases nor infection experiments have been published in goats to date (163).

Availability of preventive, therapeutic and control measures, including licensed or pipelines vaccines

Therapy in humans

Borrelia species are susceptible to several classes of antimicrobial agents, including penicillins, second- and third-generation cephalosporins, tetracyclines, macrolides and glycopeptides. On the other side they are relatively resistant to aminoglycosides, trimethoprim, rifampicin and quinolones (166,167). Treatment of human cases of LB is based on the use of a targeted antibiotic therapy. Use of first line antibiotics depends on the age of the patient and the clinical manifestation of the disease (168).

Therapy in animals

Doxycycline 10 mg/kg SID or BID is the first line antibiotic recommended for use in dogs with suspected LB because of its efficacy on *Borrelia* and on other tick-borne pathogens such as *Ehrlichia* and *Anaplasma* bacteria and purported antiarthritic and anti-inflammatory properties. β-lactams and macrolids are also antibiotic families that have proven their efficacy on the bacteria. A rapid response to the treatment occurs generally within 1-2 days, but the treatment is recommended for 4 weeks. As a matter of fact, in some cases, 4 weeks of high dose treatment (10 mg/kg doxycycline q12h) is not sufficient to clear all organisms in dogs. Relapse may be caused by coinfections or reinfections (159). Additional symptomatic treatments are recommended depending on the clinical forms observed (arthritis or nephritis).

Consistent with human guidelines, tetracyclines and β-lactam drugs are most commonly used to treat equine Lyme disease (164). However, the ideal treatment regimen for equine Lyme disease is unknown and further studies are necessary to prove the pertinence of use of those antibiotics in European horses.

Licensed or pipelined vaccines

There is actually no licensed vaccine for human prevention of LB in Europe.

An anti-*Borrelia* vaccine (Merilym 3 ND) using inactivated *B. burgdorferi* s.s., *B. garinii* and *B. afzelii* strains is available for use in dogs in Europe. The vaccine reduces the risk of skin infection by *Borrelia,* however the efficacy against the disease in dogs was not studied. This anti-*Borrelia* vaccination of dogs is actually the subject of controversies in particular because the disease remains poorly characterised in the species and infected dogs seem to respond readily to a correctly administered antibiotic treatment (159, 160, 169).

In North America, the off-label use of anti-*Borrelia* dog vaccine in horses is described. However, duration of immunity with dog vaccine used in horses seems short (170).

An oral reservoir-targeted vaccine using an OspA protein was developed and gave promising results in the USA in protecting uninfected mice from infection and reducing transmission of Bbsl bacteria (171). However, the feasibility of the use of such a vaccine as a long-term strategy to reduce hLB risk in Europe (by reducing infection of rodent reservoirs) remains uncertain taking into account the diversity of Bbsl strains, the role of birds in the epidemiological cycle and the rate of renewal of wild rodents in the field.

Other prevention measures

Prevention of tick bites in humans and domestic animals

Prevention of tick-borne diseases essentially relies on avoiding tick bite and early tick removal (147, 172-175).

Personal protective measures (wearing long trousers tucked into socks, white clothes, etc.) and use of repellents are effective to prevent tick bites. The main repellents recommended for ticks prevention in humans include DEET (N_1,N-diethyl-mtoluamide), IR3535 (ethyl butylacetyl-aminopropionate) or picardin (1-piperidinecarboxylic acid) (176). The use of permethrin for impregnation of clothes is no longer recommended, even for brief use in high exposure situations, due to skin irritation. It is also recommended to carefully check the body for ticks after coming back from outdoor activities (177). In case of bite, mechanical removal of attached tick and disinfection with antiseptics, followed by one month of observation in order to see if EM occurs are the recommendations.

In domestic animals, prevention is essentially based on tick prophylaxis i.e. acaricidal/repellent use. A high number of acaricidal specialities, developed to prevent tick infestations in domestic animals, are now available in the market of veterinary drugs. However, as 100% protection is not guaranteed, domestic animals should also be regularly checked and ticks removed in case of exposure.

Reduction of tick population or Bbsl infection, using acaricidal treatments

Many studies tested the effects of acaricidal treatments of the environment and/or wild animals (rodents, deer) on the reduction of tick density or of *Borrelia* infection (178–182). Even if an effectiveness on the reduction of the density of ticks has been demonstrated experimentally, consequences of these treatments on the environment and their acceptability by the society should be widely studied before implementation in the field (149,183).

Reduction of tick population or Bbsl infection, using hosts control

Host population control can be easily managed on domestic animals but is more complex on wild animals.

Several studies assessed the effects of ungulate management (culling or fencing, effects of deer density, habitat types, sizes of enclosed or unenclosed areas) on tick abundance (107, 110, 118, 184). *Ixodes ricinus* density tended to be lower in plots without deer than in those with deer suggesting a positive correlation between deer and tick densities (110). However, small enclosures with high deer densities were associated with reduction in tick densities as well as rodent numbers probably linked with the effect of high deer density on vegetation height (110, 118).

In Denmark, the reduction of roe deer for several years led to a reduction in LNB with a lag of 1 to 3 years, leading to the hypothesis of a reduction in the total population of ticks (Andersen *et al.*, 2018). However, in the United States, deer culling or reduction had a limited effect on tick abundance and no evidence of an effect on human disease risk reduction (126). Predators such as red fox (*Vulpes vulpes*) and stone marten (*Martes foina*) or assemblage of predators (foxes, raccoons and bobcat) were suggested as possible tick and Bbsl control measure (76, 185). They may act indirectly on Lyme disease risk and disease transmission by lowering the density of reservoir-competent mammalian hosts, such as rodents. It was also suggested that predation can change host behaviour in limiting their movement and consequently their contacts with ticks (185-187).

Effects of landscape modifications on tick population or Bbsl infection

Many methods using landscape management such as burning, mowing, leaf litter removal, herbicide treatment or desiccation were tested to reduce tick density and prevalence of Bbsl (188). Mowing (vegetation management) seems to have a reduction effect (189) while cattle grazing in forested areas was not shown to have any effect on infected nymph densities (190).

In urban areas, restoration of green areas is increasing in order to maintain human health and well-being (188). Consequences of this revegetation on vectors dynamic remain poorly understood. As people are encouraged to move around and engage in outdoor physical activities, a global study should consider the risk of tick-borne pathogen infections and implement risk prevention communication campaigns in these specific areas (191).

To implement effective control measures, it is essential to have a clear understanding of all factors that contribute to the risk of tick multiplication and Lyme disease. In the one hand, factors that may favour ticks and wild animal presence and, on the other hand, factors that could prevent

tick bites and reduce human and pet exposure (drawing channel visitor routes, developing leisure areas) should be further explored (192).

Attention should be paid to the effects of management methods on biodiversity and environment as well as the consequences on the abundance of hosts and ticks and the risk of Lyme disease (187). It would be useful to experiment the effects of all these factors with new integrated management strategies through consortia involving decision-makers, managers, scientists and citizens (183).

Disease specific recommendations

Sexual and mother-to-foetus transmission have been suggested, but never demonstrated. Pregnant women should thus be treated as the general population taking into account their status for the prescription of treatments (173). Similarly, there are actually no confirmed reported cases of *Borrelia* transmission to humans through blood transfusions or grafts (193).

Epidemiological situation at different spatial scales: past and current trends

The incidence of LB is difficult to estimate and to compare between years and countries because of a marked heterogeneity in surveillance systems, monitoring scale, case definitions, and testing methods.

In 2011, the European Concerted Action on Lyme Borreliosis (EUCALB) published a series of LB case definitions that incorporate clinical findings and essential laboratory evidence (194); however, implementation remains inconsistent in European countries. In 2023, only two countries (France and Poland) use the EUCALB cases definitions in the context their national surveillance system (195).

LNB was finally considered as the most specific form of the disease, easier to standardized and was thus chosen for future estimation of LB cases in Europe. Since 2019, ECDC added LNB to the communicable disease list with a standardized case definition. In 2023, only two countries (Bulgaria and Romania) use the ECDC LNB case definition in the context their national surveillance system of LB (195).

Past trends – results of Lyme borreliosis surveillance from 2005 to 2020

Burn *et al.* recently reviewed incidence data of LB in European countries from either national surveillance systems or systematic review for the period 2005-2020 (195,196). Taking into account all surveillance systems and considering any case definition, Burn *et al.* estimated an annual average of LB case number of 128,888 in Europe (195).

Comparison of incidences suggests (i) an increase of LB incidence at the national level in some countries of Northern and Eastern Europe from 2005 to 2020, while it remains globally constant in the majority of other countries during the same period (195,196).

Other studies suggest evidence of a geographical expansion of the disease in previously non-endemic areas as it was shown in the province of Verone, Northern Italy, using a five-years (2015-2019) sentinel surveillance (143).

Comparison of incidences between countries is difficult due to the lack of standardized surveillance system at the European level. Analysis of results of incidence data from surveillance

systems shows high reporting variations between countries. In particular, during the 2005-2020 period, Estonia, Lithuania, Slovenia, and Switzerland reported more than 100 cases/100,000 Population Per Year (PPY), France and Poland from 40-80/100,000 PPY, Finland and Latvia from 20-40/100,000 PPY while Belgium, Bulgaria, Croatia, England, Hungary, Ireland, Norway, Portugal, Romania, Scotland, and Serbia reported less than 20/100,000 cases PPY. However, at the subnational level, hotspots of infections (>100 cases/100,000 PPY) were found in some areas in particular in Belgium, Czech Republic, France, Germany, and Poland confirming the high possible variations in incidence within countries (79, 80).

Current trends – results of Lyme Neuroborreliosis surveillance according to the ECDC definition of case from 2019-2021

Since 2019, the ECDC has added the neurological form of LB called Lyme NeuroBorreliosis (LNB) to the list of communicable diseases (24).

To estimate the current epidemiological trend, we included all cases of LNB reported to the European Surveillance System Database (TESSy) during the years 2019-2021. Population denominator data were provided by the Statistical Office of the EU (Eurostat) for calculating incidence rates.

Over the 2019-2021 period, 13 EU/EEA (European Union/European Economic Area) countries reported 2645 cases of Lyme neuroborreliosis.

Analyses of the European LNB surveillance data showed that:

- The number of reported LNB cases varied considerably from one country to the other from 2019 to 2021 (Table 2) with the higher number of LNB cases reported in Czechia and Norway, followed by Poland, Italy, Denmark and Slovakia. All other countries that participate to the surveillance reported less than 40 cases during the 2019-2021 period.
- A higher number of LNB cases was reported in 2019 compared to 2020 and 2021 (Figure 6). However, the short period of surveillance and the limited number of reported cases do not allow to bring out any epidemiological trend.
- A peak of LNB cases was reached each year in the summer months (from June to September) (Figures 7 and 8).
- Two age groups – i.e., young people <20 years old (39% of cases) and adults between 50 and 79 years old (40% of cases) – represent 79% of LNB reported cases during the reporting period (Table 3, Figure 9).

The recent implementation of LNB records at the European level should lead to a cautious interpretation of the results presented here. As a matter of fact, due to the short period of reporting and the inconstant participation of European countries, it is possible that epidemiological trends in particular incidences data are underestimated in several countries.

The use of the ECDC LNB case definition in national surveillance systems in all European countries could help clarify these epidemiological trends in the future.

Table 2. Number of reported cases of *Lyme neuroborreliosis* and incidence rate (number of cases reported per country per 100,000 inhabitants) in 13 countries of the European Union and European Economic Area, 2019-2021 (data from TESSy-ECDC)

Country	N. cases	Mean incidence	Imported cases
Czechia	818	2,6	0
Denmark	157	0,9	0
Hungary	0	0,0	0
Ireland	24	0,2	2 (8%)
Italy	247	0,2	0
Lithuania	5	0,1	0
Luxembourg	2	0,1	0
Norway	806	5,0	7 (0,9%)
Poland	434	0,4	1 (0,0%)
Portugal	4	0,0	0
Romania	2	0,0	0
Slovakia	108	0,6	0
Slovenia	38	0,6	0

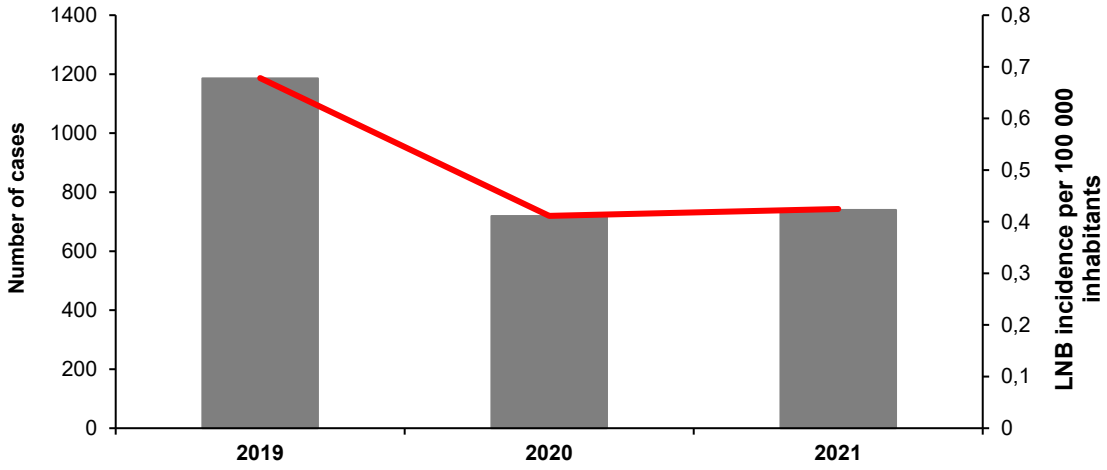

Figure 6. Total number of Lyme neuroborreliosis cases notified to ECDC during the period 2019-2021 (data from TESSy-ECDC)

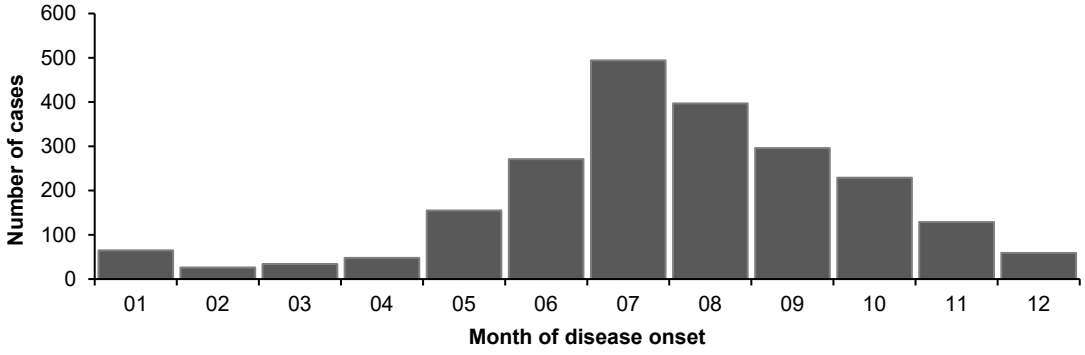

Figure 7. Number of reported Lyme neuroborreliosis cases by month of onset, 2019-2021 (data from TESSy-ECDC)

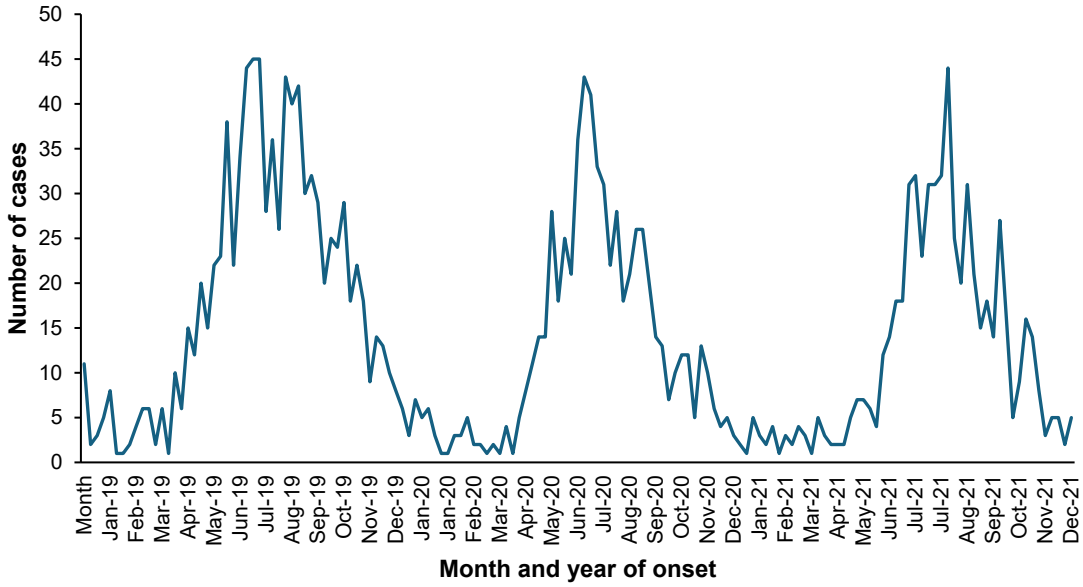

Figure 8. Number of Lyme neuroborreliosis cases by week of onset in the declaring European Union and European Economic Area countries, 2019–2021 (data from TESSy-ECDC)

Table 3. Main characteristics of reported cases of Lyme neuroborreliosis, European Union and European Economic Area countries, 2019−2021 (n = 2645) (data from TESSy-ECDC)

Characteristics	Cases		Incidence per 100,000 inhabitants
	Number	%	
Total	2645	100	0,50
Age group (years)			
0-9	738	28	NA
10-19	303	11	NA
20-29	111	4	NA
30-39	166	6	NA
40-49	213	8	NA
50-59	338	13	NA
60-69	395	15	NA
70-79	305	12	NA
≥ 80	76	3	NA
Unknown	0	NA	NA
Sex			
Female	1160	44	NA
Male	1483	56	NA
Unknown	2	NA	NA
Importation status			
Imported	10	0	NA
Locally acquired	2367	89	NA
Unknown	268	10	NA

NA: not available

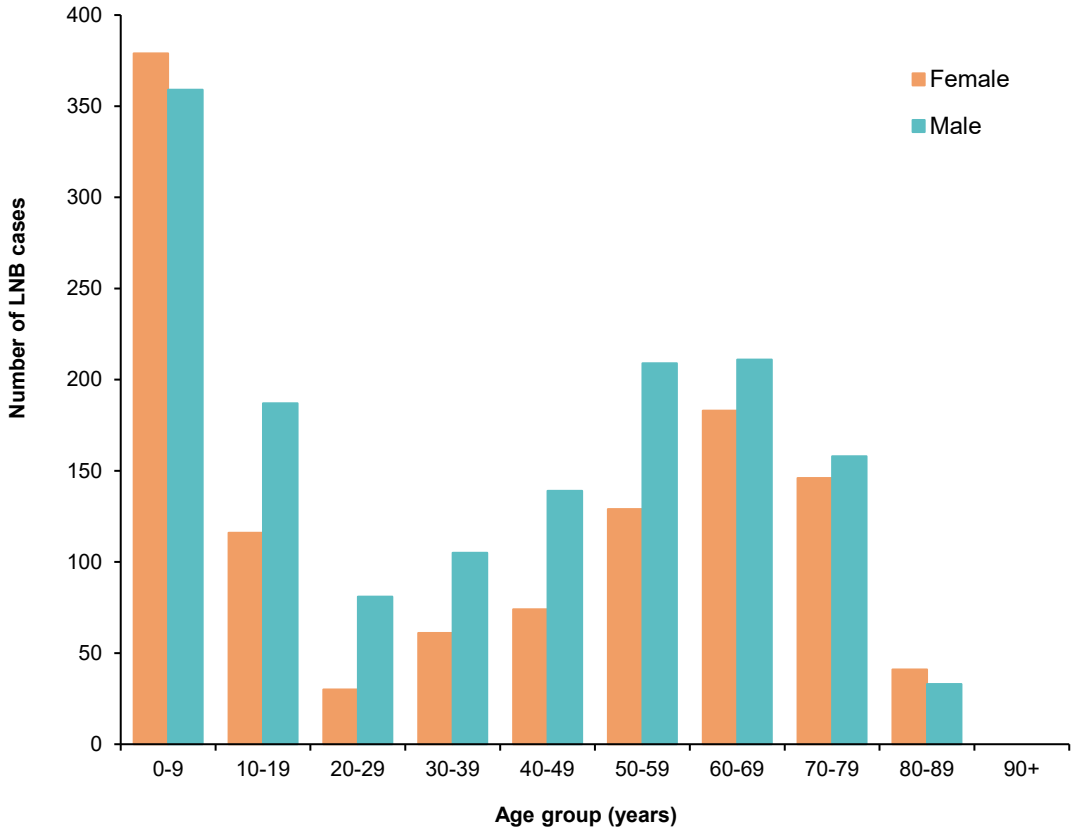

Figure 9. Number of Lyme neuroborreliosis (LNB) cases, by gender and age group in the reporting countries of European Union and European Economic Area, 2019-2021 (data from TESSy-ECDC)

Sociological and demographical dimension affecting susceptibility and exposure, including gender

Many patients with confirmed LB were exposed to ticks during leisure (142, 197) or outdoor professional activities. Main categories at risk include: farmers, forestry workers, horticulturists, hunters, rangers and veterinarians (198-209). In Poland, the seroprevalence of Bbsl infection in people with occupational risk of tick bites and forestry workers was higher than those in the control group composed of blood donors (203, 210). In Germany, military personnel showed a low but relevant risk of exposure to tick bites (211), while seroprevalence and incidence of Bbsl in football players were low (212). Recently, in Europe, an enhanced threat of exposure to tick bites has been observed in urban and peri-urban areas, green spaces and recreational parks. Infected ticks can be present in these areas and show a high risk of infection due to high density and activity of humans (213).

Several studies attempted to evaluate the clinical costs and economic outcomes of Lyme disease (214, 215). Many of these were conducted in the USA (68%) followed by Canada (9%) and the Netherlands (9%) and only one study in Sweden, Scotland and Germany (215). Lohr *et al.* estimated that the median medical cost for the hospitalization of a Lyme disease case ranged

from 2843 € for adults to 3917 € for adolescents between 2008-2011 (216). In Germany, using extrapolations, the direct costs of the disease were estimated at 23 million € annually for patient treatments and indirect costs such as loss of productivity and absence from work at 7 million €. In the Netherlands, Van den Wijngaard *et al.* estimated a mean cost (including direct and indirect costs) of about 5700 € per patient annually in 2014, resulting in a total cost of 23.5 million €/ per year (217).

Several studies reported that the general population and professionals (health care, exposed workers) have an incomplete knowledge of tick ecology and Lyme disease. Sometimes prevention practices are not adopted and knowledge of the recommendations is weak (198, 218-220). Improvement of knowledge and implementation of communication and health education actions for the public are essential, especially towards children (221-224). Leaflets, movies (225, 226), posters, information on the Internet (227), information at the entrance to a forest and mobile applications are all information and preventive tools widely used. As few studies dealt with the perception of risk by humans (218, 228, 229), new sociological studies should be performed in the future to ensure the feasibility and the acceptability of preventive measures proposed.

Diagnostic procedures and notification systems used at local, national and European scale

Diagnosis

There is currently no widely adopted standardized case definition for LB in Europe. Case definitions used for routine surveillance of LB vary by country (history of a tick bite, spectrum of clinical signs and laboratory tests used).

In laboratory LB diagnosis relies first on a suggesting clinical presentation and a history of tick exposure. Laboratory tests are often necessary to confirm infection. Culture of spirochaetes is considered as the gold-standard for confirmation of bacterial infections however it is often difficult to apply in case of LB due to the low number of viable spirochaetes usually present in patient samples and the difficulties to culture them. Thus, culture of spirochaetes is generally used only to confirm uncertain cases.

Serology is often used as a first line tool for the diagnosis of LB with an acceptable sensitivity and specificity in a majority of cases (194). However, the limits of serology should be known in particular: i) in case of negative serology in the early stages of the disease when the immune response has not yet been established; and ii) in case of positive serology in patients with successful antibiotic treatment due to the long persistence of antibodies despite recovery.

In Europe, recommended serology diagnosis relies on a two-step approach involving an initial screening test (usually ELISA, Enzyme-Linked Immunosorbent Assay), followed by a western blot for reactive and equivocal samples (168, 194). Nucleic acid amplification on infected tissues can help confirm doubtful cases. However, methods are not standardized, so test results obtained by different laboratories may show significant variability. In case of LNB, CerebroSpinal Fluid (CSF) examination is recommended.

In 2011, the EUCALB published a series of LB case definitions (Table 4) that incorporate clinical findings and essential laboratory evidence.

Table 4. Summary of clinical case definitions for Lyme borreliosis (194)

Term	Clinical case definition	Laboratory evidence: essential	Laboratory/clinical evidence: supporting
Erythema migrans	Expanding red or bluish-red patch (≥5 cm in diameter)[a], with or without central clearing. Advancing edge typically distinct, often intensely coloured, not markedly elevated.	None	Detection of Bbsl by culture and/or PCR from skin biopsy.
Borrelial lymphocytoma (rare)	Painless bluish-red nodule or plaque, usually on ear lobe, ear helix, nipple or scrotum; more frequent in children (especially on ear) than in adults.	Seroconversion or positive serology[b] Histology in unclear cases	Histology. Detection of *Bbsl* by culture and/or PCR from skin biopsy. Recent or concomitant EM.
Acrodermatitis chronica atrophicans	Long-standing red or bluish-red lesions, usually on the extensor surfaces of extremities. Initial doughy swelling. Lesions eventually become atrophic. Possible skin induration and fibroid nodules over bony prominences.	High level of specific serum IgG antibodies	Histology. Detection of Bbsl by culture and/or PCR from skin biopsy.
Lyme neuroborreliosis	In adults mainly meningo-radiculitis, meningitis; rarely encephalitis, myelitis; very rarely cerebral vasculitis. In children mainly meningitis and facial palsy.	Pleocytosis and demonstration of intrathecal specific antibody synthesis[c]	Detection of Bbsl by culture and/or PCR from CSF. Intrathecal synthesis of total IgM, and/or IgG and/or IgA. Specific serum antibodies. Recent or concomitant EM.
Lyme arthritis	Recurrent attacks or persisting objective joint swelling in one or a few large joints. Alternative explanations must be excluded.	Specific serum IgG antibodies, usually in high concentrations	Synovial fluid analysis. Detection of *Bbsl* by PCR and/or culture from synovial fluid and/or tissue.
Lyme carditis (rare)	Acute onset of atrio-ventricular (I-III) conduction disturbances, rhythm disturbances, sometimes myocarditis or pancarditis. Alternative explanations must be excluded	Specific serum antibodies	Detection of Bbsl by culture and/or PCR from endomyocardial biopsy. Recent or concomitant erythema migrans and/or neurologic disorders.
Ocular manifestations (rare)	Conjunctivitis, uveitis, papillitis, episcleritis, keratitis.	Specific serum antibodies	Recent or concomitant Lyme borreliosis manifestations. Detection of Bbsl by culture and/or PCR from ocular fluid.

[a] If <5 cm in diameter, a history of tick-bite, a delay in appearance (after the tick bite) of at least 2 days and an expanding rash at the site of the tick-bite is required.
[b] As a rule, initial and follow up samples have to be tested in parallel in order to avoid changes by inter-assay variation.
[c] In early cases intrathecally produced specific antibodies may still be absent.

For LNB, case definition has been specified in 2018 by ECDC (24) as follows:

– *Confirmed case*
  Neurological symptoms suggestive of LNB (according to European Federation of Neurological Societies (EFNS) suggested case definition) without other obvious reasons
  AND
  - Pleocytosis in cerebrospinal fluid, AND
  - Evidence of intrathecal production of LB antibodies, OR
  - Bbsl isolation, OR
  - Nucleic acid detection in cerebrospinal fluid

  OR
  - Detection of IgG LB antibodies in blood specimen only for children (age under 18) with facial palsy or other cranial neuritis and a recent (< 2 months) history of EM

– *Probable case*
  Neurological symptoms suggestive of LNB (according to European Federation of Neurological Societies (EFNS) suggested case definition) without other obvious reasons
  AND
  - Pleocytosis in cerebrospinal fluid, AND positive LB serology in cerebrospinal fluid OR
  - Specific intrathecal LB antibody production

Infrastructure capacity to identify pathogens for each Member State

Infrastructures to implement serological surveys in the general population allowed the publication of scientific articles describing the distribution of LB seroprevalence in 22 European countries, though only 14 studies are considered representative at the national scale (231). There was substantial heterogeneity among studies, which limits cross-study comparisons. Differences among studies were observed in terms of design, cohort types, periods sampled, sample sizes, and diagnostic methods (with a minority of studies using the recommended two-step serological approach described above).

More generally, LB incidence data are available for 25 European countries based on heterogeneous surveillance systems (196). Depending on the countries, these surveillance systems can be based on: i) passive or mandatory data collection; ii) defined sentinel sites or deployed at a national scale; iii) case definitions (clinical, laboratory, or both) and if relevant testing methods. Twenty-one countries for which statistics were available (84%) implemented passive surveillance. Among those different countries, only four used standardized case definitions recommended by European public health institutions.

Estimated influence of environmental change on the disease future trends

Tick population, abundance and activities are influenced by biotic and abiotic environmental factors (232). Knowledge of the ecology and epidemiology of *Ixodes* ticks and Bbsl remains a current issue to improve the control and prevention of LB (233, 234).

With climate change and global warming, *Ixodes* ticks are now reported in countries where they were not before (79,80) as in higher altitude in mountains (92,93). Global changes are susceptible to modify several key drivers that influence *Ixodes* ticks distribution, activity and survival as well as human exposure to tick bite. In particular, climate change and warming may impact: i) the life cycle of *I. ricinus*, the duration of interstadial development and mortality rates; ii) the composition and distribution of hosts on which *I. ricinus* feeds; and iii) human and animal exposures to ticks, due to changes in the spatial and temporal distributions of ticks and modifications of activities.

Several longitudinal studies were conducted in order to understand how ticks respond to environmental changes in particular weather, host density and human practices (27, 28, 46, 72–77). These studies allowed collection of data and development of models of tick activity (46, 235). In order to project future climate changes on tick population density, temperature, relative humidity or precipitation, data covering a reference period (1971-2000) were compared to similar variable in future periods (236). The future climate scenarios were applied to the near future (2012 to 2040/2050), the mid-term future (2050-2070) and the far future (2071-2100) taking into account different Representative Climate Pathways (RCP) i.e. low to extreme RCP scenarios (46,237).

In France, if the future climate followed the RCP8.5 trajectory, approximately 65% of the French surface area would have a Mediterranean climate, unfavourable for *I. ricinus* ticks by 2100 (46). In Germany, climate projections suggested that the peak of questing nymphs would shift towards the first seasons of the year and that a greater spatial heterogeneity of nymph densities would occur throughout the Germany (237). All of these projections describe situations in the near or distant future and should be taken with caution. However, recent studies have already reported perceptible changes in tick phenology in particular the presence of active ticks in winter in places where they were not before during this season (84, 238).

Anticipation of such changes in tick distribution and activity is essential since it could modify the epidemiology, seasonality and distribution of hLB cases in the future.

References

1. Adeolu M, Gupta RS. A phylogenomic and molecular marker based proposal for the division of the genus Borrelia into two genera: the emended genus Borrelia containing only the members of the relapsing fever Borrelia, and the genus Borreliella gen. nov. containing the members of the Lyme disease Borrelia (Borrelia burgdorferi sensu lato complex). Antonie Van Leeuwenhoek. 2014;105(6):1049-1072. doi:10.1007/s10482-014-0164-x
2. Gupta RS. Distinction between Borrelia and Borreliella is more robustly supported by molecular and phenotypic characteristics than all other neighbouring prokaryotic genera: Response to Margos' *et al.* "The genus Borrelia reloaded" (PLoS ONE 13(12): e0208432). *PLOS ONE*. 2019;14(8):e0221397. doi:10.1371/journal.pone.0221397
3. Margos G, Gofton A, Wibberg D, *et al.* The genus Borrelia reloaded. *PLOS ONE*. 2018;13(12):e0208432. doi:10.1371/journal.pone.0208432
4. Margos G, Marosevic D, Cutler S, *et al.* There is inadequate evidence to support the division of the genus Borrelia. *Int J Syst Evol Microbiol*. 2017;67(4):1081-1084. doi:10.1099/ijsem.0.001717
5. Krieg NR, Staley JT, Brown DR, *et al.*, eds. *Bergey's Manual® of Systematic Bacteriology*. Springer; 2010. doi:10.1007/978-0-387-68572-4
6. Murray RGE, Holt JG. The History of Bergey's Manual. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM, eds. *Bergey's Manual® of Systematic Bacteriology: Volume Two: The Proteobacteria, Part A Introductory Essays*. Springer US; 2005:1-14. doi:10.1007/0-387-28021-9_1

7. Eisen L. Vector competence studies with hard ticks and Borrelia burgdorferi sensu lato spirochetes: A review. *Ticks Tick-Borne Dis*. 2020;11(3):101359. doi:10.1016/j.ttbdis.2019.101359
8. Steinbrink A, Brugger K, Margos G, Kraiczy P, Klimpel S. The evolving story of *Borrelia burgdorferi* sensu lato transmission in Europe. *Parasitol Res*. 2022;121(3):781. doi:10.1007/s00436-022-07445-3
9. Fraser CM, Casjens S, Huang WM, *et al.* Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature*. 1997;390(6660):580-586. doi:10.1038/37551
10. Haven J, Vargas LC, Mongodin EF, *et al.* Pervasive recombination and sympatric genome diversification driven by frequency-dependent selection in Borrelia burgdorferi, the Lyme disease bacterium. *Genetics*. 2011;189(3):951-966.
11. Jacquot M, Gonnet M, Ferquel E, *et al.* Comparative population genomics of the Borrelia burgdorferi species complex reveals high degree of genetic isolation among species and underscores benefits and constraints to studying intra-specific epidemiological processes. *PloS One*. 2014;9(4):e94384-e94384.
12. Walter KS, Carpi G, Caccone A, Diuk-Wasser MA. Genomic insights into the ancient spread of Lyme disease across North America. *Nat Ecol Evol*. 2017;1(10):1569-1576. doi:10.1038/s41559-017-0282-8
13. Casjens SR, Di L, Akther S, *et al.* Primordial origin and diversification of plasmids in Lyme disease agent bacteria. *BMC Genomics*. 2018;19(1):218. doi:10.1186/s12864-018-4597-x
14. Stewart PE, Byram R, Grimm D, Tilly K, Rosa PA. The plasmids of *Borrelia burgdorferi*: essential genetic elements of a pathogen. *Plasmid*. 2005;53(1):1-13. doi:10.1016/j.plasmid.2004.10.006
15. Mongodin EF, Casjens SR, Bruno JF, *et al.* Inter- and intra-specific pan-genomes of Borrelia burgdorferi sensu lato: genome stability and adaptive radiation. *BMC Genomics*. 2013;14:693-693.
16. Casjens SR, Gilcrease EB, Vujadinovic M, *et al.* Plasmid diversity and phylogenetic consistency in the Lyme disease agent Borrelia burgdorferi. *BMC Genomics*. 2017;18(1):165. doi:10.1186/s12864-017-3553-5
17. Margos G, Gatewood AG, Aanensen DM, *et al.* MLST of housekeeping genes captures geographic population structure and suggests a European origin of *Borrelia burgdorferi*. *Proc Natl Acad Sci*. 2008;105(25):8730-8735. doi:10.1073/pnas.0800323105
18. Wang G, Liveris D, Mukherjee P, Jungnick S, Margos G, Schwartz I. Molecular Typing of *Borrelia burgdorferi*. *Curr Protoc Microbiol*. 2014;34(1):12C.5.1-12C.5.31. doi:10.1002/9780471729259.mc12c05s34
19. Jacquot M, Bisseux M, Abrial D, *et al.* High-throughput sequence typing reveals genetic differentiation and host specialization among populations of the Borrelia burgdorferi species complex that infect rodents. *PloS One*. 2014;9(2):e88581-e88581.
20. Strandh M, Råberg L. Within-host competition between Borrelia afzelii ospC strains in wild hosts as revealed by massively parallel amplicon sequencing. *Philos Trans R Soc Lond B Biol Sci*. 2015;370(1675).
21. Walter KS, Carpi G, Evans BR, Caccone A, Diuk-Wasser MA. Vectors as Epidemiological Sentinels: Patterns of Within-Tick Borrelia burgdorferi Diversity. *PLoS Pathog*. 2016;12(7):e1005759-e1005759.
22. Barbour AG. Isolation and cultivation of Lyme disease spirochetes. *Yale J Biol Med*. 1984;57(4):521-525.
23. Picken MM, Picken RN, Han D, *et al.* A two year prospective study to compare culture and polymerase chain reaction amplification for the detection and diagnosis of Lyme borreliosis. *Mol Pathol*. 1997;50(4):186-193. doi:10.1136/mp.50.4.186

24. Europe. Commission Implementing Decision (EU) 2018/945 of 22 June 2018 on the communicable diseases and related special health issues to be covered by epidemiological surveillance as well as relevant case definitions. *Official Journal of the European Union* L 170, 6/7/2018.
25. Blanchard L, Jones-Diette J, Lorenc T, Sutcliffe K, Sowden A, Thomas J. Comparison of national surveillance systems for Lyme disease in humans in Europe and North America: a policy review. *BMC Public Health*. 2022;22(1):1307. doi:10.1186/s12889-022-13669-w
26. Coipan EC, Jahfari S, Fonville M, *et al.* Spatiotemporal dynamics of emerging pathogens in questing *Ixodes ricinus*. *Front Cell Infect Microbiol*. 2013;3:36-36.
27. Hartemink N, van Vliet AJH, Gort G, *et al.* Seasonal patterns and spatial variation of *Borrelia burgdorferi* (sensu lato) infections in *Ixodes ricinus* in the Netherlands. *Parasit Vectors*. 2021;14(1):121-121.
28. Ruyts SC, Tack W, Ampoorter E, *et al.* Year-to-year variation in the density of *Ixodes ricinus* ticks and the prevalence of the rodent-associated human pathogens *Borrelia afzelii* and *B. miyamotoi* in different forest types. *Ticks Tick-Borne Dis*. 2018;9(2):141-145.
29. Richter D, Allgöwer R, Matuschka FR. Co-feeding transmission and its contribution to the perpetuation of the Lyme Disease spirochete *Borrelia afzelii*. *Emerg Infect Dis J*. 2002;8(12):1421. doi:10.3201/eid0812.010519
30. Rollend L, Fish D, Childs JE. Transovarial transmission of *Borrelia* spirochetes by *Ixodes scapularis*: a summary of the literature and recent observations. *Ticks Tick-Borne Dis*. 2013;4(1):46-51.
31. Couret J, Dyer MC, Mather TN, *et al.* Acquisition of *Borrelia burgdorferi* infection by larval *Ixodes scapularis* (Acari: Ixodidae) associated with engorgement measures. *J Med Entomol*. 2017;54(4):1055-1060. doi:10.1093/jme/tjx053
32. Hodzic E, Feng S, Freet KJ, Borjesson DL, Barthold SW. *Borrelia burgdorferi* population kinetics and selected gene expression at the host-vector interface. *Infect Immun*. 2002;70(7):3382-3388. doi:10.1128/iai.70.7.3382-3388.2002
33. Franke J, Hildebrandt A, Dorn W. Exploring gaps in our knowledge on Lyme borreliosis spirochaetes--updates on complex heterogeneity, ecology, and pathogenicity. *Ticks Tick-Borne Dis*. 2013;4(1):11-25.
34. Crippa M, Rais O, Gern L. Investigations on the mode and dynamics of transmission and infectivity of *Borrelia burgdorferi* sensu stricto and *Borrelia afzelii* in *Ixodes ricinus* ticks. *Vector Borne Zoonotic Dis Larchmt N*. 2002;2(1):3-9. doi:10.1089/153036602760260724
35. Dolan MC, Piesman J, Mbow ML, *et al.* Vector competence of *Ixodes scapularis* and *Ixodes ricinus* (Acari: Ixodidae) for three genospecies of *Borrelia burgdorferi*. *J Med Entomol*. 1998;35(4):465-470. doi:10.1093/jmedent/35.4.465
36. Gern L, Rais O. Efficient transmission of *Borrelia burgdorferi* between cofeeding *Ixodes ricinus* ticks (Acari: Ixodidae). *J Med Entomol*. 1996;33(1):189-192. doi:10.1093/jmedent/33.1.189
37. Hu CM, Wilske B, Fingerle V, Lobet Y, Gern L. Transmission of *Borrelia garinii* OspA serotype 4 to BALB/c mice by *Ixodes ricinus* ticks collected in the field. *J Clin Microbiol*. 2001;39(3):1169-1171. doi:10.1128/JCM.39.3.1169-1171.2001
38. Kahl O, Janetzki-Mittmann C, Gray JS, Jonas R, Stein J, de Boer R. Risk of infection with *Borrelia burgdorferi* sensu lato for a host in relation to the duration of nymphal *Ixodes ricinus* feeding and the method of tick removal. *Zentralblatt Bakteriol Int J Med Microbiol*. 1998;287(1-2):41-52. doi:10.1016/s0934-8840(98)80142-4
39. Pospisilova T, Urbanova V, Hes O, Kopacek P, Hajdusek O, Sima R. Tracking of *Borrelia afzelii* Transmission from Infected *Ixodes ricinus* Nymphs to Mice. *Infect Immun*. 2019;87(6).

40. Tonetti N, Voordouw MJ, Durand J, Monnier S, Gern L. Genetic variation in transmission success of the Lyme borreliosis pathogen *Borrelia afzelii*. *Ticks Tick-Borne Dis*. 2015;6(3):334-343. doi:10.1016/j.ttbdis.2015.02.007

41. van Duijvendijk G, Coipan C, Wagemakers A, *et al.* Larvae of *Ixodes ricinus* transmit *Borrelia afzelii* and *B. miyamotoi* to vertebrate hosts. *Parasit Vectors*. 2016;9:97-97.

42. Sato Y, Nakao M. Transmission of the Lyme disease spirochete, Borrelia garinii, between infected and uninfected immature Ixodes persulcatus during cofeeding on mice. *J Parasitol*. 1997;83(3):547-550.

43. Sun Y, Xu R. Ability of *Ixodes persulcatus*, *Haemaphysalis concinna* and *Dermacentor silvarum* ticks to acquire and transstadially transmit *Borrelia garinii*. *Exp Appl Acarol*. 2003;31(1-2):151-160. doi:10.1023/b:appa.0000005119.30172.43

44. Gern L, Toutoungi LN, Hu CM, Aeschlimann A. Ixodes (Pholeoixodes) hexagonus, an efficient vector of Borrelia burgdorferi in the laboratory. *Med Vet Entomol*. 1991;5(4):431-435. doi:10.1111/j.1365-2915.1991.tb00571.x

45. Lees AD. The water balance in *Ixodes ricinus* L. and certain other species of ticks. *Parasitology*. 1946;37(1-2):1-20. doi:10.1017/S0031182000013093

46. Wongnak P. *Modeling of tick population dynamics by a hierarchical Bayesian approach taking into account abiotic factors (temperature and relative humidity) in the context of adaptation to climate change*. Santé publique et épidémiologie, Université Clermont-Auvergne; 2022.

47. Hofmeester TR, Coipan EC, van Wieren SE, Prins HHT, Takken W, Sprong H. Few vertebrate species dominate the *Borrelia burgdorferi* s.l. life cycle. *Environ Res Lett*. 2016;11(4):043001. doi:10.1088/1748-9326/11/4/043001

48. Takumi K, Sprong H, Hofmeester TR. Impact of vertebrate communities on *Ixodes ricinus*-borne disease risk in forest areas. *Parasit Vectors*. 2019;12(1):434-434.

49. Wang SS, Liu JY, Wang BY, *et al.* Geographical distribution of *Ixodes persulcatus* and associated pathogens: Analysis of integrated data from a China field survey and global published data. *One Health*. 2023;16:100508. doi:10.1016/j.onehlt.2023.100508

50. Estrada-Peña A, Roura X, Sainz A, Miró G, Solano-Gallego L. Species of ticks and carried pathogens in owned dogs in Spain: Results of a one-year national survey. *Ticks Tick-Borne Dis*. 2017;8(4):443-452.

51. Szekeres S, Coipan EC, Rigó K, *et al.* Eco-epidemiology of *Borrelia miyamotoi* and Lyme borreliosis spirochetes in a popular hunting and recreational forest area in Hungary. *Parasit Vectors*. 2015;8:309-309.

52. Heylen D, Matthysen E, Fonville M, Sprong H. Songbirds as general transmitters but selective amplifiers of Borrelia burgdorferi sensu lato genotypes in Ixodes rinicus ticks. *Environ Microbiol*. 2014;16(9):2859-2868.

53. Spitalská E, Literák I, Kocianová E, Taragel'ová V. The importance of Ixodes arboricola in transmission of Rickettsia spp., Anaplasma phagocytophilum, and Borrelia burgdorferi sensu lato in the Czech Republic, Central Europe. *Vector Borne Zoonotic Dis Larchmt N*. 2011;11(9):1235-1241. doi:10.1089/vbz.2010.0210

54. Palomar AM, Portillo A, Santibáñez P, *et al.* Presence of *Borrelia turdi* and *Borrelia valaisiana* (Spirochaetales: Spirochaetaceae) in Ticks Removed From Birds in the North of Spain, 2009–2011. *J Med Entomol*. 2017;54(1):243-246. doi:10.1093/jme/tjw158

55. Nefedova VV, Korenberg EI, Fadeeva IA, Gorelova NB. (Genetic characteristics of human pathogenic *Borrelia* isolated from *Ixodes trianguliceps* Bir. and *Ixodes pavlovskyi* Pom). *Med Parazitol (Mosk)*. 2005;(2):9-12.

56. Gómez-Díaz E, Boulinier T, Sertour N, Cornet M, Ferquel E, McCoy KD. Genetic structure of marine *Borrelia garinii* and population admixture with the terrestrial cycle of Lyme borreliosis. *Environ Microbiol*. 2011;13(9):2453-2467.

57. Hanincová K, Schäfer SM, Etti S, *et al.* Association of *Borrelia afzelii* with rodents in Europe. *Parasitology*. 2003;126(1):11-20. doi:10.1017/S0031182002002548

58. Kurtenbach K, De Michelis S, Etti S, *et al.* Host association of *Borrelia burgdorferi* sensu lato – the key role of host complement. *Trends Microbiol*. 2002;10(2):74-79. doi:10.1016/S0966-842X(01)02298-3

59. Margos G, Wilske B, Sing A, *et al. Borrelia bavariensis* sp. nov. is widely distributed in Europe and Asia. *Int J Syst Evol Microbiol*. 2013;63:4284-4288.

60. Richter D, Klug B, Spielman A, Matuschka FR. Adaptation of diverse Lyme Disease spirochetes in a natural rodent reservoir host. *Infect Immun*. 2004;72(4):2442-2444. doi:10.1128/iai.72.4.2442-2444.2004

61. Richter D, Schlee DB, Matuschka FR. Reservoir competence of various rodents for the lyme disease spirochete *Borrelia spielmanii*. *Appl Environ Microbiol*. 2011;77(11):3565-3570. doi:10.1128/AEM.00022-11

62. Norte AC, da Silva LP, Tenreiro PJQ, *et al.* Patterns of tick infestation and their *Borrelia burgdorferi* s.l. infection in wild birds in Portugal. *Ticks Tick-Borne Dis*. 2015;6(6):743-750.

63. Bourdin A, Bord S, Durand J, *et al.* Forest diversity reduces the prevalence of pathogens transmitted by the tick *Ixodes ricinus*. *Front Ecol Evol*. 2022;10:891908. doi:10.3389/fevo.2022.891908

64. Richter D, Matuschka FR. Perpetuation of the Lyme Disease spirochete *Borrelia lusitaniae* by lizards. *Appl Environ Microbiol*. 2006;72(7):4627-4632. doi:10.1128/AEM.00285-06

65. Marsot M, Sigaud M, Chapuis JL, Ferquel E, Cornet M, Vourc'h G. Introduced Siberian chipmunks (*Tamias sibiricus barberi*) harbor more-diverse *Borrelia burgdorferi* sensu lato genospecies than native bank voles (*Myodes glareolus*). *Appl Environ Microbiol*. 2011;77(16):5716-5721.

66. Marsot M, Henry PY, Vourc'h G, *et al.* Which forest bird species are the main hosts of the tick, *Ixodes ricinus*, the vector of *Borrelia burgdorferi* sensu lato, during the breeding season? *Int J Parasitol*. 2012;42(8):781-788.

67. Gern L, Sell K. Isolation of Borrelia burgdorferi sensu lato from the skin of the European badger (Meles meles) in Switzerland. *Vector Borne Zoonotic Dis Larchmt N*. 2009;9(2):207-208. doi:10.1089/vbz.2008.0050

68. Telford SR, Mather TN, Moore SI, Wilson ML, Spielman A. Incompetence of deer as reservoirs of the Lyme Disease spirochete. *Am J Trop Med Hyg*. 1988;39(1):105-109. doi:10.4269/ajtmh.1988.39.105

69. Pacilly FCA, Benning ME, Jacobs F, *et al.* Blood feeding on large grazers affects the transmission of Borrelia burgdorferi sensu lato by Ixodes ricinus. *Ticks Tick-Borne Dis*. 2014;5(6):810-817.

70. Alonso S, Márquez FJ, Solano-Gallego L. *Borrelia burgdorferi* serosurvey in wild deer in England and Wales. *Vector Borne Zoonotic Dis Larchmt N*. 2012;12(6):448-455.

71. Pato FJ, Panadero R, Vázquez L, *et al.* Seroprevalence of *Borrelia burgdorferi* sensu lato in roe deer (*Capreolus capreolus*) from northwestern Spain. *J Zoo Wildl Med Off Publ Am Assoc Zoo Vet*. 2013;44(3):660-665.

72. Cull B, Hansford KM, McGinley L, *et al.* A nationwide study on *Borrelia burgdorferi* s.l. infection rates in questing *Ixodes ricinus*: a six-year snapshot study in protected recreational areas in England and Wales. *Med Vet Entomol*. Published online January 2021.

73. Hauser G, Rais O, Morán Cadenas F, Gonseth Y, Bouzelboudjen M, Gern L. Influence of climatic factors on *Ixodes ricinus* nymph abundance and phenology over a long-term monthly observation in Switzerland (2000-2014). *Parasit Vectors*. 2018;11(1):289-289.

74. Hvidsten D, Frafjord K, Gray JS, *et al.* The distribution limit of the common tick, *Ixodes ricinus*, and some associated pathogens in north-western Europe. *Ticks Tick-Borne Dis*. 2020;11(4):101388-101388.

75. Okeyo M, Hepner S, Rollins RE, *et al.* Longitudinal study of prevalence and spatio-temporal distribution of *Borrelia burgdorferi* sensu lato in ticks from three defined habitats in Latvia, 1999-2010. *Environ Microbiol*. 2020;22(12):5033-5047.

76. Ostfeld RS, Levi T, Keesing F, Oggenfuss K, Canham CD. Tick-borne disease risk in a forest food web. *Ecology*. 2018;99(7):1562-1573.

77. Taragel'ová VR, Mahríková L, Selyemová D, Václav R, Derdáková M. Natural foci of *Borrelia lusitaniae* in a mountain region of Central Europe. *Ticks Tick-Borne Dis*. 2016;7(2):350-356.

78. Beugnet F, Chalvet-Monfray K, Loukos H. FleaTickRisk: a meteorological model developed to monitor and predict the activity and density of three tick species and the cat flea in Europe. *Geospatial Health*. 2009;4(1):97-113. doi:10.4081/gh.2009.213

79. Semenza JC, Suk JE. Vector-borne diseases and climate change: a European perspective. *FEMS Microbiol Lett*. 2018;365(2):fnx244. doi:10.1093/femsle/fnx244

80. Alfredsson M, Olafsson E, Eydal M, *et al.* Surveillance of *Ixodes ricinus* ticks (Acari: Ixodidae) in Iceland. *Parasit Vectors*. 2017;10(1):466-466.

81. Estrada-Peña A, Venzal JM. Climate Niches of Tick Species in the Mediterranean Region: Modeling of Occurrence Data, Distributional Constraints, and Impact of Climate Change. *J Med Entomol*. 2007;44(6):1130-1138. doi:10.1603/0022-2585(2007)44(1130:CNOTSI)2.0.CO;2

82. Gilot B, Degeilh B, Pichot J, Doche B, Guiguen C. Prevalence of *Borrelia burgdorferi* (sensu lato) in *Ixodes ricinus* (L.) populations in France, according to a phytoecological zoning of the territory. *Eur J Epidemiol*. 1996;12(4):395-401. doi:10.1007/BF00145304

83. Lejal E, Marsot M, Chalvet-Monfray K, *et al.* A three-years assessment of *Ixodes ricinus*-borne pathogens in a French peri-urban forest. *Parasit Vectors*. 2019;12(1):551-551.

84. Wongnak P, Bord S, Jacquot M, *et al.* Meteorological and climatic variables predict the phenology of *Ixodes ricinus* nymph activity in France, accounting for habitat heterogeneity. *Sci Rep*. 2022;12(1). doi:10.1038/s41598-022-11479-z

85. Jensen PM, Danielsen F, Skarphedinsson S. Monitoring temporal trends in internet searches for "ticks" across Europe by Google trends: tick–human interaction or general interest? *Insects*. 2022;13(2):176. doi:10.3390/insects13020176

86. Danielová V, Daniel M, Schwarzová L, *et al.* Integration of a tick-borne encephalitis virus and *Borrelia burgdorferi* sensu lato into mountain ecosystems, following a shift in the altitudinal limit of distribution of their vector, *Ixodes ricinus* (Krkonoše Mountains, Czech Republic). *Vector-Borne Zoonotic Dis*. 2010;10(3):223-230. doi:10.1089/vbz.2009.0020

87. Materna J, Daniel M, Metelka L, Harčarik J. The vertical distribution, density and the development of the tick *Ixodes ricinus* in mountain areas influenced by climate changes (The Krkonoše Mts., Czech Republic). *Int J Med Microbiol*. 2008;298:25-37. doi:10.1016/j.ijmm.2008.05.004

88. Ragagli C, Mannelli A, Ambrogi C, *et al.* Presence of host-seeking *Ixodes ricinus* and their infection with *Borrelia burgdorferi* sensu lato in the Northern Apennines, Italy. *Exp Appl Acarol*. 2016;69(2):167-178.

89. Sándor AD, D'Amico G, Gherman CM, Dumitrache MO, Domșa C, Mihalca AD. Mesocarnivores and macroparasites: altitude and land use predict the ticks occurring on red foxes (*Vulpes vulpes*). *Parasit Vectors*. 2017;10(1):173. doi:10.1186/s13071-017-2113-9

90. Cadenas FM, Rais O, Jouda F, *et al.* Phenology of *Ixodes ricinus* and Infection with *Borrelia burgdorferi* sensu lato Along a North- and South-Facing Altitudinal Gradient on Chaumont Mountain, Switzerland. *J Med Entomol*. 2007;44(4):683-693. doi:10.1093/jmedent/44.4.683

91. Daniel M, Rudenko N, Golovchenko M, *et al.* The occurrence of *Ixodes ricinus* ticks and important tick-borne pathogens in areas with high tick-borne encephalitis prevalence in different altitudinal levels of the Czech Republic Part II. *Ixodes ricinus* ticks and genospecies of *Borrelia burgdorferi* sensu lato complex. *Epidemiol Mikrobiol Imunol Cas Spolecnosti Epidemiol Mikrobiol Ceske Lek Spolecnosti JE Purkyne*. 2016;65(3):182-192.
92. Garcia-Vozmediano A, Krawczyk AI, Sprong H, Rossi L, Ramassa E, Tomassone L. Ticks climb the mountains: Ixodid tick infestation and infection by tick-borne pathogens in the Western Alps. *Ticks Tick-Borne Dis*. 2020;11(5):101489-101489.
93. Akl T, Bourgoin G, Souq ML, *et al.* Detection of tick-borne pathogens in questing *Ixodes ricinus* in the French Pyrenees and first identification of *Rickettsia monacensis* in France. *Parasite Paris Fr*. 2019;26:20-20.
94. Boehnke D, Brugger K, Pfäffle M, *et al.* Estimating *Ixodes ricinus* densities on the landscape scale. *Int J Health Geogr*. 2015;14:23-23.
95. Swart A, Ibañez-Justicia A, Buijs J, *et al.* Predicting tick presence by environmental risk mapping. *Front Public Health*. 2014;2:238-238.
96. Vanwambeke SO, Van Doninck J, Artois J, Davidson RK, Meyfroidt P, Jore S. Forest classes and tree cover gradient: tick habitat in encroached areas of southern Norway. *Exp Appl Acarol*. 2016;68(3):375-385. doi:10.1007/s10493-015-0007-0
97. Vourc'h G, Abrial D, Bord S, *et al.* Mapping human risk of infection with *Borrelia burgdorferi* sensu lato, the agent of Lyme borreliosis, in a periurban forest in France. *Ticks Tick-Borne Dis*. 2016;7(5):644-652.
98. James MC, Bowman AS, Forbes KJ, Lewis F, McLeod JE, Gilbert L. Environmental determinants of *Ixodes ricinus* ticks and the incidence of *Borrelia burgdorferi* sensu lato, the agent of Lyme borreliosis, in Scotland. *Parasitology*. 2013;140(2):237-246.
99. Tack W, Madder M, Baeten L, De Frenne P, Verheyen K. The abundance of *Ixodes ricinus* ticks depends on tree species composition and shrub cover. *Parasitology*. 2012;139(10):1273-1281.
100. Hansford KM, Fonville M, Gillingham EL, *et al.* Ticks and *Borrelia* in urban and peri-urban green space habitats in a city in southern England. *Ticks Tick-Borne Dis*. 2017;8(3):353-361.
101. Mathews-Martin L, Namèche M, Vourc'h G, *et al.* Questing tick abundance in urban and peri-urban parks in the French city of Lyon. *Parasit Vectors*. 2020;13(1):576-576.
102. Paul REL, Cote M, Le Naour E, Bonnet SI. Environmental factors influencing tick densities over seven years in a French suburban forest. *Parasit Vectors*. 2016;9(1):309. doi:10.1186/s13071-016-1591-5
103. Krawczyk AI, van Duijvendijk GLA, Swart A, *et al.* Effect of rodent density on tick and tick-borne pathogen populations: consequences for infectious disease risk. *Parasit Vectors*. 2020;13(1):34-34.
104. Martello E, Mannelli A, Grego E, *et al. Borrelia burgdorferi* sensu lato and spotted fever group rickettsiae in small rodents and attached ticks in the Northern Apennines, Italy. *Ticks Tick-Borne Dis*. 2019;10(4):862-867.
105. Perez G, Bastian S, Agoulon A, *et al.* Effect of landscape features on the relationship between *Ixodes ricinus* ticks and their small mammal hosts. *Parasit Vectors*. 2016;9(1):20. doi:10.1186/s13071-016-1296-9
106. Fabri ND, Sprong H, Hofmeester TR, *et al.* Wild ungulate species differ in their contribution to the transmission of *Ixodes ricinus*-borne pathogens. *Parasit Vectors*. 2021;14(1):360. doi:10.1186/s13071-021-04860-w
107. Gilbert L, Maffey GL, Ramsay SL, Hester AJ. The effect of deer management on the abundance of *Ixodes ricinus* in Scotland. *Ecol Appl*. 2012;22(2):658-667. doi:10.1890/11-0458.1

108. Radolf JD, Caimano MJ, Stevenson B, Hu LT. Of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease spirochaetes. *Nat Rev Microbiol*. 2012;10(2):87-99.

109. Chen D, Wong H, Belanger P, Moore K, Peterson M, Cunningham J. Analyzing the correlation between deer habitat and the component of the risk for Lyme Disease in Eastern Ontario, Canada: A GIS-based approach. *ISPRS Int J Geo-Inf*. 2015;4(1):105-123. doi:10.3390/ijgi4010105

110. Hofmeester TR, Sprong H, Jansen PA, Prins HHT, Van Wieren SE. Deer presence rather than abundance determines the population density of the sheep tick, *Ixodes ricinus*, in Dutch forests. *Parasit Vectors*. 2017;10(1):433. doi:10.1186/s13071-017-2370-7

111. Medlock JM, Hansford KM, Bormane A, *et al.* Driving forces for changes in geographical distribution of Ixodes ricinus ticks in Europe. *Parasit Vectors*. 2013;6:1. doi:10.1186/1756-3305-6-1

112. Mysterud A, Hügli C, Viljugrein H. Tick infestation on medium–large-sized mammalian hosts: are all equally suitable to *Ixodes ricinus* adults? *Parasit Vectors*. 2021;14(1):254. doi:10.1186/s13071-021-04775-6

113. Klitgaard K, Kjær LJ, Isbrand A, Hansen MF, Bødker R. Multiple infections in questing nymphs and adult female Ixodes ricinus ticks collected in a recreational forest in Denmark. *Ticks Tick-Borne Dis*. 2019;10(5):1060-1065.

114. Ehrmann S, Ruyts SC, Scherer-Lorenzen M, *et al.* Habitat properties are key drivers of *Borrelia burgdorferi* (s.l.) prevalence in *Ixodes ricinus* populations of deciduous forest fragments. *Parasit Vectors*. 2018;11(1):23-23.

115. Goldstein V, Boulanger N, Schwartz D, *et al.* Factors responsible for *Ixodes ricinus* nymph abundance: Are soil features indicators of tick abundance in a French region where Lyme borreliosis is endemic? *Ticks Tick-Borne Dis*. 2018;9(4):938-944.

116. Pérez D, Kneubühler Y, Rais O, Gern L. Seasonality of *Ixodes ricinus* ticks on vegetation and on rodents and *Borrelia burgdorferi* sensu lato genospecies diversity in two Lyme borreliosis-endemic areas in Switzerland. *Vector Borne Zoonotic Dis Larchmt N*. 2012;12(8):633-644.

117. Rizzoli A, Silaghi C, Obiegala A, *et al. Ixodes ricinus* and its transmitted pathogens in urban and peri-urban areas in Europe: new hazards and relevance for public health. *Front Public Health*. 2014;2:251-251.

118. Gandy S, Kilbride E, Biek R, Millins C, Gilbert L. Experimental evidence for opposing effects of high deer density on tick-borne pathogen prevalence and hazard. *Parasit Vectors*. 2021;14(1):509. doi:10.1186/s13071-021-05000-0

119. Jaenson TG, Tälleklint L. Incompetence of roe deer as reservoirs of the Lyme borreliosis spirochete. *J Med Entomol*. 1992;29(5):813-817. doi:10.1093/jmedent/29.5.813

120. Keesing F, Ostfeld RS. Dilution effects in disease ecology. *Ecol Lett*. 2021;24(11):2490-2505. doi:10.1111/ele.13875

121. Linske MA, Williams SC, Stafford KC 3rd, Ortega IM. *Ixodes scapularis* (Acari: Ixodidae) reservoir host diversity and abundance impacts on dilution of *Borrelia burgdorferi* (Spirochaetales: Spirochaetaceae) in residential and woodland habitats in Connecticut, United States. *J Med Entomol*. 2018;55(3):681-690.

122. Ogden NH, Tsao JI. Biodiversity and Lyme Disease: Dilution or amplification? *Epidemics*. 2009;1(3):196-206. doi:10.1016/j.epidem.2009.06.002

123. Huang CI, Kay SC, Davis S, *et al.* High burdens of *Ixodes scapularis* larval ticks on white-tailed deer may limit Lyme disease risk in a low biodiversity setting. *Ticks Tick-Borne Dis*. 2019;10(2):258-268.

124. Ruyts SC, Landuyt D, Ampoorter E, *et al.* Low probability of a dilution effect for Lyme borreliosis in Belgian forests. *Ticks Tick-Borne Dis*. 2018;9(5):1143-1152.

125. Diuk-Wasser MA, VanAcker MC, Fernandez MP. Impact of land use changes and habitat fragmentation on the eco-epidemiology of tick-borne diseases. *J Med Entomol*. Published online October 2020.

126. Kugeler KJ, Jordan RA, Schulze TL, Griffith KS, Mead PS. Will culling white-tailed deer prevent Lyme Disease? *Zoonoses Public Health*. 2016;63(5):337-345.

127. Tulloch JSP, Semper AE, Brooks TJG, *et al.* The demographics and geographic distribution of laboratory-confirmed Lyme disease cases in England and Wales (2013-2016): an ecological study. *BMJ Open*. 2019;9(7):e028064-e028064.

128. Buchwald A. Buchwald A. Ein Fall von diffuser idiopathischer Haut-Atrophie. *Vierteljahr Dermatol Syph*. 1883;10:553-556.

129. Korting G. Biographische Notiz über Alfred Buchwald, den Beschreiber der diffusen idiopathischen Haut- Atrophie. *Hautarzt*. Published online 1965:324-325.

130. Afzelius A. Verdhandlungen der dermatologischen Gesellschaft zu Stockholm. Published online Oktober 1909.

131. Afzelius A. Erythema chronicum migrans. *Acta Derm- Venereol (Stockholm)*. 1921;(2):120-125.

132. Garin C, Bujadoux A. Paralysie par les tiques. *J Med Lyon*. Published online 1922:765-767.

133. Cardenas-de la Garza JA, De la Cruz-Valadez E, Ocampo-Candiani J, Welsh O. Clinical spectrum of Lyme disease. *Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol*. 2019;38(2):201-208.

134. Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP. Lyme disease-a tick-borne spirochetosis? *Science*. 1982;216(4552):1317-1319. doi:10.1126/science.7043737

135. Cook MJ. Lyme borreliosis: a review of data on transmission time after tick attachment. *Int J Gen Med*. 2015;8:1-8.

136. Hofhuis A, van de Kassteele J, Sprong H, *et al.* Predicting the risk of Lyme borreliosis after a tick bite, using a structural equation model. *PloS One*. 2017;12(7):e0181807-e0181807.

137. Wilhelmsson P, Fryland L, Lindblom P, *et al.* A prospective study on the incidence of *Borrelia burgdorferi* sensu lato infection after a tick bite in Sweden and on the Åland Islands, Finland (2008-2009). *Ticks Tick-Borne Dis*. 2016;7(1):71-79.

138. Cull B, Pietzsch ME, Gillingham EL, McGinley L, Medlock JM, Hansford KM. Seasonality and anatomical location of human tick bites in the United Kingdom. *Zoonoses Public Health*. 2020;67(2):112-121.

139. Sočan M, Blaško-Markič M, Erčulj V, Lajovic J. Socio-economic characteristics in notified erythema migrans patients. *Zdr Varst*. 2015;54(4):267-273.

140. Faulde MK, Rutenfranz M, Hepke J, Rogge M, Görner A, Keth A. Human tick infestation pattern, tick-bite rate, and associated *Borrelia burgdorferi* s.l. infection risk during occupational tick exposure at the Seedorf military training area, northwestern Germany. *Ticks Tick-Borne Dis*. 2014;5(5):594-599.

141. Hofhuis A, Herremans T, Notermans DW, *et al.* A prospective study among patients presenting at the general practitioner with a tick bite or erythema migrans in The Netherlands. *PloS One*. 2013;8(5):e64361-e64361.

142. Altpeter E, Zimmermann H, Oberreich J, Péter O, Dvořák C. Tick related diseases in Switzerland, 2008 to 2011. *Swiss Med Wkly*. 2013;143:w13725-w13725.

143. Beltrame A, Rodari P, Mauroner L, *et al.* Emergence of Lyme borreliosis in the province of Verona, Northern Italy: Five-years of sentinel surveillance. *Ticks Tick-Borne Dis*. 2021;12(2):101628-101628.

144. Strle F, Lusa L, Ružić-Sabljić E, *et al.* Clinical characteristics associated with *Borrelia burgdorferi* sensu lato skin culture results in patients with erythema migrans. *PloS One*. 2013;8(12):e82132-e82132.

145. Berglund J, Eitrem R, Ornstein K, *et al.* An epidemiologic study of Lyme disease in southern Sweden. *N Engl J Med*. 1995;333(20):1319-1327. doi:10.1056/NEJM199511163332004

146. Huppertz HI, Böhme M, Standaert SM, Karch H, Plotkin SA. Incidence of Lyme Borreliosis in the Würzburg Region of Germany. *Eur J Clin Microbiol Infect Dis*. 1999;18(10):697-703. doi:10.1007/s100960050381

147. Figoni J, Chirouze C, Hansmann Y, *et al.* Lyme borreliosis and other tick-borne diseases. Guidelines from the French Scientific Societies (I): prevention, epidemiology, diagnosis. *Med Mal Infect*. 2019;49(5):318-334.

148. Oschmann P, Dorndorf W, Hornig C, Schäfer C, Wellensiek HJ, Pflughaupt KW. Stages and syndromes of neuroborreliosis. *J Neurol*. 1998;245(5):262-272. doi:10.1007/s004150050216

149. Eisen L, Dolan MC. Evidence for personal protective measures to reduce human contact with blacklegged ticks and for environmentally based control methods to suppress host-seeking blacklegged ticks and reduce infection with lyme disease spirochetes in tick vectors and rodent reservoirs. *J Med Entomol*. 2016;53(5):1063-1092.

150. Kostić T, Momčilović S, Perišić ZD, *et al.* Manifestations of Lyme carditis. *Int J Cardiol*. 2017;232:24-32.

151. Van der Linde MR. Lyme carditis: clinical characteristics of 105 cases. *Scand J Infect Suppl*. 1991;77:81-84.

152. Obel N, Dessau RB, Krogfelt KA, *et al.* Long term survival, health, social functioning, and education in patients with European Lyme neuroborreliosis: nationwide population based cohort study. *BMJ*. 2018;361:k1998-k1998.

153. Dzięgiel B, Adaszek Ł, Carbonero A, *et al.* Detection of canine vector-borne diseases in eastern Poland by ELISA and PCR. *Parasitol Res*. 2016;115(3):1039-1044.

154. Laus F, Veronesi F, Passamonti F, *et al.* Prevalence of tick borne pathogens in horses from Italy. *J Vet Med Sci*. 2013;75(6):715-720.

155. Teodorowski O, Kalinowski M, Winiarczyk D, Janecki R, Winiarczyk S, Adaszek Ł. Molecular surveillance of tick-borne diseases affecting horses in Poland-Own observations. *Vet Med Sci*. Published online February 2021.

156. Tsachev I, Pantchev N, Marutsov P, Petrov V, Gundasheva D, Baymakova M. Serological evidence of *Borrelia burgdorferi*, *Anaplasma phagocytophilum* and *Ehrlichia* Spp. infections in horses from Southeastern Bulgaria. *Vector Borne Zoonotic Dis Larchmt N*. 2018;18(11):588-594.

157. Žákovská A, Schánilec P, Treml F, Dušková M, Agudelo RCF. Seroprevalence of antibodies against *Borrelia burgdorferi* s. l. and *Leptospira interrogans* s. l. in cats in district of Brno and its environs, the Czech Republic. *Ann Agric Environ Med AAEM*. 2020;27(3):356-360.

158. Appel MJ, Allan S, Jacobson RH, *et al.* Experimental Lyme disease in dogs produces arthritis and persistent infection. *J Infect Dis*. 1993;167(3):651-664. doi:10.1093/infdis/167.3.651

159. Littman MP, Gerber B, Goldstein RE, Labato MA, Lappin MR, Moore GE. ACVIM consensus update on Lyme borreliosis in dogs and cats. *J Vet Intern Med*. 2018;32(3):887-903. doi:10.1111/jvim.15085

160. Pantchev N, Pluta S, Huisinga E, *et al.* Tick-borne Diseases (Borreliosis, Anaplasmosis, Babesiosis) in German and Austrian Dogs: status quo and review of distribution, transmission, clinical findings, diagnostics and prophylaxis. *Parasitol Res*. 2015;114:S19-54.

161. Gerber B, Eichenberger S, Wittenbrink MM, Reusch CE. Increased prevalence of *Borrelia burgdorferi* infections in Bernese mountain dogs: a possible breed predisposition. *BMC Vet Res*. 2007;3:15. doi:10.1186/1746-6148-3-15

162. Gehlen H, Inerle K, Bartel A, *et al.* Seroprevalence of *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* Infections in German horses. *Anim Open Access J MDPI*. 2023;13(12):1984. doi:10.3390/ani13121984

163. Springer A, Glass A, Topp AK, Strube C. Zoonotic tick-borne pathogens in temperate and cold Regions of Europe - A review on the prevalence in domestic animals. *Front Vet Sci*. 2020;7:604910-604910.

164. Divers TJ, Gardner RB, Madigan JE, *et al. Borrelia burgdorferi* infection and Lyme Disease in North American horses: a consensus statement. *J Vet Intern Med*. 2018;32(2):617-632. doi:10.1111/jvim.15042

165. Grandi G, Aspán A, Pihl J, *et al.* Detection of tick-borne pathogens in lambs undergoing prophylactic treatment against ticks on two Swedish farms. *Front Vet Sci*. 2018;5:72-72.

166. Preac-Mursic V, Wilske B, Schierz G. European *Borrelia burgdorferi* isolated from humans and ticks culture conditions and antibiotic susceptibility. *Zentralblatt Für Bakteriol Mikrobiol Hyg Ser Med Microbiol Infect Dis Virol Parasitol*. 1986;263(1-2):112-118. doi:10.1016/S0176-6724(86)80110-9

167. Wormser GP, Dattwyler RJ, Shapiro ED, *et al.* The clinical assessment, treatment, and prevention of Lyme Disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis*. 2006;43(9):1089-1134. doi:10.1086/508667

168. Jaulhac B, Saunier A, Caumes E, *et al.* Lyme borreliosis and other tick-borne diseases. Guidelines from the French scientific societies (II). Biological diagnosis, treatment, persistent symptoms after documented or suspected Lyme borreliosis. *Med Mal Infect*. 2019;49(5):335-346.

169. Vogt NA, Stevens CPG. Why the Rationale for Canine *Borrelia burgdorferi* vaccination is unpersuasive. *Front Vet Sci*. 2021;8:719060. doi:10.3389/fvets.2021.719060

170. Guarino C, Asbie S, Rohde J, Glaser A, Wagner B. Vaccination of horses with Lyme vaccines for dogs induces short-lasting antibody responses. *Vaccine*. 2017;35(33):4140-4147. doi:10.1016/j.vaccine.2017.06.052

171. Voordouw MJ, Tupper H, Önder Ö, *et al.* Reductions in human Lyme disease risk due to the effects of oral vaccination on tick-to-mouse and mouse-to-tick transmission. *Vector Borne Zoonotic Dis Larchmt N*. 2013;13(4):203-214.

172. Černý J, Lynn G, Hrnková J, Golovchenko M, Rudenko N, Grubhoffer L. Management options for *Ixodes ricinus*-Associated pathogens: a review of prevention strategies. *Int J Environ Res Public Health*. 2020;17(6).

173. Gocko X, Lenormand C, Lemogne C, *et al.* Lyme borreliosis and other tick-borne diseases. Guidelines from the French scientific societies. *Med Mal Infect*. 2019;49(5):296-317.

174. Hofmann H, Fingerle V, Hunfeld KP, *et al.* Cutaneous Lyme borreliosis: Guideline of the German Dermatology Society. *Ger Med Sci GMS E-J*. 2017;15:Doc14-Doc14.

175. Rahlenbeck S, Fingerle V, Doggett S. Prevention of tick-borne diseases: an overview. *Br J Gen Pract J R Coll Gen Pract*. 2016;66(650):492-494.

176. Žákovská A, Nejezchlebová H, Bartoňková N, *et al.* Activity of the tick *Ixodes ricinus* monitored in a suburban park in Brno, Czech Republic, in association with the evaluation of selected repellents. *J Vector Ecol J Soc Vector Ecol*. 2013;38(2):295-300.

177. van der Heijden A, Mulder BC, Poortvliet PM, van Vliet AJH. Social-cognitive determinants of the tick check: a cross-sectional study on self-protective behavior in combatting Lyme disease. *BMC Public Health*. 2017;17(1):900-900.

178. Bharadwaj A, Stafford KC 3rd, Behle RW. Efficacy and environmental persistence of nootkatone for the control of the blacklegged tick (Acari: Ixodidae) in residential landscapes. *J Med Entomol*. 2012;49(5):1035-1044.

179. Dolan MC, Schulze TL, Jordan RA, *et al.* Evaluation of Doxycycline-Laden oral bait and topical fipronil delivered in a single bait box to Control *Ixodes scapularis* (Acari: Ixodidae) and reduce *Borrelia burgdorferi* and *Anaplasma phagocytophilum* infection in small mammal reservoirs and host-seeking ticks. *J Med Entomol*. 2017;54(2):403-410.

180. Pelletier J, Rocheleau JP, Aenishaenslin C, *et al.* Evaluation of fluralaner as an oral acaricide to reduce tick infestation in a wild rodent reservoir of Lyme disease. *Parasit Vectors*. 2020;13(1):73-73.

181. Van Wieren SE, Braks MAH, Lahr J. 19. Effectiveness and environmental hazards of acaricides applied to large mammals for tick control. In: Braks MAH, Van Wieren SE, Takken W, Sprong H (Ed.). *Ecology and control of vector-borne diseases*. Vol 4. Wageningen Academic Publishers; 2016. p. 265-278. doi:10.3920/978-90-8686-838-4_19

182. Williams SC, Stafford KC, Molaei G, Linske MA. Integrated control of nymphal *Ixodes scapularis*: effectiveness of white-tailed deer reduction, the entomopathogenic fungus *Metarhizium anisopliae*, and fipronil-based rodent bait boxes. *Vector-Borne Zoonotic Dis*. 2018;18(1):55-64. doi:10.1089/vbz.2017.2146

183. Cosson JF. (Ecology of Lyme disease). *Sante Publique Vandoeuvre--Nancy Fr*. Published online May 2019:73-87.

184. Andersen NS, Skarphédinsson S, Knudtzen FC, Olesen CR, Jensen TG, Jensen PM. Reduction in human Lyme neuroborreliosis associated with a major epidemic among roe deer. *Ticks Tick-Borne Dis*. 2018;9(2):379-381.

185. Hofmeester TR, Jansen PA, Wijnen HJ, *et al.* Cascading effects of predator activity on tick-borne disease risk. *Proc Biol Sci*. 2017;284(1859).

186. Levi T, Kilpatrick AM, Mangel M, Wilmers CC. Deer, predators, and the emergence of Lyme disease. *Proc Natl Acad Sci U S A*. 2012;109(27):10942-10947.

187. Terraube J. Can protected areas mitigate Lyme Disease risk in Fennoscandia? *EcoHealth*. 2019;16(2):184-190.

188. Millins C, Gilbert L, Medlock J, Hansford K, Thompson DB, Biek R. Effects of conservation management of landscapes and vertebrate communities on Lyme borreliosis risk in the United Kingdom. *Philos Trans R Soc Lond B Biol Sci*. 2017;372(1722).

189. Del Fabbro S. Fencing and mowing as effective methods for reducing tick abundance on very small, infested plots. *Ticks Tick-Borne Dis*. 2015;6(2):167-172.

190. Sprong H, Moonen S, van Wieren SE, Hofmeester TR. Effects of cattle grazing on Ixodes ricinus-borne disease risk in forest areas of the Netherlands. *Ticks Tick-Borne Dis*. 2020;11(2):101355. doi:10.1016/j.ttbdis.2019.101355

191. Quine CP, Barnett J, Dobson ADM, *et al.* Frameworks for risk communication and disease management: the case of Lyme disease and countryside users. *Philos Trans R Soc Lond B Biol Sci*. 2011;366(1573):2010-2022.

192. Heylen D, Lasters R, Adriaensen F, Fonville M, Sprong H, Matthysen E. Ticks and tick-borne diseases in the city: Role of landscape connectivity and green space characteristics in a metropolitan area. *Sci Total Environ*. 2019;670:941-949.

193. Gerber MA, Shapiro ED, Krause PJ, Cable RG, Badon SJ, Ryan RW. The risk of acquiring Lyme disease or babesiosis from a blood transfusion. *J Infect Dis*. 1994;170(1):231-234.

194. Stanek G, Fingerle V, Hunfeld KP, *et al.* Lyme borreliosis: Clinical case definitions for diagnosis and management in Europe. *Clin Microbiol Infect*. 2011;17(1):69-79. doi:10.1111/j.1469-0691.2010.03175.x

195. Burn L, Tran TMP, Pilz A, *et al.* Incidence of Lyme Borreliosis in Europe from national surveillance systems (2005–2020). *Vector Borne Zoonotic Dis*. 2023;23(4):156-171. doi:10.1089/vbz.2022.0071

196. Burn L, Vyse A, Pilz A, *et al.* Incidence of Lyme Borreliosis in Europe: a systematic review (2005–2020). *Vector Borne Zoonotic Dis*. 2023;23(4):172-194. doi:10.1089/vbz.2022.0070

197. Bušová A, Dorko E, Feketeová E, *et al.* Association of seroprevalence and risk factors in Lyme disease. *Cent Eur J Public Health*. 2018;26:S61-S66.

198. Pańczuk A, Tokarska-Rodak M, Mikuľáková W, Kendrová L, Magurová D. Exposure to ticks and undertaking Lyme borreliosis prevention activities among students from Poland and Slovakia. *Ann Agric Environ Med AAEM*. 2019;26(2):217-221.

199. Bartosik K, Sitarz M, Szymanska J, Buczek A. Tick bites on humans in the agricultural and recreational areas in south-eastern Poland. *Ann Agric Env Med*. 2011;18(1):151-157.

200. Bura M, Bukowska A, Michalak M, *et al.* Exposure to hepatitis E virus, hepatitis A virus and *Borrelia* spp. infections in forest rangers from a single forest district in western Poland. *Adv Clin Exp Med Off Organ Wroclaw Med Univ*. 2018;27(3):351-355.

201. De Keukeleire M, Robert A, Luyasu V, Kabamba B, Vanwambeke SO. Seroprevalence of *Borrelia burgdorferi* in Belgian forestry workers and associated risk factors. *Parasit Vectors*. 2018;11(1):277-277.

202. De Keukeleire M, Robert A, Kabamba B, Dion E, Luyasu V, Vanwambeke SO. Individual and environmental factors associated with the seroprevalence of *Borrelia burgdorferi* in Belgian farmers and veterinarians. *Infect Ecol Epidemiol*. 2016;6:32793-32793.

203. Kiewra D, Szymanowski M, Zalewska G, *et al.* Seroprevalence of *Borrelia burgdorferi* in forest workers from inspectorates with different forest types in Lower Silesia, SW Poland: preliminary study. *Int J Environ Health Res*. 2018;28(5):502-510.

204. Letrilliart L, Ragon B, Hanslik T, Flahault A. Lyme disease in France: a primary care-based prospective study. *Epidemiol Infect*. 2005;133(5):935-942. doi:10.1017/S0950268805004413

205. Lewandowska A, Kruba Z, Filip R. Epidemiology of Lyme disease among workers of forest inspectorates in Poland. *Ann Agric Environ Med AAEM*. 2013;20(2):329-331.

206. Lledó L, Giménez-Pardo C, Gegúndez MI. Screening of forestry workers in Guadalajara Province (Spain) for antibodies to Lymphocytic Choriomeningitis virus, Hantavirus, *Rickettsia* spp. and *Borrelia burgdorferi*. *Int J Environ Res Public Health*. 2019;16(22).

207. Rojko T, Ruzić-Sabljić E, Strle F, Lotric-Furlan S. Prevalence and incidence of Lyme borreliosis among Slovene forestry workers during the period of tick activity. *Wien Klin Wochenschr*. 2005;117(5-6):219-225. doi:10.1007/s00508-004-0306-7

208. Thorin C, Rigaud E, Capek I, *et al.* Séroprévalence de la borréliose de Lyme et de l'encéphalite à tiques chez des professionnels exposés dans le Grand Est de la France. *Médecine Mal Infect*. 2008;38(10):533-542. doi:10.1016/j.medmal.2008.06.008

209. Tomao P, Ciceroni L, D'Ovidio MC, *et al.* Prevalence and incidence of antibodies to *Borrelia burgdorferi* and to tick-borne encephalitis virus in agricultural and forestry workers from Tuscany, Italy. *Eur J Clin Microbiol Infect Dis*. 2005;24(7):457-463. doi:10.1007/s10096-005-1348-0

210. Chmielewska-Badora J, Moniuszko A, Żukiewicz-Sobczak W, Zwoliński J, Piątek J, Pancewicz S. Serological survey in persons occupationally exposed to tick-borne pathogens in cases of co-infections with *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Bartonella* spp. and *Babesia microti*. *Ann Agric Environ Med AAEM*. 2012;19(2):271-274.

211. Sammito S, Müller-Schilling L, Gundlach N, Faulde M, Böckelmann I. Workplace-related risk of tick bites in military personnel stationed in Northern Germany. *Int Arch Occup Environ Health*. 2019;92(7):1061-1065.

212. Breitbart P, Meister S, Meyer T, Gärtner BC. Incidence and Prevalence of *Borrelia burgdorferi* antibodies in male professional football players. *Clin J Sport Med Off J Can Acad Sport Med*. Published online May 2019.

213. Sormunen JJ, Kulha N, Klemola T, Mäkelä S, Vesilahti EM, Vesterinen EJ. Enhanced threat of tick-borne infections within cities? Assessing public health risks due to ticks in urban green spaces in Helsinki, Finland. *Zoonoses Public Health*. 2020;67(7):823-839.

214. Mac S, da Silva SR, Sander B. The economic burden of Lyme disease and the cost-effectiveness of Lyme disease interventions: A scoping review. *PloS One*. 2019;14(1):e0210280-e0210280.

215. Mattingly TJ 2nd, Shere-Wolfe K. Clinical and economic outcomes evaluated in Lyme disease: a systematic review. *Parasit Vectors*. 2020;13(1):341-341.

216. Lohr B, Müller I, Mai M, Norris DE, Schöffski O, Hunfeld KP. Epidemiology and cost of hospital care for Lyme borreliosis in Germany: lessons from a health care utilization database analysis. *Ticks Tick-Borne Dis*. 2015;6(1):56-62.

217. van den Wijngaard CC, Hofhuis A, Simões M, *et al.* Surveillance perspective on Lyme borreliosis across the European Union and European Economic Area. *Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull*. 2017;22(27).

218. Beaujean D, Bults M, van Steenbergen JE, Voeten HACM. Study on public perceptions and protective behaviors regarding Lyme disease among the general public in the Netherlands: implications for prevention programs. *BMC Public Health*. 2013;13:225-225.

219. Bord S, Dernat S, Ouillon L, *et al.* Tick ecology and Lyme borreliosis prevention: a regional survey of pharmacists' knowledge in Auvergne-Rhône-Alpes, France. *Ticks Tick-Borne Dis*. 2022;13(3):101932. doi:10.1016/j.ttbdis.2022.101932

220. Puppo C, Préau M. (Prevention and management of Lyme disease: On complexity and the need to take into consideration various psycho-social factors). *Sante Publique Vandoeuvre--Nancy Fr*. Published online May 2019:65-71.

221. Beaujean D, Gassner F, Wong A, Steenbergen van JE, Crutzen R, Ruwaard D. Determinants and protective behaviours regarding tick bites among school children in the Netherlands: a cross-sectional study. *BMC Public Health*. 2013;13:1148-1148.

222. Mowbray F, Amlôt R, Rubin GJ. Predictors of protective behaviour against ticks in the UK: a mixed methods study. *Ticks Tick-Borne Dis*. 2014;5(4):392-400.

223. San Martin Rodriguez M, Kaier K, Hehn M, Borde JP. Knowledge, habits and attitudes towards TBE and other tick-borne diseases in German forestry trainees. *Ticks Tick-Borne Dis*. 2020;11(1):101307-101307.

224. Shadick NA, Zibit MJ, Nardone E, DeMaria AJ, Iannaccone CK, Cui J. A School-based intervention to increase Lyme Disease preventive measures among elementary school-aged children. *Vector Borne Zoonotic Dis Larchmt N*. 2016;16(8):507-515.

225. Beaujean D, Crutzen R, Gassner F, *et al.* Comparing the effect of a leaflet and a movie in preventing tick bites and Lyme disease in The Netherlands. *BMC Public Health*. 2016;16:495-495.

226. Yiannakoulias N, Tooby R, Sturrock SL. Celebrity over science? An analysis of Lyme disease video content on YouTube. *Soc Sci Med 1982*. 2017;191:57-60.

227. Scheerer C, Rüth M, Tizek L, Köberle M, Biedermann T, Zink A. Googling for ticks and Borreliosis in Germany: nationwide google search analysis from 2015 to 2018. *J Med Internet Res*. 2020;22(10):e18581-e18581.

228. Dernat S, Johany F. Tick Bite Risk as a Socio-spatial representation—An exploratory study in Massif Central, France. *Land*. 2019;8(3):46. doi:10.3390/land8030046

229. Marcu A, Uzzell D, Barnett J. Making sense of unfamiliar risks in the countryside: the case of Lyme disease. *Health Place*. 2011;17(3):843-850.

230. Stanek G, Wormser GP, Gray J, Strle F. Lyme borreliosis. *Lancet Lond. Engl.* 2012;379:461-473. doi:10.1016/S0140-6736(11)60103-7

231. Burn L, Pilz A, Vyse A, *et al.* Seroprevalence of Lyme Borreliosis in Europe: results from a systematic literature review (2005–2020). *Vector-Borne Zoonotic Dis*. 2023;23(4):195-220. doi:10.1089/vbz.2022.0069

232. Dobson ADM, Randolph SE. Modelling the effects of recent changes in climate, host density and acaricide treatments on population dynamics of Ixodes ricinus in the UK: Ixodes ricinus population change model. *J Appl Ecol*. 2011;48(4):1029-1037. doi:10.1111/j.1365-2664.2011.02004.x

233. Medlock JM, Hansford KM, Bormane A, *et al.* Driving forces for changes in geographical distribution of *Ixodes ricinus* ticks in Europe. *Parasit Vectors*. 2013;6(1):1. doi:10.1186/1756-3305-6-1

234. Ogden NH, Lindsay LR. Effects of climate and climate change on vectors and vector-borne diseases: ticks are different. *Trends Parasitol*. 2016;32(8):646-656. doi:10.1016/j.pt.2016.04.015

235. Beck HE, Zimmermann NE, McVicar TR, Vergopolan N, Berg A, Wood EF. Present and future Köppen-Geiger climate classification maps at 1-km resolution. *Sci Data*. 2018;5(1):180214. doi:10.1038/sdata.2018.214

236. Intergovernmental Panel On Climate Change. *Climate Change 2021 – The physical science basis: Working Group I contribution to the sixth assessment report of the Intergovernmental Panel on Climate Change*. 1st ed. Cambridge University Press; 2023. doi:10.1017/9781009157896

237. Nolzen H, Brugger K, Reichold A, Brock J, Lange M, Thulke HH. Model-based extrapolation of ecological systems under future climate scenarios: The example of *Ixodes ricinus* ticks. Shahid S, ed. *PLOS ONE*. 2022;17(4):e0267196. doi:10.1371/journal.pone.0267196

238. Kjellander P, Bergvall UA, Chirico J, Ullman K, Christensson M, Lindgren PE. Winter activity of Ixodes ricinus in Sweden. *Parasit Vectors*. 2023;16(1):229. doi:10.1186/s13071-023-05843-9

TICK-BORNE ENCEPHALITIS

Valentina Tagliapietra (a), Annapaola Rizzoli (a), Maria Bellenghi (b), Claudia Cataldo (b), Francesca Dagostin (a), Simon Dellicour (c, d), Timothee Dub (e), Henna Mäkelä (e), Giovanni Marini (a), Luciano Toma (f), Maria Fernanda Vincenti González (d), William Wint (g), Luca Busani (b)
(a) Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige, Trento
(b) Centro di Riferimento per la Medicina di Genere, Istituto Superiore di Sanità, Rome
(c) Department of Microbiology, Immunology and Transplantation, Rega Institute, KU Leuven
(d) Spatial Epidemiology Lab (SpELL), Université Libre de Bruxelles (ULB), Brussels
(e) Department of Health Security, Finnish Institute for Health and Welfare, Helsinki
(f) Dipartimento Malattie Infettive, Istituto Superiore di Sanità, Rome
(g) Department of Biology, Environmental Research Group Oxford Ltd, Oxford

Biological, ecological and molecular features of the causative agent

Disease name

Tick-Borne Encephalitis (TBE), Russian Spring and Summer Encephalitis (RSSE), Central European Encephalitis (CEE).

Disease agent

Common, scientific and Latin name

Tick-Borne Encephalitis Virus (TBEV), *Flavivirus tick-borne encephalitis.*

Taxonomy

Family: *Flaviviridae*; Genus: *Flavivirus*; Species: *tick-borne encephalitis*

Disease agent characteristics

TBE mature virions are smooth spherical particles, 50 nm in diameter. The core consists of a NucleoCapsid (NC) approximately 30 nm in diameter, surrounded by a host-derived lipid bilayer membrane in which the viral envelope (E) and membrane (M) proteins are embedded. The NC is a positive single-stranded RNA molecule approximately 11 kb in length. It shows no discernible symmetry, but the C protein surrounds the viral genome like a cage (1). The Open Reading Frame of the genome, flanked by 5' and 3' untranslated regions, encodes one large polyprotein of approximately 3400 amino acids which is processed into 3 structural (Capsid,C, precursor of the membrane protein M,prM and Envelope, E) and 7 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) (2). Among the structural proteins, E is the major target for neutralizing antibodies and induces protective immunity. It is responsible for specific binding to a cellular receptor and penetration of the virus into the host cell and is also believed to be a determinant of TBEV virulence (3). In particular, amino acid substitutions in E protein often cause decrease in neuroinvasiveness, but not neurovirulence (4). The C protein represents a structural component of the NC with a low sequence homology between different flaviviruses. This protein accumulates on the surface of endoplasmic

reticulum-driven organelles named lipid droplets that may play multiple roles in the viral life-cycle (5). The prM protein is the precursor of the membrane protein and has a major role in virus maturation, assembly and secretion (6). Structural proteins have been proved to be the primary viral determinants of non-viraemic transmission between ticks (7). Non-structural proteins have multi functions such as virus-host interaction (NS1), viral replication (NS1, NS2A, NS4A and NS4B), translation and virion production, stress response (NS4B), interference with interferon signalling (NS2A, NS4B, and NS5), membrane re-arrangements (NS4A) and serve as RNA-dependent RNA polymerase (NS5). Moreover, NS determines the extent of the cytopathic effect in cell culture (7).

Physiochemical properties

TBEV is readily inactivated by organic solvents and detergents. Non-ionic detergents, such as Triton X, solubilize the entire envelope, releasing M and E proteins; whereas sodium deoxycholate appears to remove only E, leaving M associated with the nucleocapsid. Mature virions sediment at about 200S and have a buoyant density of about 1.19 g cm^{-3} in sucrose (8). Flaviviruses are stable at slightly alkaline pH (8.0) and low temperatures (especially at -60°C or below), but TBEV has been reported to preserve at least residual infectivity over the broader pH range 1.42-9.19. Total inactivation of virus suspended in blood or other protein solutions occurs within 30 min at 56°C. Flaviviruses are stable for at least 6 h in liquid aerosol suspension at room temperature and 23-80% humidity. On the other hand, ultra-low temperatures preserve infectivity almost indefinitely and once freeze-dried they survive almost indefinitely at room temperature. Flaviviruses are inactivated by ultraviolet light, gamma-irradiation, and disinfectants, including 3-8% formaldehyde, 2% glutaraldehyde, 2-3% hydrogen peroxide, 500-5000-ppm available chlorine, alcohol, 1% iodine, and phenol iodophors (9).

Priority level for EU

In 2011, the first attempt to collect TBE surveillance data at the EU/EEA (European Union/European Economic Area) level underlined the need for an agreed case definition and systematic data collection. Therefore, in 2012, the European Commission included TBE in the list of notifiable diseases in the EU/EEA (10). The European Centre for Disease Prevention and Control (ECDC) annually collects data from 28 countries plus Iceland and Norway, based on the EU case-definition.

Distribution of the pathogen

Tick-Borne Encephalitis Virus (TBEV) has expanded its distributional range during the last decades and shows an irregular distribution over a large geographical range with a patchy occurrence in restricted foci of limited size where it circulates among vertebrate hosts and ticks. At continental scale TBEV has a Palearctic distribution, along the 8°C isotherm, in Eurasia and Northern Africa (Tunisia). At the European scale, TBEV is reported in 25 countries, specifically 22 EEA countries, 2 EFTA (European Free Trade Association) countries, 1 EU candidate countries and United Kingdom, with a distribution range from Spain and the Netherlands to the West, the Baltic countries to the east, the Southern coastal areas of Scandinavia and UK to the north and Greece to the south (Figure 1).

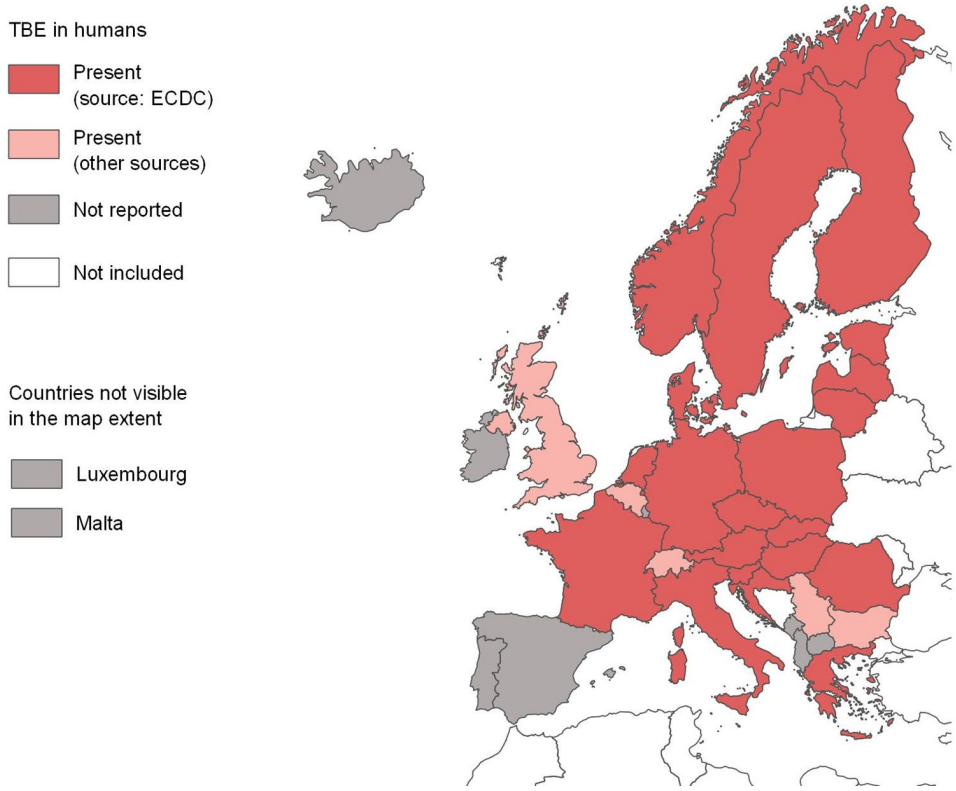

Figure 1. European Union and European Economic Area countries that reported at least one locally acquired TBE human case in the period 2010-2021. Data from TESSy-ECDC, or other sources such as national surveillance systems (Switzerland, Serbia) and published literature (24-26)

TBEV currently includes five recognized main subtypes or lineages (Figure 2):

– *Western European subtype* (TBEV-Eu)
It is endemic in rural and forest areas mainly of central, eastern and Northern Europe including the European part of Russia and South Korea and has *Ixodes ricinus* as its principal tick vector. Recently, it has been reported also in Northern Africa (Tunisia) (11). Infections are typically biphasic and are characterized by a viraemic phase with fever, malaise, headache, myalgia, leukocytopenia, thrombocytopenia and elevated liver enzymes; after a 1-week latency period, 25% of patients develop clinical signs of neurological involvement. Residual sequelae are observed in 25–50% of patients, but < 2% of cases are fatal (12).

– *Far Eastern subtype* (TBEV-FE)
It is endemic in far-eastern Russia and in forest regions in China and Japan and has *I. persulcatus* as its principal tick vector (13,14). Cases infected with TBEV-FE were reported in Estonia and Latvia (15). This subtype causes the most severe central nervous system disorder with a development of focal meningoencephalitis or polyencephalitis accompanied by loss of consciousness and fatigue during recovery. Major damage to neurons in different parts of the brain and spinal cord is possible. A fatality rate of 20-60% and an absence of chronic forms are reported.

– *Siberian subtype* (TBEV-Sib)
It is endemic in the Ural region, Siberia, far-eastern Russia and some areas in north-eastern Europe (Estonia and Finland) and has *Ixodes persulcatus* as its principal tick vector (16).

TBEV infections in Siberian-Ural regions present as a less severe disease with a high prevalence of the non-paralytic febrile form of encephalitis, while chronic forms seem to be more frequent. Case fatality rates rarely exceed 6-8%.

– *Baikalian subtype* (TBEV-Bkl)
This subtype was detected in the Irkutsk region (Ekhirit-Bulagatskiy district), Buryat Republic (Bichurskiy and Barguzinskiy district) and Transbaikalia (National Park Alkhanay, Duldurginskiy district and Krasnochikoiskiy district). The amino acid sequences of 886-84 subtypes confirmed that its genetic structure is a unique mixture of the three common subtypes. Thirty unique substitutions were detected. Genetic difference from the three common subtypes (TBEV-Eu, TBEV-FE, and TBEV-Sib) is more than 12%. The pathogenic potential is high (17-19).

– *Himalayan subtype* (TBEV-Him)
The strains (Himalaya-1 and Himalaya-2) have been detected in *Marmota himalayana* in Qinghai-Tibet Plateau, China (20). Phylogenetic analysis demonstrated that this could be considered as a new member of TBEV-FE group. At amino acid level, the diversity of the E protein was less than 2.2% within the subtype and 3.6-5.6% between the subtypes. TBEV-Him differed by 5.0-7.3% from the other subtypes, while polyprotein diverged of 4.8-7.4%. Him-TBEV displayed 69 amino acid substitutions in complete polyprotein of which 36 were unique. The profile of pathogenic associated amino acid substitution of Him-TBEV is similar to low virulence strain Oshima (5-10) (TBEV-FE).

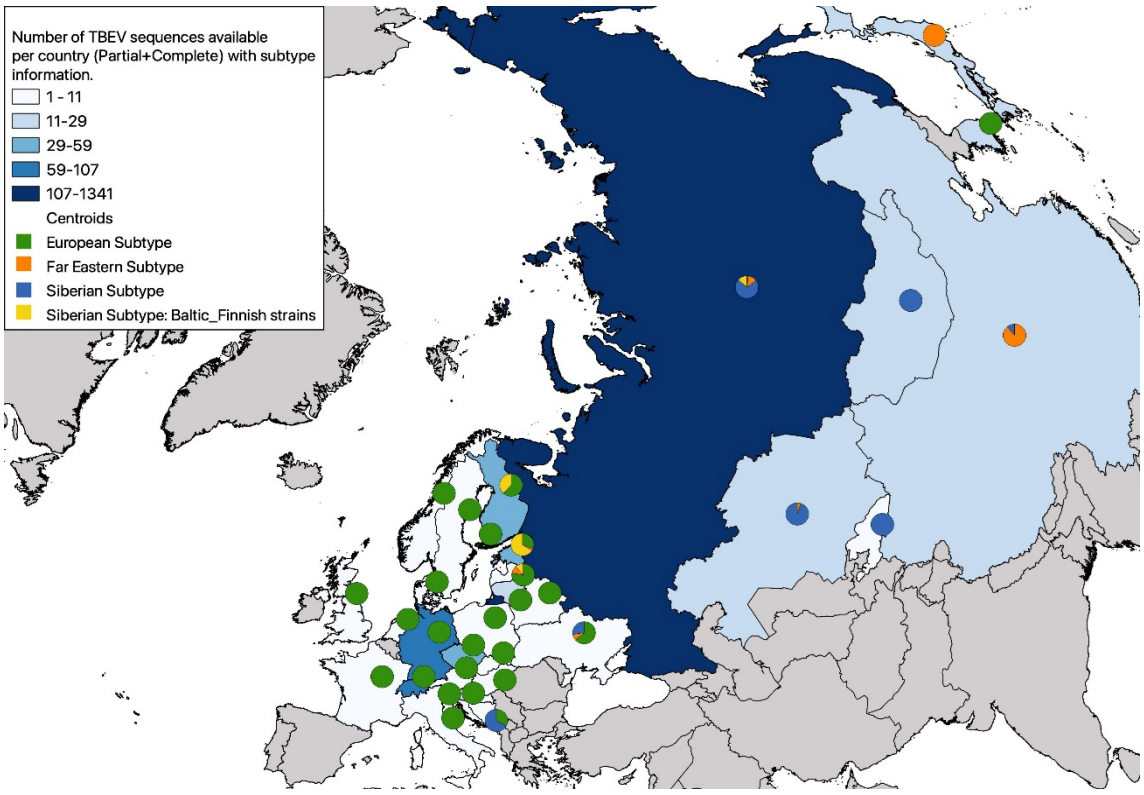

Figure 2. Eurasian distribution and abundance of the main TBEV subtypes sequences (number of sequences used= 1928) (data sources: https://www.ncbi.nlm.nih.gov/labs/virus/vssi/#/ and https://www.bv-brc.org/).

Other TBEV subtypes differing from the main ones namely Sallandse strain from the Netherlands (21) and 178-179 single strain from Irkutsk (22) are still under review.

In some countries, two or three subtypes co-circulate (e.g., Baltic States, Siberia, Ukraine) (23) (*see* Figure 2).

Ecology and transmission routes

TBEV circulates in natural cycles involving several vertebrate hosts and vectors. A map showing evidence of TBE virus occurrence in vector or host is provided in Figure 3. Ticks are both vectors and main reservoirs of the virus remaining infected throughout their life cycle due to the transstadial and transovarial transmission. The tick life-cycle needs three blood meals to be completed and the same number of hosts. The larvae mainly feed on small mammals, nymphs feed on small-and medium-sized mammals, birds, and reptiles, and adults on large animals such as ungulates.

Uninfected ticks can also acquire the infection while feeding on a viraemic competent host (systemic transmission). However, especially in case of TBEV-Eu, the most effective amplification route is through co-feeding where viral transmission takes place when naïve ticks at different developmental stages (particularly nymphs and larvae) co-feed with infected ticks on the same animal host. According to this mechanism, the virus is transmitted non-systemically, even in an immune host, to the next tick generation (27).

For an effective TBEV-Eu transmission and amplification, synchronous activity of larvae and nymphs supported by specific climatic conditions (autumnal cooling and spring warming) are needed (28, 29). However, in areas with ineffective co-feeding transmission, such as in the northern distribution areas (30) and at high altitudes, other mechanisms for maintaining virus circulation, as the transmission from infected host to ticks, in natural foci become relatively more important.

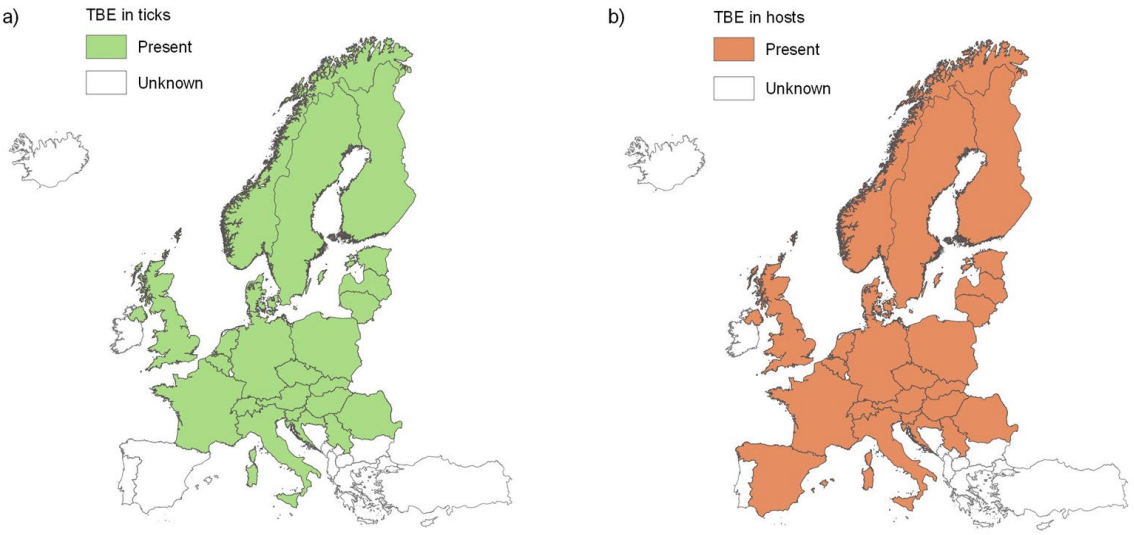

Figure 3. Distribution of TBEV presence in vectors (a) and vertebrate hosts (b) (derived from a literature review updated to 2021)

Vectors

Ticks are the main vectors and reservoirs of TBEV, although it has also been isolated from mosquitoes (TBEV-FE in *Aedes vexans*) (31). All the tick species involved in the eco-epidemiological cycle of TBEV belong to the Ixodidae family. The most common and widespread species are *Ixodes ricinus* (also main vector for the TBEV-EU subtype) and *I. persulcatus*. *I. ricinus* is widely distributed throughout Europe, West to East from Ireland to the Urals, and North to South from Northern Sweden to North Africa (Figure 4a), while *I. persulcatus* is predominant in Northern Europe (Figure 4b). The 14 species of ticks listed in Table 1 are proven to transmit TBEV.

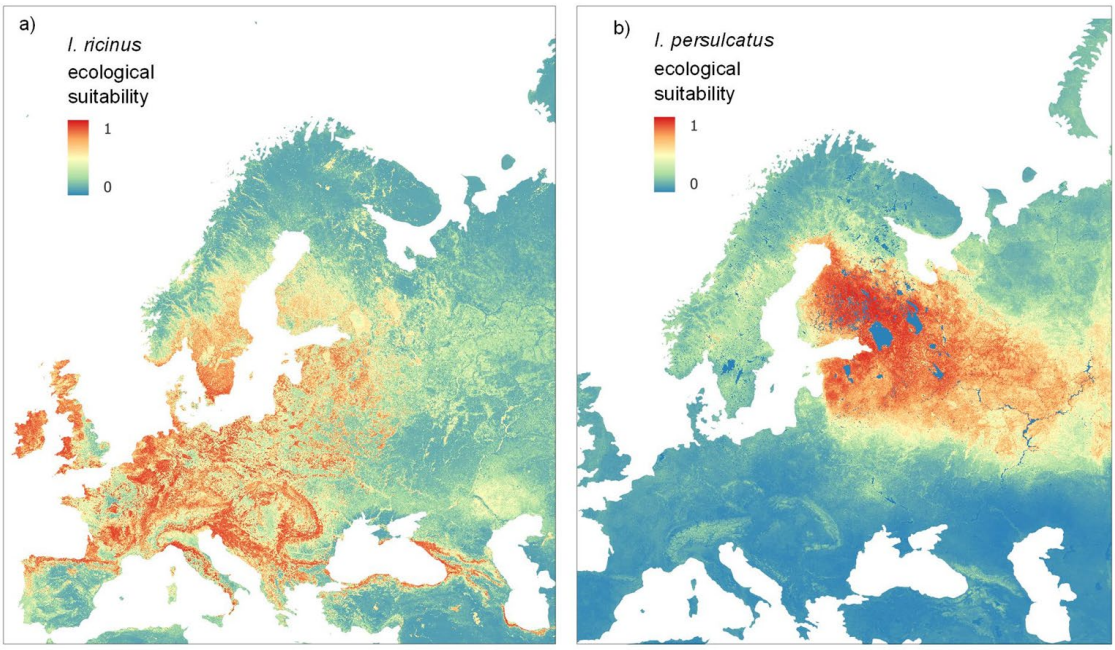

Figure 4. Current 1-km ecological suitability estimates for *I. ricinus* and *I. persulcatus* across Europe, produced using random forest and boosted regression trees analyses (source: ERGO group)

Table 1. List of *Ixodidae* species that transmit TBEV

Genus	Species	Reference
Ixodes	*ricinus*	(32,33)
Ixodes	*arboricola*	(34)
Ixodes	*hexagonus*	(35,36)
Ixodes	*trianguliceps*	(37)
Ixodes	*persulcatus*	(33)
Ixodes	*ovatus*	(38)
Dermacentor	*marginatus*	(39-41)
Dermacentor	*reticulatus*	(39, 40, 42, 43)
Haemaphysalis	*concinna*	(44)
Haemaphysalis	*inermis*	(40)
Haemaphysalis	*punctata*	(41)
Haemaphysalis	*longicornis*	(45)
Haemaphysalis	*flava*	(45)
Haemaphysalis	*nipponensis*	(45)

Hosts

Various animal species are susceptible and can be infected with TBEV.

However, their ecological role in viral transmission can vary. They can act as dead-end hosts or maintain and amplify the virus (reservoir hosts), with or without evident symptoms of the disease.

In some species, the viremia is followed by viral shedding in urine and milk, therefore enhancing the risk of transmission through milk and milk products.

In other cases, although being incompetent as reservoir for the virus, they participate in maintaining or amplifying the tick population and therefore affecting indirectly the viral transmission.

Rodents and insectivores are considered the main reservoir hosts for TBEV maintenance and circulation. Once infected with TBEV, they are supposed to develop a chronic infection, although the duration of viremia and thus their infectivity to ticks are commonly considered short (two to nine days).

A recent experimental study in bank voles (*Myodes glareolus*) showed that viremia might last up to 28 days, but infectivity to ticks was not tested (46). Also, the possibility exists that low viral replication in latently infected rodent hosts may occur under the influence of fluctuations in stress of sexual hormone levels during their life cycle (30, 47, 48). In laboratory mice, sexual transmission has also been reported (49).

Domestic ruminants, such as goats, sheep and cattle, rarely show any clinical symptom, although they develop a detectable viremia that can be a source of infection either directly or indirectly (milk, milk products and blood), although not sufficient to be uptaken by feeding ticks (50).

The role of birds as TBEV reservoirs still needs to be demonstrated, although they contribute to the dispersal of the virus including the introduction into new areas by carrying infected ticks.

TBEV was isolated occasionally from *Turdus pilaris*, *T. iliacus*, other *Turdus* spp., *Corvus monedula*, *C. corone*, *Pica pica*, *Sturnus vulgaris*, *Lanius collurio*, *Fringilla montifringilla*, *F. coelebs*, *Loxia curvirostra*, *Carduelis flammea*, *Anthus trivialis*, *Erithacus rubecula*, *Motacilla alba*, *M. flava*, *Emberiza* spp., *Jynx torquilla*, *Bonasa bonasia*, *Crex crex*, *Scolopax rusticola*, *Clangula hyemalis*, *Melanitta fusca*, *Anas querquedula*, *Fulica atra*.

TBEV-infected larvae were collected from *Anthus trivialis*, *Phoenicurus phoenicurus*, *Erithacus rubecula* and *Turdus philomelos* (51), while a potential for transovarial transmission by isolation of the virus from the eggs, was demonstrated in *T. iliacus*, *T. pilaris*, *T. ruficollis*, *T. pallidus*, *Lanius cristatus*, *Emberiza fucata*, *Troglodytes troglodytes*, *Accipiter gentilis* (52).

Wild ungulates are important hosts for the maintenance of tick populations, by providing a sufficient blood meal to adult stages, although they are not competent for viral transmission (53).

Carnivores can be locally important, specifically, the role of red foxes (*Vulpes vulpes*) deserves more investigations as they are exposed to TBEV both from infected tick bites and from eating TBE-infected preys (54-56).

In Table 2 the vertebrate species for which TBEV symptoms or viremia or both are reported.

Table 2. List of vertebrate species that reported TBE symptoms and/or developed viremia

Species (*Latin name*)	Symptoms (ref.)	Viremia
Goat (*Capra hircus*)	No	Yes
Sheep (*Ovis aries*)	Yes (57)	Yes
Cow (*Bos taurus*)	No	Yes
Dog (*Canis lupus familiaris*)	Yes (58)	Yes
Horse (*Equus caballus*)	Yes (59, 60)	Yes
Roe deer (*Capreolus capreolus*)	Yes (61)	Yes
Red deer (*Cervus elaphus*)	No	Yes
Fallow deer (*Dama dama*)	No	Yes
Wild boar (*Sus scrofa*)	No	Yes
White-tailed deer (*Odocoileus virginianus*)	No	Yes
Moose (*Alces alces*)	No	Yes
Mouflon (*Ovis ammon musimon*)	Yes (62)	Yes
European bison (*Bison bonasus bonasus*)	No	Yes
Red fox (*Vulpes vulpes*)	No	Yes
Racoon dog (*Nyctereutes procyonoides*)	No	Yes
Mountain hare (*Lepus timidus*)	No	Yes
European hare (*Lepus europaeus*)	No	Yes
Bank vole (*Myodes glareolus*)	No	Yes
Grey-sided vole (*Myodes rufocanus*)	No	Yes
Northern red-backed vole (*Myodes rutilus*)	No	Yes
Field vole (*Microtus agrestis*)	No	Yes
Common vole (*Microtus arvalis*)	No	Yes
Hedgehog (*Erinaceus europaeus*)	Yes (63)	Yes
Northern white-breasted hedgehog (*Erinaceus roumanicus*)	No	Yes
Eastern hedgehog (*Erinaceus concolor*)	No	Yes
Yellow-necked mouse (*Apodemus flavicollis*)	No	Yes
Wood mouse (*Apodemus sylvaticus*)	No	Yes
Striped field mouse (*Apodemus agrarius*)	No	Yes
Large japanese field mouse (*Apodemus speciosus*)	No	Yes
Small japanese field mouse (*Apodemus argenteus*)	No	Yes
Red squirrel (*Sciurus vulgaris*)	No	Yes
Eastern grey squirrel (*Sciurus carolinensis*)	No	Yes
Brown rat (*Rattus norvegicus*)	No	Yes
Northern birch mouse (*Sicista betulina*)	No	Yes
European mole (*Talpa europaea*)	No	Yes
European polecat (*Mustela putorius*)	No	Yes
Common shrew (Sorex araneus)	No	Yes
Barbary macaque (*Macaca sylvanus*)	Yes (64)	Yes

Drivers of disease emergence and spread

Ecological drivers

TBE epidemiology typically shows a profound fluctuation in the annual cases reported and a patchy distribution. Explanation could be found in the complex interplay of biotic and abiotic factors which characterize the transmission chain of the TBE virus (65).

In Table 3, the ecological and environmental covariates included in the sixty-two studies selected are reported.

Table 3. List of ecological and environmental covariates included in the selected studies in decreasing order according to the number of studies including them

Environmental drivers	N. of papers (n. 62)	% of impact
Temperature (average daily, monthly seasonal and annual temperature, autumnal cooling rate, spring warming rate, mean diurnal temperature range)	40	65%
Land use (forest, agriculture, vegetation cover)	19	31%
Precipitation (total daily, monthly, seasonal and annual precipitation)	15	24%
Deers (roe deer, red deer, fallow deer, moose, white-tailed deer abundance or density)	13	21%
Vegetation (Mean NDVI, EVI, Beech fructification index)	12	19%
Humidity (daily, monthly, annual mean relative humidity)	11	18%
Rodents (yellow-necked mouse, bank vole abundance or density)	10	16%
Topography (distance from forest or meadow, distance to sea or water course, latitude, exposition, slope)	9	15%
Altitude	8	13%
Feeding ticks (number or density of feeding and co-feeding ticks)	8	13%
Time (year and season)	7	11%
Questing ticks (density of questing larvae and nymphs)	6	10%
Foxes (abundance or density)	5	8%
Atmosphere (Scandinavian Index, NAO Index)	5	8%
Hares (European hare, mountain hare abundance or density)	5	8%
Wild boars (abundance or density)	4	6%
Soil (mean soil temperature and humidity, soil types)	4	6%
Saturation deficit	4	6%
Snow cover	3	5%
Other hosts (lynx, racoon abundance or density)	3	5%
Tick ecology (birth and mortality rate, average egg production.)	2	3%
Light (daily, annual sunshine hours)	2	3%

NDVI: Normalized Difference Vegetation Index; EVI: Enhanced Vegetation Index; NAO: North Atlantic Oscillation

Climate change is generally considered a primary driver of TBE trend and temporal fluctuations, although its complex role in affecting the zoonotic system or human behaviour is still debated (66). One of the most efficient mechanisms of TBEV transmission involves the co-feeding of larvae and nymphs through the seasonal synchrony of the different tick life stages that is in turn affected by climatic variables. In particular, it has been shown that a high rate of autumnal cooling (i.e., a rapid decrease in late summer temperatures) induces a behavioural diapause in larvae and nymphs therefore reducing the delay between nymphal and larval activity during the following spring (28, 29, 67-70). Similarly, rapidly rising temperatures in spring, referred to as "spring warming rate", cause larvae to become active at the same time as nymphs (71, 72).

Abiotic factors directly affect off-host stages of the ticks, in particular temperature and relative humidity. The known temperature threshold values for tick activity range between 5 and 10 °C (73-76), with significant differences found for larvae (6°C) and for nymphs and adults (5°C) (77). Regarding Relative air Humidity (RH), a peak in activity for larvae has been reported at 75-85% RH and for nymphs and adults between 50-100% RH (77). It has been demonstrated that tick abundance is positively correlated with temperature and relative humidity, which affects the hydration of tick's body (78,79), but negatively with saturation deficit (an index combining temperature and relative humidity) (72,80). The effect of severe winters on annual nymphal

density (81) and on subsequent TBE incidence (82) has also been proved. The latitudinal and altitudinal expansion of ticks and their increase of the activity range have consequences on increased probability of contacts between humans and parasites.

Density of animal host's population and/or their tick load are key drivers for TBEV circulation. Small rodents (mainly *Apodemus* spp. and *Myodes* spp.) play a crucial role both as a source of bloodmeal for immature ticks and as bridge hosts for the virus (83). The TBE incidence was repeatedly demonstrated to be correlated with their 1-year lagged population density (84). Among medium-sized and large mammals, deer and hares are reported to affect the presence of TBE foci. In particular, ungulates are able to greatly amplify tick abundances, but at the same time divert tick bites from competent hosts, and thus may cause a dilution effect of TBEV prevalence in ticks above certain threshold densities (85, 86). Hares and red foxes might also contribute to such dilution effect, as high host species diversity and high population numbers weaken the incidence of TBE-infected ticks on any given host (87). A similar relationship has been found between the number of co-feeding tick groups and *A. flavicollis* density, with a decline in the number of co-feeding ticks after a threshold of approximately 10 mice/ha (70). Sylvatic carnivores may have an indirect effect on TBEV incidence through predation of deer and small mammals. Other hosts such as ruminants are considered non-competent, but due to their long-term persistent viremia and antibody response, they are often used as sentinels. Birds, especially migratory, contribute to the dispersal of the ticks over long distances.

Regarding the environment, forests, especially well-connected coniferous, broad-leaf and mixed forest patches with open areas, provide suitable habitat and resources for ungulates, rodents, and ticks, thus favouring their encounter and boosting the risk of occurrence of TBE human cases (88-93). Evidence has been found for a significant correlation between TBE incidence and an increasing ratio of high stand forest to coppice cover, which corresponds to an increase in habitat suitability for small mammals, especially *Apodemus* spp. (94). Landscape characteristics also affect human risk to tick exposure, as people engaged in recreational or occupational outdoor activities in a risk area are at increased risk of tick bites. Therefore, proxies for forest accessibility, such as forest road length (95, 96) and the maximum distance from forests or meadows (97) are associated with higher TBE risk. Greener and healthier vegetation, which can be evaluated by the satellite derived Normalized Difference Vegetation Index (NDVI) and Enhanced Vegetation Index (EVI), promotes tick presence, availability of food resources for primary consumers and their coexistence, in turn reflecting in a higher TBE suitability (93, 97).

In the current context of global biodiversity loss, recent efforts (mostly focused on Lyme disease) were made to understand the effect of biodiversity on vector-borne diseases transmission, with contrasting conclusions. Species diversity might be important in the ecology of infectious diseases, particularly vector-borne zoonosis: on one hand, high species diversity in vertebrate hosts may play a beneficial role by acting as competent or not competent reservoirs of the pathogen (98); on the other hand, high diversity of vertebrate hosts may help generalist vectors or pathogens to avoid local extinction and therefore may play a role in increasing the disease risk to humans. At broad spatial scales, habitat alteration can also influence disease risk (99, 100): habitat destruction and the fragmentation of landscapes into small, isolated units are known to cause reduction or elimination of some vertebrate species and therefore diversity (101, 102). Often, species that occupy high trophic levels are the most sensitive to such habitat destruction. Loss of these species, which are generally non competent reservoirs for vector-borne zoonosis, may increase disease risk both via reduction of diverted blood meals from these incompetent hosts and via the loss of a regulatory "predator" effect on typically more reservoir-competent hosts.

Environmental variables are not the only ones that can explain the spatio-temporal distribution of TBE cases. Human activity and behaviour can act in synergy with them by increasing the chances of coming in contact with infected ticks. Low economic status has been identified as a

significant risk factor for TBE infection in some parts of Europe, forcing people to exploit the rural and natural habitat in search for food products or wood. For example, for eight central and eastern European countries, the differential degree of TBE upsurge in the early 1990s was significantly positively correlated with contemporary poverty indicators, including the percentage of total household expenditure spent on food (103). An increase in TBE incidence has been also reported in Europe in 2020. A possible explanation could be an enhanced exposure to people to infected ticks as a consequence of the post lock-down human behaviour.

Natural history of disease in humans and animals, including symptoms, morbidity and mortality

Brief history of the pathogen and disease

In the early 1920s, residents and workers of villages in the Taiga Forest from the far Eastern region of Russia, reported a severe form of brain disease. An expedition led by Prof. Lev A. Zilber was sent in 1937 to Khabarovsk Territory to investigate the origins and causes of this disease. Despite extremely difficult conditions of the remote taiga location and the absence of infrastructures, the expedition successfully isolated the virus from patients, vertebrates and ticks. Furthermore, the pioneer researchers expounded on basic eco-epidemiology of the disease and provided some useful prophylactic information on how to avoid infection (104). The first cases in China were reported in 1943 and virus isolation from patients in 1944 (105). The first documented TBEV isolation in Europe was made from *I. ricinus* ticks in Belarus in 1939 (strain 256), while the second was reported in former Czechoslovakia in 1948 (strain Hanzalova) from both patients and ticks in Beroun near Prague and in Moravia (106). The alimentary route of infection was first identified in the European part of Russia between 1947 and 1951 (9). In the early 1990's, non-viraemic transmission of TBEV between co-feeding *I. ricinus* ticks was proved by prof. Labuda and collaborators from the Institute of Zoology SAS (Slovakia) in collaboration with the Institute of Virology NERC (Oxford, UK), using ticks and TBE virus strain from Central Bohemia (107). The European TBE vaccine was made available in 1976 (108).

Disease in humans

Humans are dead-end accidental hosts for TBEV. The infection can be primarily contracted through the TBEV-infected saliva injected from the bite of a tick. Among the most relevant developmental stages involved in human transmission, nymphs of *I. ricinus* are the most abundant, aggressive and less host specific, while for *I. persulcatus* the adult tick's stage is considered the most important. It is assumed that dairy unpasteurized products from infected animals (cattle, sheep, goats) stand for 1% of all TBE cases and has been reported mostly in eastern Europe and the Balkans; however, small outbreaks of foodborne TBE have also been reported in central and western Europe and Russia (109-113) (Figure 5). Other non-vectorial routes of infection include: single cases of tick-borne encephalitis after slaughter of probably viraemic goats, blood transfusions, solid organ transplantation (114), breastfeeding and laboratory investigations (115, 116).

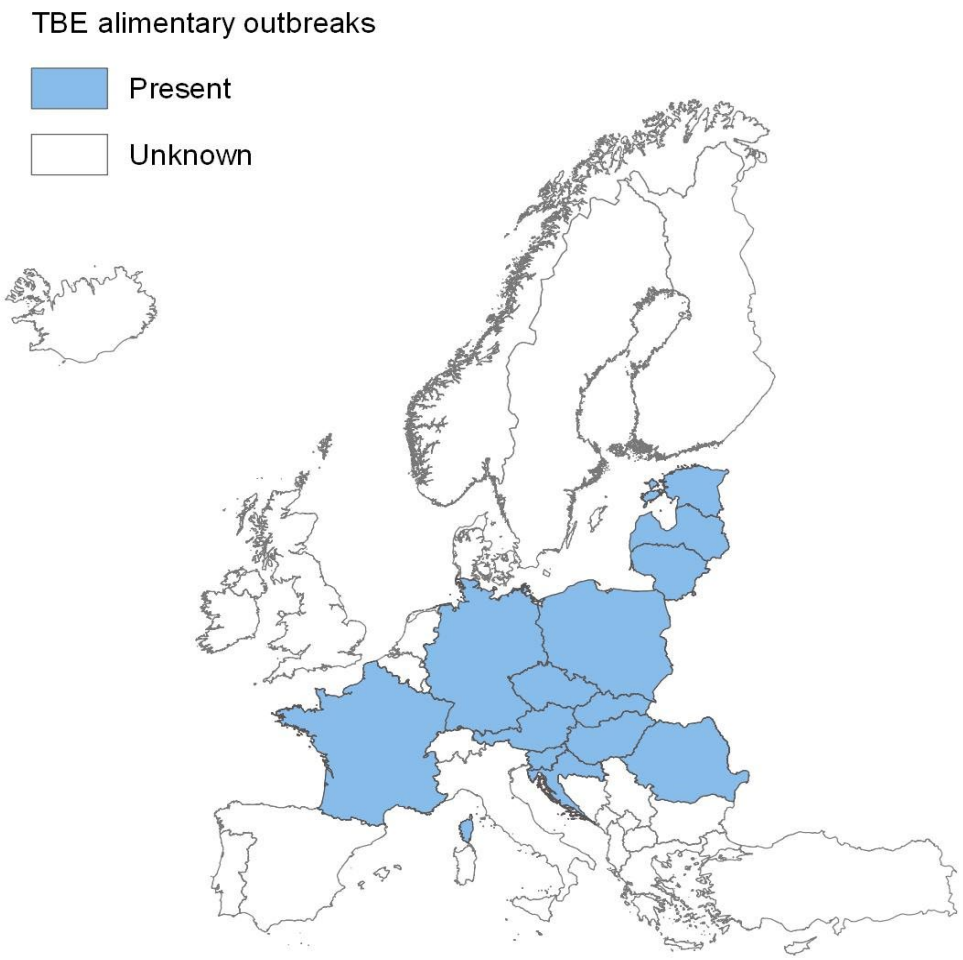

Figure 5. European countries with reported cases of TBE foodborne infections (literature review updated to 2021)

Most of the infections (between 70 and 98%) with TBE virus strains of the European subtype are either sub-clinical or asymptomatic. Clinically, the course of infection may depend on the virus subtype, the inoculation dose, the age and immune status of the host. The European subtype typically take a biphasic course (about two-thirds of patients), characterized by a first stage with non-specific symptoms and a second one with additional neurological involvement. Symptomatic infection without central nervous system (CNS) involvement is defined as "abortive TBE". The incubation period ranges from 2 to 28 days and is usually 7-14 days. Foodborne TBEV transmission has a shorter incubation period (3 to 4 days) and exhibits a monophasic course of the disease in 50% of recorded cases.

When symptoms of TBE infection progress in two phases, the initial one correlates with viremia and usually presents with non-specific symptoms such as moderate fever, headache, body pain (myalgia and arthralgia), fatigue, general malaise, anorexia, nausea, and others. This phase lasts for 2-7 days and is followed by amelioration or even an asymptomatic interval that usually lasts for about 1 week (range 1-21 days). If a second phase appears, it presents as meningitis in

approximately 50% of adult patients, as meningoencephalitis in about 40%, and in meningoencephalomyelitis in around 10%. Meningitis typically manifests with high fever, headache, nausea, and vomiting; many patients have photophobia, and some vertigo. Encephalitis can be manifested by impaired consciousness ranging from somnolence to stupor and, in rare cases, coma. Other manifestations comprise personality changes, behavioural disorders, concentration and cognitive function disturbances, tongue fasciculations and tremor of extremities; very rarely focal or generalized seizures, delirium and psychosis develop. Flaccid paresis, which are a typical characteristic of meningoencephalomyelitis, usually arise during the febrile phase of the disease, and are occasionally preceded by severe pain in the affected muscle groups.

Viral genotypes or the apparent virulence of a particular TBEV subtype and infection dose can determine different degrees of disease severity. TBEV-Eu infection usually results in a rather mild form of TBE. TBEV-Sib infection generally results in a mild illness associated with a non-paralytic febrile form of encephalitis (9), with a tendency towards persistent TBE caused by chronic viral infection. TBEV-FE infection is believed to cause the most severe forms of TBE (117). In addition, in Russia, haemorrhagic forms of TBE and chronic (or progressive) forms have been reported (9, 117-119). Other factors affecting the severity of the symptoms include age (TBE tends to be more severe in elderly people); presence of comorbidities; immunosuppressed individuals and co-infection with other tick-borne pathogens (120-127). Genetic predisposition is currently under study, polymorphisms in genes such as CCR5D32, TLR3, OAS2, OAS3, IL28B, IL10, CD209 and MMP-9 are associated with more severe forms. Finally, the monophasic course of the infection is usually more severe.

Many patients with neurologic forms of TBE are left with chronic sequelae of infection with significant consequences for daily activities and the quality of life. The most frequently reported signs are neuropsychiatric disorders, such as apathy, irritability, memory and concentration dysfunction, sensory disturbances, persistent flaccid paresis, or paralysis and altered sleep patterns. A particular form following infection by TBEV-FE and -Sib is reported in Russia. Some patients may develop a chronic (progressive) TBE that manifests after several months or years under certain circumstances (hypothermia, physical or psychological trauma, overwhelming physical labor, alcohol intoxication, abortion, labour or even physiotherapy) or as a continuously progressive form with an increase in focal CNS lesions, leading to patient death after 9-20 years post infection (23).

Studies on the three main TBEV genotypes showed the following mortality rates: TBEV-Eu <2%; TBEV-Sib 3–6%; TBEV-FE 20–40%.

Disease in animals

Animals can get TBEV from the bite of an infected tick and develop neurological symptoms similarly to humans. Usually, the veterinary aspects of this disease are poorly recognized even in domestic animals:

– *TBE in dogs*
  Canine TBEV infection is possible in endemic areas and seroprevalence varies from country to country ranging from 0.1% in Belgium to 53.6% in Germany. Results are of difficult comparison due to different diagnostic factors considered and type of tests performed. In general, infection can have an acute course with complete remission within 1-2 weeks (31-59%), although a longer period is possible (12-25%). Late sequelae like paresis, muscle atrophy, epileptic seizures or blindness are described. A definite diagnosis is rarely achieved due to low sensitive PCRs availability. Virus detection is very difficult,

due to the fast immunological virus clearance from the brain and CSF or the quick fatal outcome (58).

– *TBE in horses*
Horses are prone to TBE infection although they remain mostly asymptomatic. Few cases have been reported so far (59,60,128,129) and the clinical signs mainly depend on the affected brain region(s). The outcome displays a broad spectrum of neurological symptoms such as ataxia, tonic-clonic seizures, apathy, and stupor, inappetence, mydriasis, convulsions of the legs, skittishness, bruxism and altered reaction to environmental stimuli.

– *TBE in ruminants (sheep, roe deer and mouflon)*
Domestic and wild ruminants are important amplifier hosts for tick populations and capable of developing a detectable viremia in the blood that is not transmissible to feeding ticks although it can pass on the milk and eventually milk products. Few cases of sub-clinical and clinical infections have been reported, all with neurological signs and fatal outcomes. A lamb and a roe deer suffered from ataxia, torticollis, tremor, nystagmus, hypersalivation and finally somnolence with inappetence and recumbency, while a muflon was found moribund before suppression (57,61,62).

– *TBE in other animals*
Insectivores are known to be reservoirs of TBEV, but records of clinical signs are rare. A study screening the presence of TBEV in several European hedgehogs, detected antibodies and virus RNA from an animal that contemporarily showed neurological symptoms (63). Monkeys have been used as animal models to test the efficacy of vaccines. For viruses such as TBEV, clinical manifestation and pathomorphological lesions in the Central Nervous System in the monkeys are similar to humans (64).

Availability of preventive, therapeutic and control measures, including licensed or pipelines vaccines

Therapy in humans

No specific antivirals are approved for the treatment of TBE in Europe (23), and patient care is mainly symptomatic and supportive, including intensive care interventions in severe cases. Specific anti-TBEV immunoglobulins, nonspecific immunoglobulins or recombinant anti-TBEV immunoglobulins are used or tested for prophylaxis or treatment. Vaccines can also be used in a therapeutic context as well. Specific small molecule antivirals are not yet recognized or widely used.

Therapy in animals

Dogs and horses develop similar clinical symptoms as humans. There is no licensed anti-TBE vaccine for dogs. Symptomatic therapy is strongly recommended for dogs with TBE. Sedation and relaxation are necessary in the case of seizures. Steroid use is controversial, as immunosuppression may prolong the presence of the virus, although in dogs with marked cerebrospinal fluid pleocytosis, steroids seem to be mandatory to effectively protect the brain tissue from further fulminant immune response.

In cases of muscle atrophy and paresis, physiotherapy as early as possible has been shown to improve the general outcome and shorten the time of rehabilitation. TBE in horses is a rare event. Regarding therapeutic options and prognosis, a horse with recumbent status due to TBE has a poor prognosis if it is not possible to force the horse to stand up again.

Licensed or pipelined vaccines

Currently, all six licensed vaccines (two from Europe, three from Russia and one from China) are based on inactivated whole viruses containing various strains of the European or Far-Eastern TBEV subtype. The two European vaccines are based on the Austrian isolate Neudoerfl (FSME-IMMUN®, Pfizer, USA) approved in 1976 (130) and the German isolate K23 (Encepur®, GlaxoSmithKline) approved in 1991 (131, 132). Sucrose is used as a stabilizer in Encepur®, whereas human serum albumin in FSME-IMMUN®. Both vaccines have the pediatric formulations namely FSME-IMMUN® (Junior) and Encepur-K®. Data from clinical studies and post-marketing surveillance show that FSME-IMMUN® and Encepur® are safe, efficacious (with seroconversion rates reaching 92-100% after complete vaccination) and interchangeable (133-135). Three vaccines are produced in the Russian Federation: TBE vaccine Moscow and Tick-E-Vac (Klesch-E-Vac) are both produced by the Chumakov FSC R&D IBP RAS in Moscow and are based on the Sofjin strain of TBEV-FE; EnceVir® vaccine is produced by Microgen in Tomsk using strain 205 of TBEV-FE (23, 136) (Table 4). In China, SenTaiBao based on the Chinese TBEV-FE strain Sen-Zhang is approved as a TBEV vaccine (137).

Table 4. TBE vaccines licensed in Europe and Russia (23)

Name	Strain	Amount of antigen
FSME-IMMUN® (Pfizer)	Neudoerfl (TBEV-Eu)	2.4 µg (adults)/1.2 µg (children)
Encepur® (GSK)	K23 (TBEV-Eu)	1.5 µg (adults)/0.75 µg (children)
TBE Moscow (FSBSI "Chumakov FSC R&D IBP RAS")	Sofjin (TBEV-FE)	1.0 ± 0.5 µg/mL (dose 0.5 mL for children from 3 years old and adults)
Tick-E-Vac/Klesch-E-Vac (FSBSI "Chumakov FSC R&D IBP RAS")	Sofjin (TBEV-FE)	1.0 ± 0.5 µg/mL (dose 0.25 mL for children 1-13 years old; 0.5 mL for adults)
EnceVir® (Microgen)	205 (TBEV-FE)	2.0-2.5 µg

Other prevention measures

Other than active immunization as a protective measure against infection with TBEV, those preventing tick bites are also very important. These include: the use of chemical repellents with DEET or picaridin; wear light-coloured protective clothing; tuck pant legs into socks; avoid tick-infested areas and most importantly careful body check after visiting tick infested areas. The virus in tick saliva increases ten to 100-fold during feeding, therefore early removal of ticks does not prevent disease.

Disease specific recommendations

TBEV is transiently present in the blood, therefore it could be hypothetically transmitted through blood transfusion or organ transplantation. A cluster with a fatal outcome has been recently reported (114) recognising the fragility of transplant recipients and the need to screen organ donors who live or have recently visited TBE-endemic areas.

The course of acute TBEV infections during pregnancy has not yet been investigated systematically. Moreover, the transmission and impact on foetal development is not fully understood. According to the few cases reported from literature, intrauterine transmitted infection doesn't occur (138), while trans placental transmission of IGg maternal antibodies has been detected with decreasing titres over time, where they no longer could be found after nine months of the infants (139). Finally, also breast-feeding transmission should be carefully considered especially in areas with high TBEV-endemicity (140).

Epidemiological situation at different spatial scales: past and current trends

Since 2012, the ECDC requires all 27 EU Member States, plus Iceland and Norway, to annually report their TBE data to TESSy repository. More detailed information on surveillance systems is available in the ECDC Annual Epidemiological Report (AER) (141).

Past trends

The number of TBE cases reported in Europe, excluding Russia, increased over the years 1990-1994, probably reflecting the start of surveillance in many countries (33). Over the following 15 years (1995-2009), the trend was stable with an annual number of TBE cases fluctuating between 2,000 and 4,000 cases. Peaks occurred when a set of countries reported unusually high numbers of TBE cases, e.g. 2006 and 2009 (33). An analysis carried out in eight European countries suggested that human behaviour in response to good weather conditions, e.g. increased outdoor recreational activities, was the main explanation for the 2006 spike rather than tick abundance (142).

Current trends

In 2012, TBE became a notifiable disease in Europe and uniform clinical and laboratory data were adopted as case definition. To analyse the current epidemiological trend, we included all cases reported to the TESSy during the years 2012-2020. Population denominator data were provided by the Statistical Office of the EU (Eurostat) for calculating incidence rates. Annual rates of change and their 95% confidence intervals (CI) were estimated using a log-linear regression, while the goodness of fit was assessed using F statistics, according to (15). We used RStudio software (R Core Team 2021) for all data management and statistical analyses.

Over the 2012-2020 period, 25 EU/EEA countries reported 25,825 TBE cases, of which 24,088 (93.0%) were confirmed cases and 1737 (7.0%) probable cases (Table 5). We excluded 59 cases with unknown classification. Cyprus, Iceland, Malta, and Portugal had no TBE surveillance. In the same period, the annual incidence increased with an annual variation of 5.4% ($p = 0.05$) (Table 5).

Table 5. Number of reported cases of tick-borne encephalitis, incidence (number of cases per 100,000 inhabitants), imported cases and trend, in 25 countries of the European Union and European Economic Area, 2012-2020 (data from TESSy-ECDC)

Country	2012-2020			Trend	
	N. cases	Mean incidence	Imported cases*	Annual variation (%)	95% CI
Austria	1043	1.33	13 (1%)	15.3	6 to 25
Belgium	2	0.00	2 (100%)	NA	NA
Bulgaria	6	0.01	NA	NA	NA
Croatia	63	0.17	NA	8.74	-75 to 93
Czechia	5552	5.83	29 (1%)	6.5	-1 to 14
Denmark	4	0.01	NA	NA	NA
Estonia	897	7.55	6 (1%)	-8.3	-14 to -3
Finland	574	1.16	NA	10.4	6 to 15
France	121	0.02	20 (17%)	29.3	-4 to 63
Germany	3670	0.50	139 (4%)	12.3	4 to 21
Greece	4	0.00	2 (50%)	19.1	-39 to 77
Hungary	255	0.29	4 (2%)	-11.7	-21 to -3
Ireland	1	0.00	1 (100%)	NA	NA
Italy	210	0.04	6 (3%)	34.3	-12 to 81
Latvia	1692	9.53	0	-0.8	-7 to 6
Lithuania	4551	17.59	42 (1%)	5.9	-2 to 14
Luxembourg	1	0.02	1 (100%)	NA	NA
Netherlands	21	0.01	9 (43%)	3.8	-31 to 39
Norway	165	0.35	16 (10%)	23.0	16 to 30
Poland	1943	0.57	8 (%)	0.8	-7 to 9
Romania	12	0.01	3 (25%)	-1.8	-49 to 45
Slovakia	1215	2.49	6 (%)	4.6	-6 to 15
Slovenia	1270	6.82	1 (%)	-2.2	-18 to 13
Spain	1	0.00	1 (100%)	NA	NA
Sweden	2552	2.86	43 (2%)	4.0	-3 to 11
EU/EEA	**25825**	**0.65**	**352 (11%)**	**5.4**	**0 to 11**

Such a trend is mainly driven by a constant and significant increase in TBE mean annual incidence, which increased of 33% between the period 2012-2016 to 2017-2020 from 0.75/100,000 to 0.56/100,000, respectively 24,355 cases reported the date of onset of symptoms. Among these, 22,601 (93%) were recorded between April-October, with a peak in June and July, while the minimum (290 cases; 1.2%) in December-March (Figure 6).

The weekly distribution of the cases over the whole period reveals the seasonal intra and inter-annual heterogeneity of this disease (Figure 7).

In 2020, 3,808 TBE cases were reported to TESSy from 17 EU/EEA countries with an incidence rate of 0.72 cases/100,000. Top five incidence rate countries were: Lithuania 25.7/100,000, Slovenia 8.5/100,000, Czechia with 7.9/100.000, Latvia 7.2/100,000 and Estonia 5.1/100,000. In 2019, the average mortality rate in Europe was 0.7%.

From the total cases recorded, 72.9% had a known vaccination status. Among these 18,821 cases, 17,881 (95 %) were not vaccinated, 392 received one or two doses (2%), 411 (2.2%) three doses or more and 137 (0.7%) an unknown number of doses (Table 6).

In conclusion, the European TBE surveillance data highlighted an increasing trend over the years since the disease became notifiable. This is mainly driven by a few countries reporting the majority of cases. For example, three countries (Czech Republic, Germany, and Lithuania) accounted for 53% of all reported TBE cases, while two countries (Estonia, Hungary), which accounted for 4% of all reported TBE cases, showed a significant negative trend in the period 2012-2020.

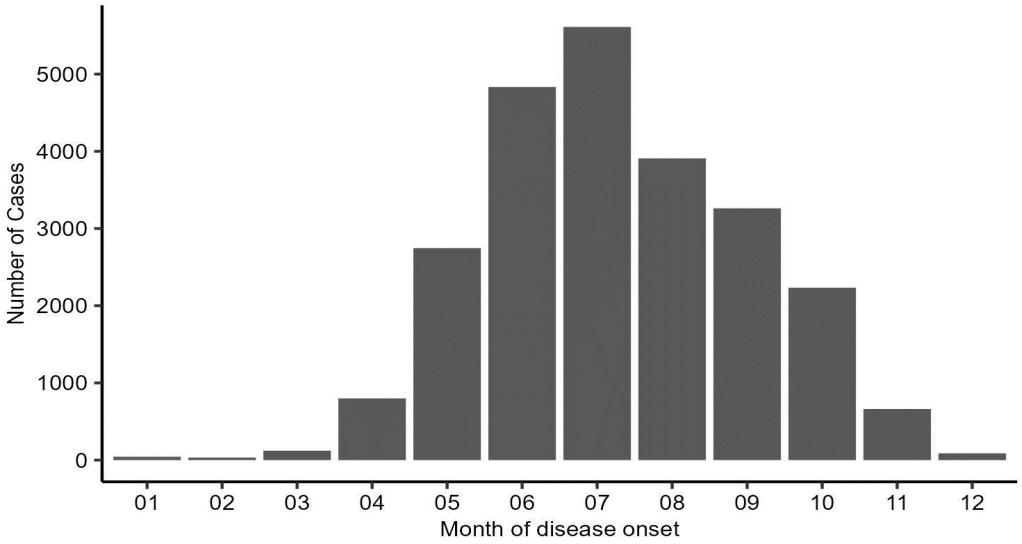

Figure 6. Number of reported tick-borne encephalitis cases by month of onset, 2012-2020 (data from TESSy-ECDC)

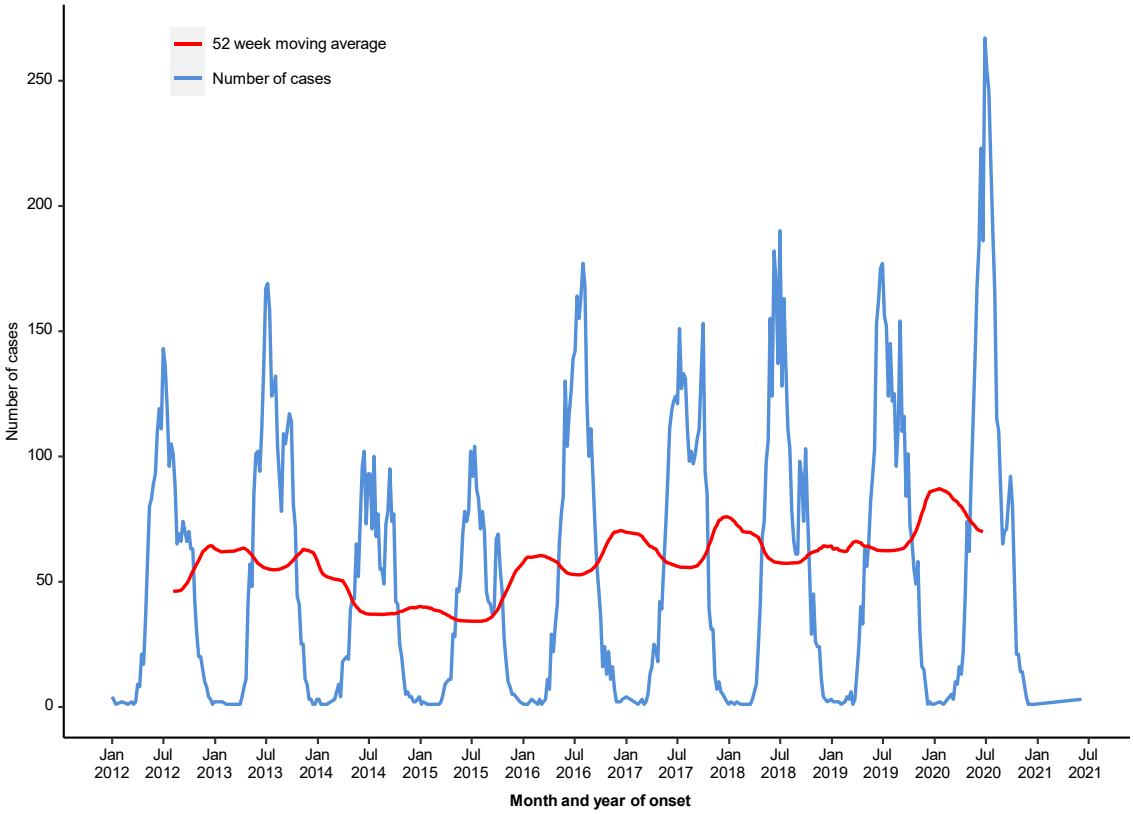

Figure 7. Number of reported tick-borne encephalitis cases by week of onset, and 52-week moving average in the European Union and European Economic Area countries, 2012-2020 (data from TESSy-ECDC)

Table 6. Main characteristics of reported cases of tick-borne encephalitis, European Union and European Economic Area countries, 2012−2020 (n = 25825) (data from TESSy-ECDC)

Characteristics	N. cases	%	Incidence rate per 100,000 persons
Total	**25,825**	**100**	**5.81**
Age group (years)			
<20	3,043	12%	0.68
20-29	2,332	9%	0.52
30-39	3,271	13%	0.74
40-49	4,344	17%	0.98
50-59	5,140	20%	1.16
60-69	4,470	17%	1.01
70-79	2,589	10%	0.58
≥ 80	629	2%	0.14
Unknown	7	NA	NA
Sex			
Female	10,439	40%	0.00
Male	15,375	60%	0.00
Unknown	11	NA	NA
Importation status			
Imported	352	1%	NA
Locally acquired	24,320	94%	NA
Unknown	1153	4%	NA
Vaccination status			
Four doses	185	1%	NA
Three doses	226	1%	NA
Two doses	192	1%	
One dose	200	1%	NA
Vaccinated unknown doses	137	1%	NA
Not vaccinated	17,881	69%	NA
Unknown	7,004	27%	NA

The reported TBE cases followed a pronounced seasonality with most cases occurring during the warmer months May-October, which correspond to the period when people tend to spend a greater amount of time outdoors (48) and tick populations are more active.

No vaccination data are available at European level.

Finland

In Finland, TBE is endemic, with a mean of 64 cases reported annually and an average incidence of 1.16 per 100,000 inhabitants over the period 2012-2020 (Figure 8a).

The total number of cases reported is 574. The trend is generally increasing. TBE cases affect mainly males both in adult and older age (Figure 8b).

The place of infection was reported to TESSy from 2017 onwards. Since then, the most cases were reported from the Åland Islands (Figure 9).

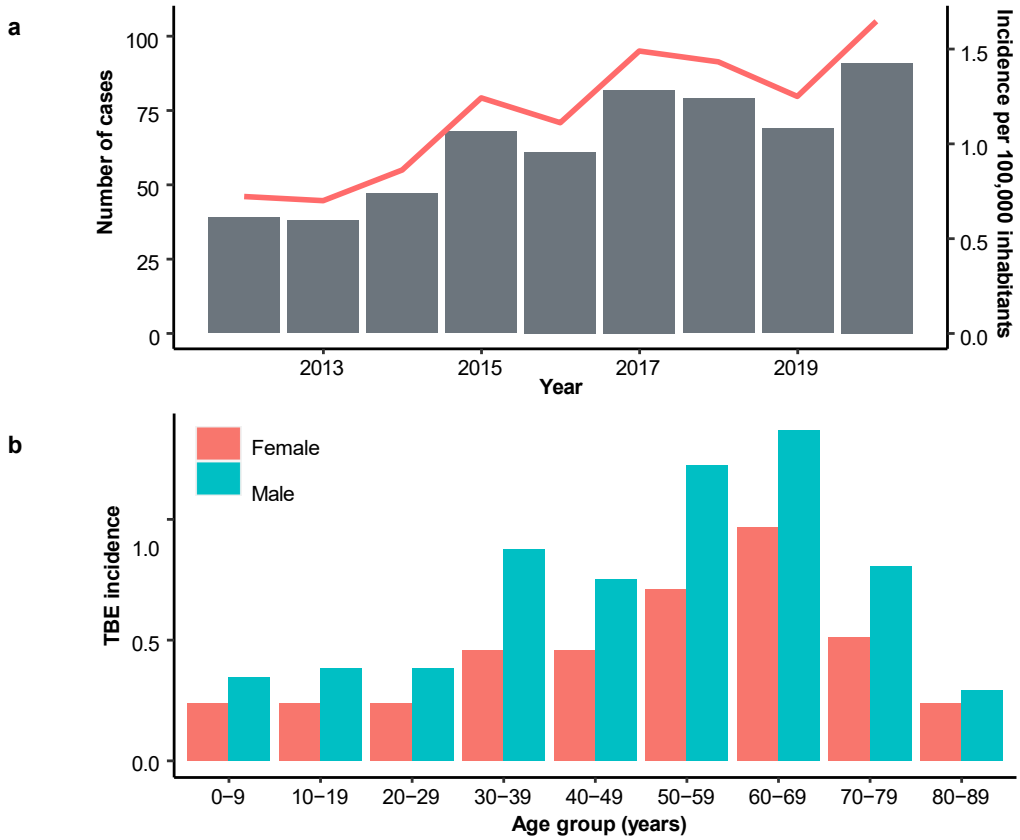

Figure 8. FINLAND - TBE cases (n. 574, period 2012-2020): yearly number of cases and incidence per 100,000 inhabitants (a) and distribution of TBE incidence by sex and age group (b) (data from TESSy-ECDC)

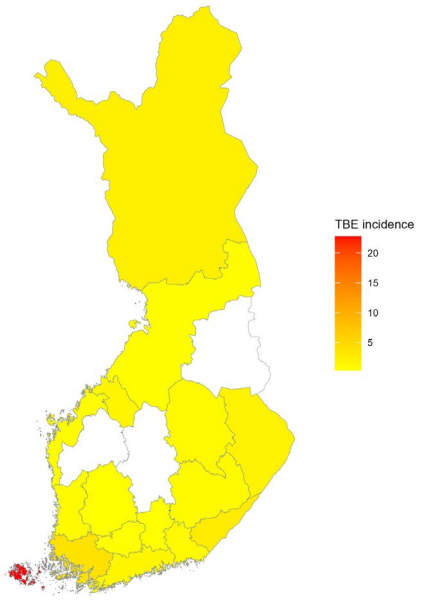

Figure 9. FINLAND - TBE average incidence rate per 100,000 inhabitants by NUTS3 (n. 257, period 2017-2020) (data from TESSy-ECDC)

France

A total of 121 cases of TBE are reported in France from 2012 to 2020, with a mean of 15 cases reported annually and an average incidence of 0.02 per 100 000 inhabitants (Figure 10a). Age and gender distribution includes mainly males from 50 to 69, but also categories from 30 to 49 are affected (Figure 10b).

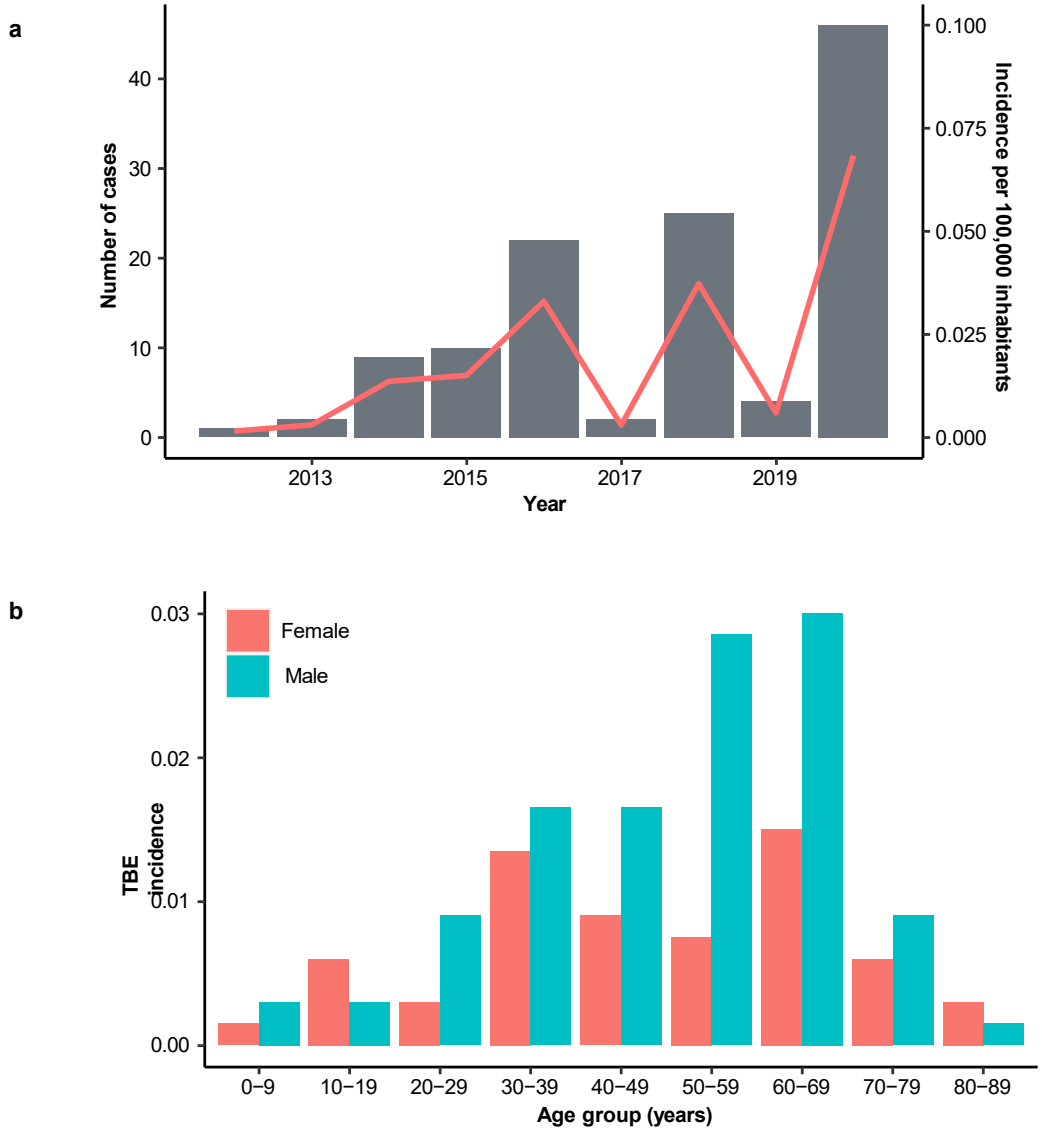

Figure 10. FRANCE - TBE cases (n. 121, period 2012-2020): yearly number of casesand incidence per 100,000 inhabitants (a) and distribution of TBE incidence by sex and age group (b) (data from TESSy-ECDC)

The place of infection was reported to TESSy from 2017 onwards. In 2020 there was a steep increase in the number of reported cases (n. 46) with respect to the previous year (n. 4), mostly due to an alimentary outbreak located in the eastern part of France (Figure 11) (143).

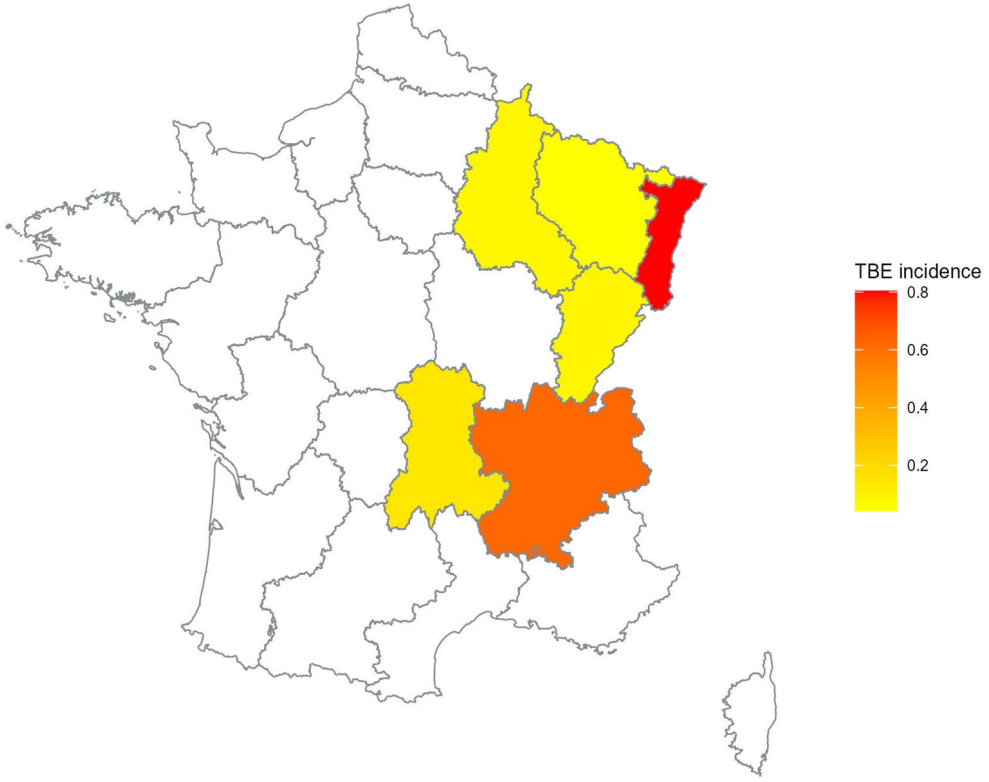

Figure 11. FRANCE - TBE average annual incidence rate per 100 000 inhabitants by NUTS2 (n. 64, period 2017-2020) (data from TESSy-ECDC)

Italy

The total number of cases reported from Italy over the period 2012-2020 is 210, with a mean of 35 cases reported annually and an average incidence of 0.04 per 100 000 inhabitants (Figure 12a). It involves mainly males included in the 40-49 to 70-79 age groups (Figure 12b).

The place of infection was reported to TESSy from 2017 onwards, evidencing that TBE incidence is mainly located in the north-eastern part of the country (Figure 13).

Spain and Serbia

At the current date there is no evidence of locally acquired TBE human cases in Spain, as during the period 2012-2020 Spain reported only 1 imported case.

Information about TBE human infections in Serbia were provided by the Department for Communicable Diseases Prevention and Control of National Public Health Institute "Dr Milan Jovanovic-Batut". Data are available for the years 2017-2018 only, with a total of 18 reported cases.

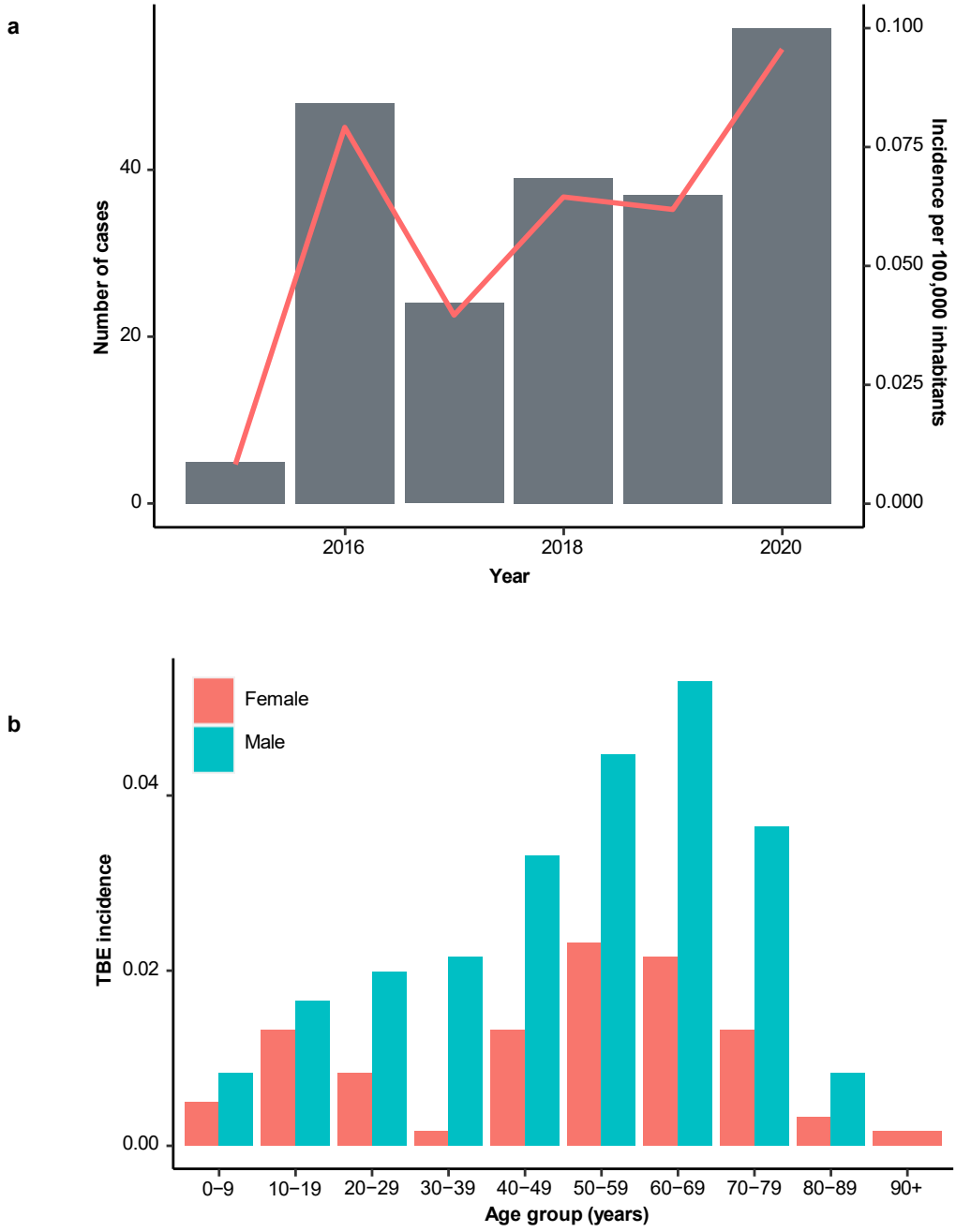

Figure 12. ITALY - TBE cases (n. 210, period 2012-2020): a) Yearly number of cases (bars) and incidence per 100 000 inhabitants (line) and b) distribution of TBE incidence by gender and age group (data from TESSy-ECDC)

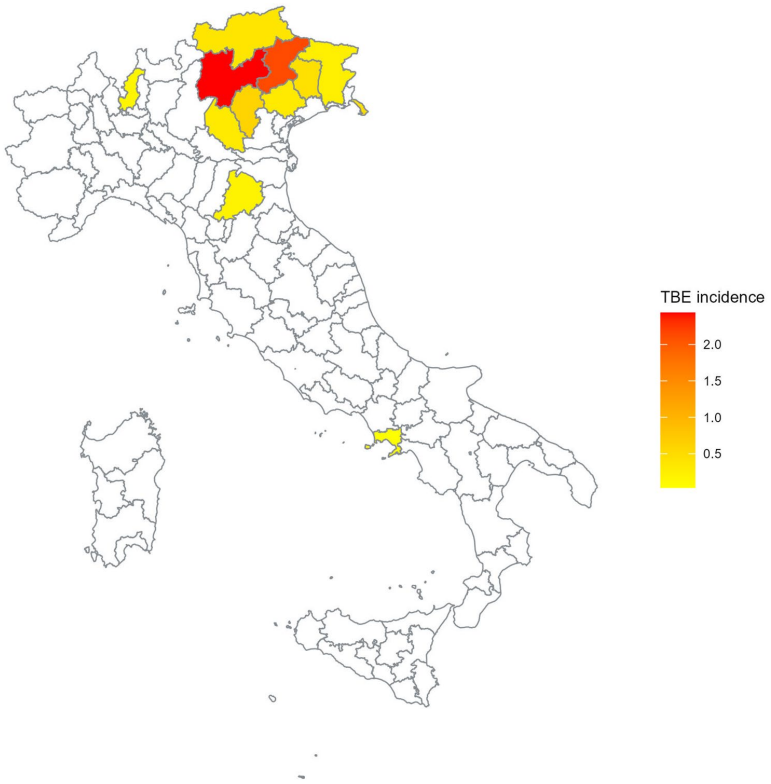

Figure 13. ITALY - TBE average annual incidence rate per 100 000 inhabitants by NUTS3 (n. 123, period 2017-2020) (data from TESSy-ECDC)

Sociological and demographical dimension affecting susceptibility and exposure, including gender

Human infections arise principally through tick bites to which people are exposed as they enter the forests for occupation and recreation. Socio-economic conditions have been identified as part of a network of independent but synergistic factors significantly correlating with TBE incidence. These conditions may operate with different force and at different time-scales depending on the cultural, societal and political contexts characteristic of each country. Abrupt socio-economic transitions could explain the TBE upsurge in ex-communist countries, while slower increases in western countries may have happened such as more retired people engaging in outdoor activities. Furthermore, changes in public health services (144) and the recent economic crisis (2009) (145) have been shown to explain annual spikes in incidence via their effects on human behaviour.

TBE has been observed with a higher incidence among risk groups such as: agricultural and forest workers, hunters, and in general people spending time outdoors such as hikers, bikers, foragers of mushrooms and berries, etc. In Europe 25,818 cases recorded in TESSy between 2012 and 2020 had information on sex (*see* Table 6). TBE was more common in males, with a male-to-female ratio of 1.5:1 (*see* Table 6). This proportion could be explained by the fact that men are less prone to use protective measures and have a lower risk perception (146-148) than women. Moreover, they present a 5-fold higher risk of neurological/neurocognitive sequelae than women (OR: 5017, 95%CI: 1199-20,987) (149).

With respect to the demographic distribution, 25,814 cases between 2012 and 2020 reported information on age (*see* Table 6) with 13,954 cases (54%) in the 40-69 years old group. TBE incidence increased with age in both sexes, peaking at 0.66 cases per 100,000 population in males aged 50-59 years, and then decreasing in older age groups (Figure 14).

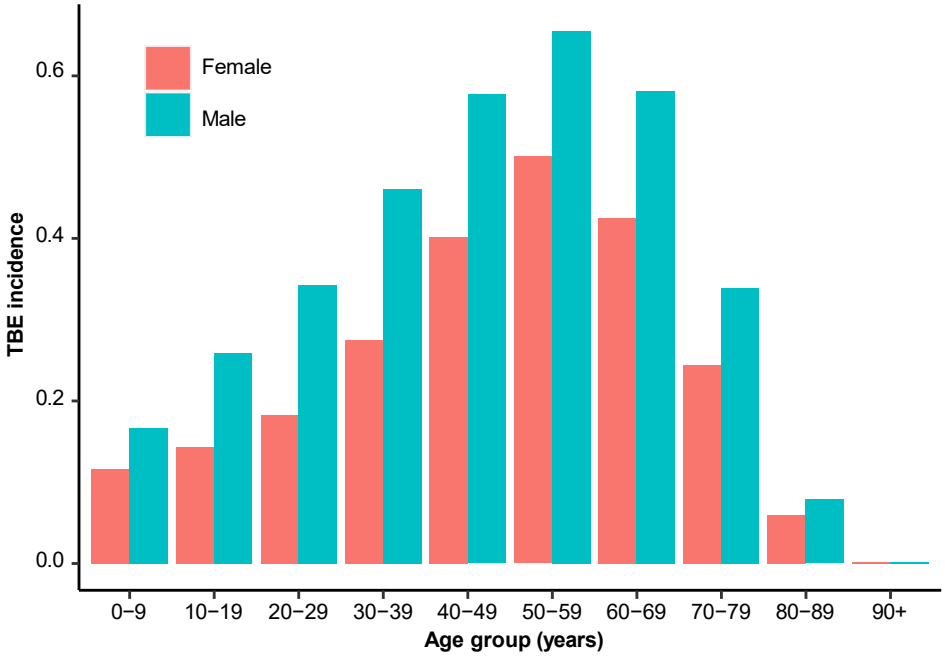

Figure 14. Incidence of tick-borne encephalitis per 100,000 population, by gender and age group in the European Union and European Economic Area countries, 2012-2020 (data from TESSy-ECDC)

The burden of TBE is mostly represented by its incidence and is generally not expressed in Disability-Adjusted Life Years (DALYs) in Global Burden Diseases studies, nonetheless it's long-term sequelae. DALYs measures the burden of diseases combining information on incidence, mortality and sequelae associated with them. As far as we know, only Slovenia performed such analysis revealing the importance of this approach in terms of vaccination policy (150, 151).

Diagnostic procedures and notification systems used at local, national and European scale

Diagnosis

ECDC publishes weekly and annual epidemiological reports regarding communicable diseases of concern to the EU. Data provided by EU Member States are uploaded into TESSy which is a flexible database for collection, validation, cleaning, analysis and dissemination of data for public health actions. All European Union Member States (28) and EEA countries (3) report their available data on communicable diseases as described in Decision No 2119/98/EC, to the system. TBE reporting and data retrieving is yet not homogeneous (Table 7). For 2020, 24 EU/EEA

countries reported data on TBE, among these 19 used various versions of the EU case definition for TBE (2008, 2012 and 2018), three reported using another case definition and two did not specify which case definition was used. 19 countries over 24 compulsory report TBE.

Cases are notified on a case-based level for all countries except Belgium and Bulgaria, and different administrative geographical units (NUTS) are used.

TBE is a notifiable disease in European Union since 2012 (152). The criteria considered are:

– *Case definition*
  A confirmed case is defined as any person meeting the clinical i.e. symptoms of inflammation of the central nervous system AND laboratory criteria i.e. at least one of the following five:
  1. TBE specific IgM AND IgG antibodies in blood;
  2. TBE specific IgM antibodies in CSF;
  3. Seroconversion or four-fold increase of TBE-specific antibodies in paired serum samples;
  4. Detection of TBE viral nucleic acid in a clinical specimen;
  5. Isolation of TBE virus from clinical specimen.

  A probable case is defined as any person meeting the clinical criteria and the laboratory criteria for a probable case i.e. detection of TBE-specific IgM-antibodies in a unique serum sample OR any person meeting the clinical criteria with exposure to a common source (unpasteurised dairy products).
– *Anamnestic*
  Patient medical records should include: age, gender, vaccination coverage for TBEV, consciousness of tick bite, Charlson Comorbidity Index (CCI), type of course (biphasic or monophasic), clinical manifestations, and professional exposure.
– *Clinical*
  Regarding the TBE European subtype, a series of symptoms are possible including: fever, headache, asthenia, arthromyalgia, nausea, vomiting, ataxia, constipation, thremor, diarrhea, abdominal pain, visual disorders, limb palsy, disorientation, paresthesia, ideomotor slowing, hearing disorders, syncope, lymphadenopathy, urinary retention, sore throat, aphasia, dizziness, stupor, and amnesia.
– *Laboratory*
  TBEV can be detected in blood samples during the first febrile phase and in brain tissue during the phase involving CNS symptoms. Therefore RT-PCR is a valuable diagnostic tool when there's the need to confirm an infection of a febrile illness following a tick bite or as a confirmation in fatal cases or in immunosuppressed patients. RT-PCR can detect viral RNA in urine samples for up to 19 days after the start of neurological symptoms. During the initial phase, leukopenia as well as thrombocytopenia are found in about 70% of patients (153). During the second phase, elevated white blood cell count may be present, C-reactive protein (CRP) concentration and erythrocyte sedimentation rate (ESR) may be elevated, especially in long-lasting severe cases. Analysis of cerebrospinal fluid (CSF) usually shows pleocytosis and a moderately raised protein level (153).
  Changes in blood cells count during clinical manifestations include mostly neutropenia, lymphocytopenia, monocytosis, leukocytosis and thrombocytopenia. C-reactive protein (CRP) concentration and Erythrocyte Sedimentation Rate (ESR) are elevated. Pleocytosis and raised protein levels are found in cerebrospinal fluid. Other laboratory findings are: high levels of bilirubin, Gamma-GlutamylTransferase (GGT) or transaminase level, electrolyte disorders, increase of fibrinogen and amylase, creatine phosphokinase and lactate dehydrogenase (149). Computed Tomography (CT), Magnetic Resonance Imaging (MRI) and ElectroEncephaloGram (EEG) abnormalities could be found.

Table 7. Surveillance system overview for tick-borne encephalitis

Country	Data source	Type of surveillance			Data reported by				Case definition used
		Cp, V, O	A, P	C, A	Laboratories	Physicians	Hospitals	Others	
Austria	AT-Epidemiegesetz	Cp	P	C	Y	Y	Y	Y	EU-2012
Belgium	BE-REFLAB	V	P	A	Y	N	N	N	EU-2018
Bulgaria	BG-NATIONAL_SURVEILLANCE	Cp	P	A	Y	Y	Y	Y	EU-2018
Croatia	HR-CNIPH	.	.	C	.	.	.	.	Not specified/ unknown
Czechia	CZ-ISIN	Cp	A	C	Y	Y	Y	N	EU-2008
Estonia	EE-NAKIS	Cp	P	C	Y	Y	Y	N	EU-2012
Finland	FI-NIDR	Cp	P	C	Y	N	N	N	EU-2012
France	FR-NATIONAL_REFERENCE_CENTRES	V	P	C	Y	N	N	N	EU-2012
Germany	DE-SURVNET@RKI-7.1/6	Cp	P	C	Y	Y	Y	Y	Other
Greece	EL-NOTIFIABLE_DISEASES	Cp	P	C	Y	Y	Y	.	EU-2018
Hungary	HU-EFRIR	Cp	P	C	Y	Y	Y	N	EU-2012
Ireland	IE-CIDR	Cp	P	C	Y	Y	Y	N	EU-2012
Italy	IT-ARBO	Cp	P	C	N	Y	Y	.	Other
Latvia	LV-BSN	Cp	P	C	Y	Y	Y	N	EU-2012
Lithuania	LT-COMMUNICABLE_DISEASES	Cp	P	C	Y	Y	N	N	EU-2018
Luxembourg	LU-SYSTEM1	V	.	C	N	N	N	N	Not specified/ unknown
Netherlands	NL-TBE	V	P	C	Y	N	N	N	EU-2012
Norway	NO-MSIS_A	Cp	P	C	Y	Y	Y	N	EU-2012
Poland	PL-NATIONAL_SURVEILLANCE	Cp	P	C	Y	Y	Y	N	EU-2012
Romania	RO-RNSSy	Cp	P	C	N	N	Y	N	EU-2018
Slovakia	SK-EPIS	Cp	A	C	Y	Y	Y	N	EU-2012
Slovenia	SI-SURVIVAL	Cp	P	C	Y	Y	Y	N	EU-2008
Spain	ES-STATUTORY_DISEASES	Cp	P	C	Y	Y	Y	N	EU-2018
Sweden	SE-SMINET	Cp	P	C	Y	Y	N	N	Other

(from: https://www.ecdc.europa.eu/en/publications-data/surveillance-systems-overview-2020).

Cp Compulsory; **V** voluntary, **O** other
A Active, **P** Passive
C Case-based; **A** Aggregated

Infrastructure capacity to identify pathogens for each Member State

In 2007, the EU-funded dedicated surveillance network for enteric pathogens subsequently broadened to cover 21 food- and waterborne diseases and zoonoses, the European Food- and Waterborne Diseases and Zoonoses Network (FWD-Net). FWD-Net is coordinated by ECDC with the support of a coordination committee (CC). Each Member States nominated an Institute to be referred to as National Focal Point (Table 8).

Table 8. List of National Focal Points of the European Food- and Waterborne Diseases and Zoonoses Network (FWD-Net – ECDC) (link to websites included)

Country	Institute	Address
Austria	Austrian Agency for Health and Food Safety	Beethovenstraße 6, AT-8010 Graz
Belgium	Sciensano	Rue Juliette Wytsmanstraat 14, 1050 Brussels
Bulgaria	National Centre of Infectious and Parasitic Diseases	26 Yanko Sakazov Blvd, 1504 Sofia
Croatia	Croatian National Institute of Public Health	Rockefellerova 7, 10000 Zagreb
Cyprus	Directorate of Medical and Public Health Services	1, Prodromou str, CY-1448 Nicosia
Czech Republic	National Institute of Public Health/ Regional Public Health Authority Moravian-Silesian Region in Ostrava	Šrobárova 48, 100 42 Praha 10/ Na Belidle 7, 702 00 Ostrava
Denmark	Statens Serum Institut	5 Artillerivej, DK-2300 Copenhagen S
Estonia	Health Board	81 Paldiski Mnt, 10617 Tallinn
Finland	National Institute for Health and Welfare	Mannerheimintie 166, (00)271 Helsinki
France	Institute for Public Health Surveillance	12 rue du Val d'Osne, FR-94410 Saint-Maurice cedex
Germany	Robert Koch Institute	Seestraße 10, 13353 Berlin
Greece	National Public Health Organization	3-5 Agrafon St., EL-15123 Athens
Hungary	National Center for Epidemiology	Gyali ut 2-6, Budapest
Iceland	Centre of Health Security and Communicable Disease Prevention	Barónsstíg 47, IS - 101 Reykjvík
Ireland	Health Protection Surveillance Centre	25-27 Middle Gardiner Street, IR-1 Dublin
Italy	National Institute of Health	Viale Regina Elena 299, 00161 Rome
Latvia	Centre for Disease Prevention and Control	Duntes 22, 1005 Riga
Liechtenstein	Principality of Liechtenstein	Äulestrasse 51, 9490 Vaduz
Lithuania	Centre for Communicable Diseases and AIDS	Nugaletoju st. 14D, LT-10105 Vilnius
Luxembourg	Ministry of Health	1, rue Charles Darwin L-1433 Luxembourg
Malta	Superintendence of Public Health	37-39 Rue D'Argens, MT-5 Msida MSD

Country	Institute	Address
Netherlands	National Institute for Public Health and the Environment (RIVM)	Antonie van Leeuwenhoeklaan 9, PO Box 1, 3720BA Bilthoven
Norway	Norwegian Institute of Public Health	PO Box 4404 Nydalen, (0)403 Oslo
Poland	National Institute of Public Health/National Institute of Hygiene	24 Chocimska Street, (00)791 Warsaw
Portugal	Directorate General of Health	Alameda D. Afonso Henriques, 45- 2º, 1049-005 Lisbon
Romania	National Institute of Public Health	Dr. Leonte Anastasievici 1-3, (0)50463 Bucharest
Slovakia	Public Health Authority of the Slovak Republic	Trnavská cesta 52, SK-826 45 Bratslava
Slovenia	National Institute of Public Health (NIJZ)	Trubarjeva 2, SL - 1000 Ljubljana
Spain	National Centre of Epidemiology, Health Institute Carlos III/ Instituto de Salud Carlos III: National Centre for Microbiology	Monforte de Lemos, 5, ES-28029 Madrid/ Ctra. Majadahonda a Pozuelo, Km. 2, 28220 Madrid
Sweden	Public Health Agency of Sweden	Nobels väg 18, Solna, 17182 Stockholm

Estimated influence of environmental change on the disease future trends

TBEV circulation in nature require the co-occurrence of several biotic components (competent tick vectors, competent reservoirs, and feeding hosts for ticks) that interplay in a complex way and whose abundance, temporal and spatial dynamics are affected or modulated by abiotic factors (e.g. climatic condition, habitat structure, food availability, etc.) other than by biotic and socio-economic factors (trophic interaction, wildlife management, etc.). Climate change is expected to affect all these components, starting from the spatial and temporal distribution of *I. ricinus* complex (154-157), and consequently with an expected increase of the probability of TBE occurrence at higher altitude and northern latitude, beyond the current edges of its range (158, 159). The northern shift and increased tick activity are related to milder winters and longer spring and autumn seasons combined with increased vegetative cover and rise of ungulates abundance, as already observed in Sweden, Norway and in the Baltic countries (160). Similarly, in the Alps and in the Carpathian Mountains of Central Europe, an altitudinal shift of *I. ricinus* from about 700 to 1200 m asl has also been already reported (161, 162). Similarly, forest expansion and altered synchronized seed crops that affect density and distribution of rodents and wild ungulates and that in turn drive up the density of infected ticks has been reported (94, 163-165). In some cases, enhanced human exposure to infected ticks driven by changes in socio-economical condition have been considered the most important driver of upsurge in TBE cases (71). Estimating and predicting the transmission risk of TBEV under a global change scenario is essential for planning public health interventions including vaccination programs. Most of the proposed models infer TBE morbidity against some indicative environmental variables (e.g., mean annual temperature, precipitation, NDVI, etc.) or population estimates of key hosts (e.g., rodents or ticks), or most commonly combining both (166-169), in order to get an estimate of future trends.

TBE interannual fluctuations in the number of cases are not yet well parametrized, due to the difficulties to obtain information such as the vaccination coverage and human behaviour. The actual trend is toward an increasing, facilitated by the fact that the human population is ageing and growing in numbers and with a higher probability of exposure. Moreover, the expected extension of tick questing season, the periodic variations in food availability for key hosts and the consequent increase of infected ticks through non-systemic transmission (co-feeding) (168), support this trend. At the same time, it has also been reported that climate warming will to some extent reduce TBE incidence in Southern and lowland areas (170) as well as the impact of extreme meteorological events or prolonged drought conditions on ticks' occurrence (171).

On a large European scale, the affected countries might exhibit different and contrasting oscillations of TBE human cases according to the matching of several cycles (e.g., 2 to 6 years for the tick *I. ricinus*, 2 to 5 years for *muridae* rodents, 4-6 years for beech masting production, quasi-decadal for large and medium-sized herbivores, some insectivores and predators, quasi-biennial for some climatic oscillations, (172).

The complexity of the disease system, at this large-scale, makes a long-term prediction too uncertain to be realistic therefore the use of validated covariates that can forecast the disease trend 1-2 years in advance would be enough for the health system to be prepared.

Finally, to assess the actual effective occurrence of TBE and therefore make more robust predictions of the potential future trend under a climate change scenario, quantitative data related to the aforementioned biotic factors (host community composition, host density etc.) and other abiotic factors (such as land use) should be retrieved at a high-resolution spatial scale.

References

1. Pulkkinen LIA, Butcher SJ, Anastasina M. Tick-borne encephalitis virus: A structural view. *Viruses*. 2018;10(7):1-20.
2. Heinz FX, Mandl CW. The molecular biology of tick-borne encephalitis virus. Review article. *APMIS Acta Pathol Microbiol Immunol Scand*. 1993;101(10):735-45.
3. Gritsun TS, Holmes EC, Gould EA. Analysis of flavivirus envelope proteins reveals variable domains that reflect their antigenicity and may determine their pathogenesis. *Virus Res*. 1995;35(3):307-21.
4. McMinn PC. The molecular basis of virulence of the encephalitogenic flaviviruses. *J Gen Virol*. 1997;78(Pt 11):2711-22.
5. Kofler RM, Heinz FX, Mandl CW. Capsid protein C of tick-borne encephalitis virus tolerates large internal deletions and is a favorable target for attenuation of virulence. *J Virol*. 2002;76(7):3534-43.
6. Yoshii K, Yokozawa K, Kariwa H, *et al.* A conserved region in the prM protein is a critical determinant in the assembly of flavivirus particles. *J Gen Virol*. 2012;93(1):27-38.
7. Khasnatinov MA, Tuplin A, Gritsun DJ, *et al.* Tick-borne encephalitis virus structural proteins are the primary viral determinants of non-viraemic transmission between ticks whereas non-structural proteins affect cytotoxicity. *PLoS ONE*. 2016;11(6).
8. King AMQ, Adams MJ, Carstens EB, *et al. Virus taxonomy, classification and nomenclature of viruses. Ninth report of the International Committee of Taxonomic of Viruses.* Amsterdam: Elsevier, 2011.
9. Gritsun TS, Lashkevich VA, Gould EA. Tick-borne encephalitis. *Antiviral Res.* 2003;57(1-2):129-46.
10. Amato-Gauci A, Zeller H. Tick-borne encephalitis joins the diseases under surveillance in the European Union. *Euro Surveill*. 2012;17(42):20299.
11. Fares W, Dachraoui K, Cherni S, *et al.* Tick-borne encephalitis virus in Ixodes ricinus (Acari: Ixodidae) ticks, Tunisia. *Ticks Tick-Borne Dis*. 2020; 12:101606.

12. Růžek D, Dobler G, Mantke OD. Tick-borne encephalitis: Pathogenesis and clinical implications. *Travel Med Infect Dis*. 2010;8(4):223-32.
13. Leonova GN, Belikov SI. Phylogenetic analysis and distribution of far eastern tick-borne encephalitis virus subtype (Flaviridae, Flavirus, TBEV-FE) from Asia. *Vopr Virusol*. 2019;64(5):250-6.
14. Kovalev SY, Kokorev VS, Belyaeva I V. Distribution of Far-Eastern tick-borne encephalitis virus subtype strains in the former Soviet Union. *J Gen Virol*. 2010;91(12):2941-6.
15. Beauté J, Spiteri G, Warns-Petit E, *et al.* Tick-borne encephalitis in europe, 2012 to 2016. *Euro Surveill*. 2018;23(45).
16. Tkachev SE, Babkin I V, Belokopytova PS, *et al.* Genetic diversity and geographical distribution of the Siberian subtype of the tick-borne encephalitis virus. *Ticks Tick Borne Dis*. 2020;11(2):1877-9603.
17. Adelshin R V., Sidorova EA, Bondaryuk AN, *et al.* "886-84-like" tick-borne encephalitis virus strains: Intraspecific status elucidated by comparative genomics. *Ticks Tick-Borne Dis.* 2019;10(5):1168-72.
18. Kovalev SY, Mukhacheva TA. Reconsidering the classification of tick-borne encephalitis virus within the Siberian subtype gives new insights into its evolutionary history. *Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis*. 2017;20171100(55):159-65.
19. Kozlova V, Demina V, Tkachev E, *et al.* Characteristics of the Baikal subtype of tick-borne encephalitis virus circulating in eastern Siberia. *Acta Biomed Sci*. 2018;3(4):53-60.
20. Dai XY, Shang GB, Lu S, *et al.* A new subtype of eastern tick-borne encephalitis virus discovered in Qinghai-Tibet Plateau, China. *Emerg Microbes Infect.* 2018;25(7):1751-2222.
21. Weststrate AC, Knapen D, Laverman GD, *et al.* Increasing evidence of tick-borne encephalitis (TBE) virus transmission, the Netherlands, June 2016. *Euro Surveill.* 2017;22(11):1560-7917.
22. Demina T V., Dzhioev YP, Verkhozina MM, *et al.* Genotyping and characterization of the geographical distribution of tick-borne encephalitis virus variants with a set of molecular probes. *J Med Virol.* 2010;82(6):965-76.
23. Ruzek D, Avšič Županc T, Borde J, *et al.* Tick-borne encephalitis in Europe and Russia: Review of pathogenesis, clinical features, therapy, and vaccines. *Antiviral Research.* 2019; 164, p. 23-51.
24. Stoefs A, Heyndrickx L, De Winter J, *et al.* Autochthonous cases of tick-borne encephalitis, Belgium, 2020. *Emerg Infect Dis*. 2021 Aug;27(8):2179-82.
25. Mohareb E, Christova I, Soliman A, *et al.* Tick-borne encephalitis in Bulgaria, 2009 to 2012. *Euro Surveill*. 2013;14(18):1560-7917.
26. Mansbridge CT, Osborne J, Holding M, *et al.* Autochthonous tick-borne encephalitis in the United Kingdom: A second probable human case and local eco-epidemiological findings. *Ticks Tick-Borne Dis*. 2022;13(1):101853.
27. Labuda M, Kozuch O, Zuffová E, *et al.* Tick-borne encephalitis virus transmission between ticks cofeeding on specific immune natural rodent hosts. *Virology*. 1997;235(1):138-43.
28. Randolph SE, Miklisova D, Lysy J, *et al.* Incidence from coincidence: patterns of tick infestations on rodents facilitate transmission of tick-borne encephalitis virus. *Parasitology*. 1999;118(Pt 2):177-86.
29. Randolph SE, Green RM, Peacey MF, *et al.* Seasonal synchrony: the key to tick-borne encephalitis foci identified by satellite data. *Parasitology*. 2000;121(01):15-23.
30. Tonteri E, Jaaskelainen Anu E, Tikkakoski T, *et al.* Tick-borne encephalitis virus in wild rodents in winter, Finland, 2008-2009. *Emerg Infect Dis*. 2011;17(1):72-5.
31. Pukhovskaya NM, Morozova O V, Vysochina NP, *et al.* Tick-borne encephalitis virus in arthropod vectors in the Far East of Russia. *Ticks Tick-Borne Dis*. 2018;9(4):824-33.
32. Benda R. The common tick Ixodes ricinus L. as a reservoir and vector of tick-borne encephalitis virus, I, II. *J Hyg Epidemiol Microbiol Immunol*. 1958;2:314-44.

33. Süss J. Tick-borne encephalitis 2010: epidemiology, risk areas, and virus strains in Europe and Asia-an overview. *Ticks Tick-Borne Dis*. 2011;2(1):2-15.
34. Lichard M, Kozuch O. Persistence of tick-borne encephalitis virus in nymphs and adults of Ixodes arboricola and its transmission to white mice. *Acta Virol.* 1967;11(5):480.
35. Krivanec K, Kopecký J, Tomková E, *et al.* Isolation of TBE virus from the tick Ixodes hexagonus. *Folia Parasitol* (Praha). 1988;35(3):273-6.
36. Jemeršić L, Deždek D, Brnić D, *et al.* Detection and genetic characterization of tick-borne encephalitis virus (TBEV) derived from ticks removed from red foxes (Vulpes vulpes) and isolated from spleen samples of red deer (Cervus elaphus) in Croatia. *Ticks Tick-Borne Dis*. 2014;5(1):7-13.
37. Valarcher JF, Hagglund S, Juremalm M, *et al.* Tick-borne encephalitis. *Rev Sci Tech.* 2015;34(2):453-66.
38. Takeda T, Ito T, Chiba M, *et al.* Isolation of tick-borne encephalitis virus from Ixodes ovatus (Acari: Ixodidae) in Japan. *J Med Entomol.* 1998;35(3):227-31.
39. Kozuch O, Nosek J. Transmission of tick-borne encephalitis (TBE) virus by Dermacentor marginatus and D. reticulatus ticks. *Acta Virol.* 1971;15(4):334.
40. Nosek J. The ecology and public health importance of Dermacentor marginatus and D. reticulatus ticks in Central Europe. *Folia Parasitol* (Praha). 1972;19(1):93-102.
41. Abdiyeva K, Turebekov, N Yegemberdiyeva, *et al.* Vectors, molecular epidemiology and phylogeny of TBEV in Kazakhstan and central Asia. *Parasit Vectors*. 2020;1(13):504.
42. Lickova M, Fumacova Havlikova S, Slavikova M, *et al.* Dermacentor reticulatus is a vector of tick-borne encephalitis virus. *Ticks Tick Borne Dis*. 2020;11(4):1877-9603.
43. Chitimia-Dobler L, Lemhofer G, Dobler G, *et al.* Repeated isolation of tick-borne encephalitis virus from adult Dermacentor reticulatus ticks in an endemic area in Germany. *Parasit Vectors.* 2019;12(12):1756-3305.
44. Kozuch O, Nosek J. Experimental transmission of tick-borne encephalitis (TBE) virus by Haemaphysalis concinna ticks. Vol. 24, *Acta virologica.* Slovakia; 1980. p. 377.
45. Yun SM, Song BG, Choi W, *et al.* Prevalence of tick-borne encephalitis virus in ixodid ticks collected from the republic of Korea during 2011-2012. *Osong Public Health Res Perspect*. 2012;3(4):213-21.
46. Michelitsch A, Tews BA, Klaus C, *et al.* In vivo characterization of tick-borne encephalitis virus in bank voles (Myodes glareolus). *Viruses*. 2019;11(11):1069.
47. Lindquist L. Tick-borne encephalitis. *Handb Clin Neurol*. 2014;123:531-59.
48. Lindquist L, Vapalahti O. Tick-borne encephalitis. *The Lancet*. 2008;371(9627):1861-71.
49. Gerlinskaya LA, Bakhvalova VN, Morozova O V, *et al.* Sexual transmission of tick-borne encephalitis virus in laboratory mice. *Bull Exp Biol Med*. 1997;123(3):283-4.
50. Knap N, Avšič-Županc T. Factors affecting the ecology of tick-borne encephalitis in Slovenia. *Epidemiology and Infection.* 2015;143:2059-67.
51. Waldenström J, Lundkvist A, Falk KI, *et al.* Migrating birds and tickborne encephalitis virus. *Emerg Infect Dis.* 2007;13(8):1215-8.
52. Hubálek Z, Rudolf I. Tick-borne viruses in Europe. *Parasitol Res*. 2012;111(1):9-36.
53. Jaenson TGT, Petersson EH, Jaenson DGE, *et al.* The importance of wildlife in the ecology and epidemiology of the TBE virus in Sweden: incidence of human TBE correlates with abundance of deer and hares. *Parasit Vectors*. 2018;29(11):1756-3305.
54. Rieger MA, Nübling M, Müller W, *et al.* Foxes as indicators for TBE endemicity - A comparative serological investigation. *Zentralblatt Bakteriol*. 1999;289(5-7):610-8.

55. Haemig PD, Lithner S, de Luna SS, *et al.* Red fox and tick-borne encephalitis (TBE) in humans: Can predators influence public health? *Scand J Infect Dis.* 2008;40(6-7):527-32.
56. Radda A. Die Zeckenenzephalitis in Europa. *Z FürAngewandte Zool*. 1973;60:409-61.
57. Böhm B, Schade B, Bauer B, *et al.* Tick-borne encephalitis in a naturally infected sheep. *BMC Vet Res*. 2017; 22;13(1):1-6.
58. Pfeffer M, Dobler G. Tick-borne encephalitis virus in dogs - is this an issue? *Parasit Vectors*. 2011;59:1756-3305.
59. Fouché N, Oesch S, Ziegler U, *et al.* Clinical Presentation and laboratory diagnostic work-up of a horse with tick-borne encephalitis in Switzerland. *Viruses*. 2021;13(8).
60. Conze TM, Bagó Z, Revilla-Fernández S, *et al.* Tick-Borne Encephalitis Virus (TBEV) infection in two horses. *Viruses*. 2021;13(9).
61. Da Rold G, Obber F, Monne I, *et al.* Clinical tick-borne encephalitis in a roe deer (Capreolus capreolus L.). *Viruses*. 2022;14(2):300.
62. Bagó Z, Bauder B, Kolodziejek J, *et al.* Tickborne encephalitis in a mouflon (Ovis ammon musimon). *Vet Rec*. 2002;150(7):218-20.
63. Schönbächler K, Hatt JM, Silaghi C, *et al.* Confirmation of tick-borne encephalitis virus in an european hedgehog (Erinaceus europaeus). *Schweiz Arch Tierheilkd.* 2018;161(1):23-31.
64. Süss J, Gelpi E, Klaus C, *et al.* Tickborne encephalitis in naturally exposed monkey (Macaca sylvanus). *Emerg Infect Dis J*. 2007;13(6):905.
65. Dagostin F, Tagliapietra V, Marini G, *et al.* Ecological and environmental factors affecting the risk of tick-borne encephalitis in Europe, 2017 to 2021. *Euro Surveill*. 2023;28(42).
66. Nuttall PA. Climate change impacts on ticks and tick-borne infections. *Biologia* (Bratisl). 2022;77:1503-12.
67. Labuda M, Randolph SE. Survival strategy of tick-borne encephalitis virus: cellular basis and environmental determinants. *Zentralblatt Bakteriol Int J Med Microbiol*. 1999;289:5-7.
68. Perkins SE, Cattadori IM, Tagliapietra V, *et al.* Empirical evidence for key hosts in persistence of a tick-borne disease. *Int J Parasitol.* 2003;33(9).
69. Carpi G, Cagnacci F, Neteler M, *et al.* Tick infestation on roe deer in relation to geographic and remotely sensed climatic variables in a tick-borne encephalitis endemic area. *Epidemiol Infect.* 2008;136(10):1416-24.
70. Rosà R, Tagliapietra V, Manica M, *et al.* Changes in host densities and co-feeding pattern efficiently predict tick-borne encephalitis hazard in an endemic focus in northern Italy. *Int J Parasitol*. 2019;20190900(49):779-87.
71. Sumilo D, Asokliene L, Bormane A, *et al.* Climate Change Cannot Explain the Upsurge of Tick-borne Encephalitis in the Baltics. *PLoS One*. 2007;6(2):1932-6203.
72. Andreassen A, Jore S, Cuber P, *et al.* Prevalence of tick borne encephalitis virus in tick nymphs in relation to climatic factors on the southern coast of Norway. *Parasit Vectors*. 2012;22(5):1756-3305.
73. Randolph SE. Tick-borne encephalitis incidence in Central and Eastern Europe: consequences of political transition. *Microbes Infect*. 2008;10(3):209-16.
74. Jaenson TGT, Eisen L, Comstedt P, *et al.* Risk indicators for the tick Ixodes ricinus and Borrelia burgdorferi sensu lato in Sweden. *Med Vet Entomol*. 2009;23(3):226-37.
75. Barandika Jesus F, Hurtado A, Juste Ramon A, *et al.* Seasonal dynamics of Ixodes ricinus in a 3-year period in northern Spain: first survey on the presence of tick-borne encephalitis virus. *Vector Borne Zoonotic Dis*. 2010;10(10):1027-35.

76. Daniel M, Malý M, Danielová V, *et al.* Abiotic predictors and annual seasonal dynamics of Ixodes ricinus, the major disease vector of Central Europe. *Parasit Vectors*. 2015; 8(478):1-12.
77. Cuber P, Andreassen Å, Vainio K, *et al.* Risk of exposure to ticks (Ixodidae) and the prevalence of tick-borne encephalitis virus (TBEV) in ticks in Southern Poland. *Ticks Tick-Borne Dis.* 2015;6(3):356-63.
78. Randolph SE. Dynamics of tick-borne disease systems: minor role of recent climate change. *Rev Sci Tech Int Off Epizoot*. 2008;27(2):367-81.
79. Burri C, Bastic V, Maeder G, *et al.* Microclimate and the zoonotic cycle of tick-borne encephalitis virus in Switzerland. *J Med Entomol.* 2011;48(3):615-27.
80. Tagliapietra V, Rosà R, Arnoldi D, *et al.* Saturation deficit and deer density affect questing activity and local abundance of Ixodes ricinus (Acari, Ixodidae) in Italy. *Vet Parasitol*. 2011;183(1-2).
81. Brugger K, Schiffner-Rohe J, Walter M, *et al.* Modeling the Distribution of Tbev-Infected Ticks in Germany To Estimate Tbe-Infections in Humans. *Value Health*. 2018;21(1):S379.
82. Daniel M, Kriz B, Danielova V, *et al.* Correlation between meteorological factors and tick-borne encephalitis incidence in the Czech Republic. *Parasitol Res*. 2008;103(1):113-932.
83. Rosà R, Tagliapietra V, Manica M, *et al.* Changes in host densities and co-feeding pattern efficiently predict tick-borne encephalitis hazard in an endemic focus in northern Italy. *Int J Parasitol.* 2019;49(10):779-87.
84. Tkadlec E, Václavík T, Široký P. Rodent host abundance and climate variability as predictors of tickborne disease risk 1 year in advance. *Emerg Infect Dis.* 2019;25(9):1738-41.
85. Cagnacci F, Bolzoni L, Rosà R, *et al.* Effects of deer density on tick infestation of rodents and the hazard of tick-borne encephalitis. I: Empirical assessment. *Int J Parasitol.* 2012;42(4).
86. Bolzoni L, Rosa R, Cagnacci F, *et al.* Effect of deer density on tick infestation of rodents and the hazard of tick-borne encephalitis. II: population and infection models. *Int J Parasitol*. 2012;42(4):373-81.
87. Palo RT. Tick-borne encephalitis transmission risk: Its dependence on host population dynamics and climate effects. *Vector-Borne Zoonotic Dis*. 2014 May 1;14(5):346-52.
88. Vanwambeke SO, Sumilo DS, Bormane A, *et al.* Landscape predictors of tick-borne encephalitis in Latvia: land cover, land use, and land ownership. *Vector-Borne Zoonotic Dis.* 2010;10(5):497-506.
89. Kiffner C, Vor T, Hagedorn P, *et al.* Factors affecting patterns of tick parasitism on forest rodents in tick-borne encephalitis risk areas, Germany. *Parasitol Res.* 2011;108(2):323-35.
90. Knap N, Avsic-Zupanc T. Factors affecting the ecology of tick-borne encephalitis in Slovenia. *Epidemiol Infect*. 2015;143(10):2059-67.
91. Kriz B, Daniel M, Benes C, *et al.* The role of game (wild boar and roe deer) in the spread of tick-borne encephalitis in the Czech Republic. *Vector-Borne Zoonotic Dis*. 2014;14(11):801-7.
92. Kolář J, Potůčková M, Štefanová E. Tick-born encephalitis risk assessment based on satellite data. *Acta Univ Carol Geogr*. 2016;51(2):155-67.
93. Uusitalo R, Siljander M, Pellikka P, *et al.* Modelling habitat suitability for occurrence of human tick-borne encephalitis (TBE) cases in Finland. *Ticks Tick Borne Dis.* 2020;11(5):1877-9603.
94. Rizzoli A, Hauffe HC, Tagliapietra V, *et al.* Forest structure and roe deer abundance predict Tick-borne encephalitis risk in Italy. *PLoS ONE.* 2009;4(2).
95. Zeimes CB, Olsson GE, Hjertqvist M, *et al.* Shaping zoonosis risk: Landscape ecology vs. landscape attractiveness for people, the case of tick-borne encephalitis in Sweden. *Parasit Vectors*. 2014;7(1).
96. Stefanoff P, Rubikowska B, Bratkowski J, *et al.* A predictive model has identified tick-borne encephalitis high-risk areas in regions where no caseswere reported previously, Poland, 1999-2012. *Int J Environ Res Public Health*. 2018 Apr 4;15(4).

97. Kjaer Lene J, Klitgaard K, Bodker R, *et al.* Predicting and mapping human risk of exposure to ixodes ricinus nymphs using climatic and environmental data, Denmark, Norway and Sweden, 2016. *Euro Surveill*. 2019;24(9):1560-7917.
98. Keesing F, Ostfeld RS. Impacts of biodiversity and biodiversity loss on zoonotic diseases. *Proc Natl Acad Sci U S A.* 2021;118(17):1-8.
99. McCallum H, Dobson A. Disease, habitat fragmentation and conservation. *Proc Biol Sci.* 2002;269(1504):2041-9.
100. Allan BF, Keesing F, Ostfeld RS. Effect of forest fragmentation on Lyme Disease risk. *Conserv Biol.* 2003;17(1):267-72.
101. Krauss J, Bommarco R, Guardiola M, *et al.* Habitat fragmentation causes immediate and time-delayed biodiversity loss at different trophic levels. *Ecol Lett.* 2010;13(5):597-605.
102. Wilkinson DA, Marshall JC, French NP, *et al.* Habitat fragmentation, biodiversity loss and the risk of novel infectious disease emergence. *J R Soc Interface.* 2018;15(149).
103. Sumilo D, Bormane A, Asokliene L, *et al.* Socio-economic factors in the differential upsurge of tick-borne encephalitis in Central and Eastern Europe. *Rev Med Virol.* 2008;18(2):81-95.
104. Zlobin VI, Pogodina V V., Kahl O. A brief history of the discovery of tick-borne encephalitis virus in the late 1930s (based on reminiscences of members of the expeditions, their colleagues, and relatives). *Ticks and Tick-borne Dis*. 2017; 8(6):813-20.
105. Yoshii K, Song JY, Park SB, *et al.* Tick-borne encephalitis in Japan, Republic of Korea and China. *Emerg Microbes Infect.* 2017;20(6):1751-2222.
106. Daniel M, Rudenko N, Golovchenko M, *et al.* The occurrence of Ixodes ricinus ticks and important tick-borne pathogens in areas with high tick-borne encephalitis prevalence in different altitudinal levels of the Czech Republic Part II. Ixodes ricinus ticks and genospecies of Borrelia burgdorferi sensu lato complex. *Epidemiol Mikrobiol Imunol.* 2016;65(3):182-192.
107. Labuda M, Danielova V, Jones LD, *et al.* Amplification of tick-borne encephalitis virus infection during co-feeding of ticks. *Med Vet Entomol*. 1993;7(4):339-42.
108. Kunz C. Vaccination against TBE in Austria: the success story continues. *Int J Med Microbiol*. 2002;291 Suppl:56-7.
109. Kohl O, Kožuch O, Elečková E, *et al.* Family outbreak of alimentary tick-borne encephalitis in Slovakia associated with a natural focus of infection. *Eur J Epidemiol*. 1996;12(4):373-5.
110. Gresíková M, Sekeyová M, Stúpalová S, *et al.* Sheep milk-borne epidemic of tick-borne encephalitis in Slovakia. *Intervirology*. 1975;5(1-2):57-61.
111. Holzmann H, Aberle SW, Stiasny K, *et al.* Tick-borne encephalitis from eating goat cheese in a mountain region of Austria. *Emerg Infect Dis.* 2009;15(10):1671-3.
112. Caini S, Szomor K, Ferenczi E, *et al.* Tick-borne encephalitis transmitted by unpasteurised cow milk in western Hungary, September to October 2011. *Euro Surveill*. 2012;22(12):17-21.
113. Hudopisk N, Korva M, Janet E, *et al.* Tick-borne encephalitis associated with consumption of raw goat milk, Slovenia, 2012. *Emerg Infect Dis.* 2013;19(5):806-8.
114. Lipowski D, Popiel M, Perlejewski K, *et al.* A cluster of fatal Tick-borne encephalitis virus infection in organ transplant setting. *J Infect Dis.* 2017;215(6):896-901.
115. Ličková M, Havlíková SF, Sláviková M, *et al.* Alimentary infections by tick-borne encephalitis virus. *Viruses*. 2022;14(1).
116. Martello E, Gillingham EL, Phalkey R, *et al.* Systematic review on the non-vectorial transmission of Tick-borne encephalitis virus (TBEv). *Ticks Tick-Borne Dis*. 2022;13(6).
117. Gritsun TS, Frolova TV, Zhankov AI, *et al.* Characterization of a siberian virus isolated from a patient with progressive chronic tick-borne encephalitis. *J Virol.* 2003;77(1):25-36.

118. Ternovoi VA, Kurzhukov GP, Sokolov YV, *et al.* Tick-borne encephalitis with hemorrhagic syndrome, Novosibirsk region, Russia, 1999. E*merg Infect Dis*. 2003;9(6):743-6.

119. Mickiene A, Laiskonis A, Günther G, *et al.* Tickborne encephalitis in an area of high endemicity in lithuania: disease severity and long-term prognosis. *Clin Infect Dis.* 2002;35(6):650-8.

120. Mickiene A, Pakalniene J, Nordgren J, *et al.* Polymorphisms in chemokine receptor 5 and Toll-like receptor 3 genes are risk factors for clinical tick-borne encephalitis in the Lithuanian population. *PloS One*. 2014;9(9):1932-6203.

121. Kaiser R. Tick-borne encephalitis: Clinical findings and prognosis in adults. *Wien Med Wochenschr.* 2012;162(11-12):239-43.

122. Zajkowska J, Czupryna P, Pancewicz S, *et al.* Fatal outcome of tick-borne encephalitis - a case series. *Neurol Neurochir Pol. 2011;45(4):402-6.*

123. Barkhash AV, Voevoda MI, Romaschenko AG. Association of single nucleotide polymorphism rs3775291 in the coding region of the TLR3 gene with predisposition to tick-borne encephalitis in a Russian population. *Antiviral Res*. 2013;99(2):136-8.

124. Barkhash AV, Babenko VN, Voevoda MI, *et al.* Association of IL28B and IL10 gene polymorphism with predisposition to tick-borne encephalitis in a Russian population. *Ticks Tick Borne Dis*. 2016;7(5):808-12.

125. Barkhash AV, Perelygin AA, Babenko VN, *et al.* Variability in the 2'-5'-oligoadenylate synthetase gene cluster is associated with human predisposition to tick-borne encephalitis virus -induced disease. *J Infect Dis.* 2010;15(202):1813-8.

126. Barkhash AV, Perelygin AA, Babenko VN, *et al.* Single nucleotide polymorphism in the promoter region of the CD209 gene is associated with human predisposition to severe forms of tick-borne encephalitis. *Antiviral Res.* 2012;93(1):64-8.

127. Barkhash AV, Yurchenko AA, Romaschenko AG, *et al.* A matrix metalloproteinase 9 (MMP9) gene single nucleotide polymorphism is associated with predisposition to tick-borne encephalitis virus - induced severe central nervous system disease. *Ticks Tick Borne Dis*. 2018;(9):763-7.

128. Rushton JO, Lecollinet S, Hubalek Z, *et al.* Tick-borne encephalitis virus in horses, Austria, 2011. *Emerg Infect Dis.* 2013;19(4):635-7.

129. Müller K, König M, Thiel HJ. (Tick-borne encephalitis (TBE) with special emphasis on infection in horses). *DTW Dtsch Tierarztl Wochenschr.* 2006;113(4):147-51.

130. Barrett PN, Schober-Bendixen S, Ehrlich HJ. History of TBE vaccines. *Vaccine*. 2003;21(SUPPL. 1):S41-9.

131. Harabacz I, Bock H, Jüngst C, *et al.* A randomized phase II study of a new tick-borne encephalitis vaccine using three different doses and two immunization regimens. *Vaccine*. 1992;10(3):145-50.

132. Girgsdies OE, Rosenkranz G. Tick-borne encephalitis: development of a paediatric vaccine. A controlled, randomized, double-blind and multicentre study. *Vaccine*. 1996;14(15):1421-8.

133. Zavadska D, Anca I, Andre F, *et al.* Recommendations for tick-borne encephalitis vaccination from the Central European Vaccination Awareness Group (CEVAG). *Hum Vaccines Immunother*. 2013;9(2):362-74.

134. Zavadska D, Odzelevica Z, Liepina L, *et al.* Tick-borne encephalitis: A 43-year summary of epidemiological and clinical data from Latvia (1973 to 2016). *PloS One*. 2018;13(11):1932-6203.

135. Demicheli V, Debalini MG, Rivetti A. Vaccines for preventing tick-borne encephalitis. *Cochrane Database Syst Rev*. 2009;1(59):1469-493X.

136. Kollaritsch H, Paulke-Korinek M, Holzmann H, *et al.* Vaccines and vaccination against tick-borne encephalitis. *Expert Rev Vaccines*. 2012;11(9):1103-19.

137. Xing Y, Schmitt HJ, Arguedas A, *et al.* Tick-borne encephalitis in China: A review of epidemiology and vaccines. *Vaccine*. 2017;1(35):1227-37.

138. Divé I, Veje M, Dobler G, *et al.* Tick-borne encephalitis virus (TBEV) infection in pregnancy: Absence of virus transmission to the fetuses despite severe maternal disease - A case study. *Ticks Tick-Borne Dis.* 2020;11(5).

139. Bjonholm E, Soderholm S, Stephansson O, *et al.* Tick-borne encephalitis in pregnant women: A mini narrative review. *New Microbes New Infect.* 2022;48:101017.

140. Kerlik J, Avdičová M, Musilová M, *et al.* Breast milk as route of tick-borne encephalitis virus transmission from mother to infant. *Emerg Infect Dis*. 2022;28(5):1060-1.

141. European Centre for Disease Prevention and Control. Tick-borne encephalitis. In: ECDC. Annual epidemiological report for 2020. European Centre for Disease Prevention and Control. Stockholm; 2022.

142. Randolph SE, Asokliene L, Avsic-Zupanc T, *et al.* Variable spikes in tick-borne encephalitis incidence in 2006 independent of variable tick abundance but related to weather. *Parasit Vectors*. 2008;1(1):1-18.

143. Gonzalez G, Bournez L, Moraes RA, *et al.* A One-Health Approach to Investigating an Outbreak of Alimentary Tick-Borne Encephalitis in a Non-endemic Area in France (Ain, Eastern France): A Longitudinal Serological Study in Livestock, Detection in Ticks, and the First Tick-Borne Encephalitis Virus. *Front Microbiol.* 2022;13.

144. Šumilo D, Bormane A, Vasilenko V, *et al.* Upsurge of tick-borne encephalitis in the Baltic States at the time of political transition, independent of changes in public health practices. *Clin Microbiol Infect.* 2009;15(1):75-80.

145. Godfrey ER, Randolph SE. Economic downturn results in tick -borne disease upsurge. *Parasit Vectors*. 2011;15(4):1756-3305.

146. Lalani T, Yun H, Tribble D, *et al.* A comparison of compliance rates with anti-vectorial protective measures during travel to regions with dengue or chikungunya activity, and regions endemic for Plasmodium falciparum malaria. *J Travel Med.* 2016;23(5).

147. Hasler T, Fehr J, Held U, *et al.* Use of repellents by travellers: A randomised, quantitative analysis of applied dosage and an evaluation of Knowledge, Attitudes and Practices (KAP). *Travel Med Infect Dis*. 2019;28:27-33.

148. Borșan SD, Trif SR, Mihalca AD. Recreational behaviour, risk perceptions, and protective practices against ticks: a cross-sectional comparative study before and during the lockdown enforced by the COVID-19 pandemic in Romania. *Parasit Vectors.* 2021;14(1):1-15.

149. Barp N, Di Nuzzo M, Contini C, *et al.* Clinical and laboratory findings in tick-borne encephalitis virus infection. *Parasite Epidemiol Control*. 2020;10(19):2405-6731.

150. Šmit R, Postma MJ. The burden of tick-borne encephalitis in disability-adjusted life years (DALYs) for Slovenia. *PLoS ONE*. 2015;10(12).

151. Fafangel M, Cassini A, Colzani E, *et al.* Estimating the annual burden of tick-borne encephalitis to inform vaccination policy, Slovenia, 2009 to 2013. *Euro Surveill*. 2017;22(16).

152. Europe. Commission Implementing Decision (EU) 2018/945 of 22 June 2018 on the communicable diseases and related special health issues to be covered by epidemiological surveillance as well as relevant case definitions. *Official Journal of the European Union* L 170, 6/7/2018.

153. Bogovic P, Strle F. Tick-borne encephalitis : A review of epidemiology, clinical characteristics, and management. *World J Clin Cases*. 2015;16(3):430-41.

154. Jaenson TGT, Jaenson DGE, Eisen L, *et al.* Changes in the geographical distribution and abundance of the tick Ixodes ricinus during the past 30 years in Sweden. *Parasit Vectors*. 2012;10(8):1756-3305.

155. Medlock JM, Hansford KM, Bormane A, *et al.* Driving forces for changes in geographical distribution of Ixodes ricinus ticks in Europe. *Parasit Vectors*. 2013;6(1) 1-11.

156. Alkishe AA, Peterson AT, Samy AM. Climate change influences on the potential geographic distribution of the disease vector tick Ixodes ricinus. *PLoS ONE*. 2017;12(12):1932-6203.

157. Rizzoli A. Changes expected in Ixodes ricinus temporal and spatial distribution in Europe. In: Nuttall P (Ed.). *Climate, ticks and diseases*. Wallingford, UK: CABI; 2021. p. 171-5.

158. Danielova V, Kliegrova S, Daniel M, *et al.* Influence of climate warming on tickborne encephalitis expansion to higher altitudes over the last decade (1997-2006) in the Highland Region (Czech Republic). *Cent Eur J Public Health*. 2008;16(1):4-11.

159. Suss J. Tick-borne encephalitis in Europe and beyond--the epidemiological situation as of 2007. *Euro Surveill.* 2008;26(13):1560-7917.

160. Lindgren E, Talleklint L, Polfeldt T. Impact of climatic change on the northern latitude limit and population density of the disease-transmitting European tick Ixodes ricinus. *Environ Health Perspect.* 2000;108(2):119-23.

161. Garcia-Vozmediano A, Krawczyk AI, Sprong H, *et al.* Ticks climb the mountains: ixodid tick infestation and infection by tick-borne pathogens in the Western Alps. *Ticks Tick-Borne Dis*. 2020;1(5):101489.

162. Daniel M, Danielova V, Kriz B, *et al.* Shift of the tick Ixodes ricinus and tick-borne encephalitis to higher altitudes in Central Europe. *Eur J Clin Microbiol Infect Dis.* 2003;22(5):327-8.

163. Krawczyk AI, Duijvendijk GLA van, Swart A, *et al.* Effect of rodent density on tick and tick-borne pathogen populations: consequences for infectious disease risk. *Parasit Vectors.* 2020;13(1):34.

164. Bregnard C, Rais O, Voordouw MJ. Climate and tree seed production predict the abundance of the European Lyme disease vector over a 15-year period. *Parasit Vectors.* 2020;13(1):1-12.

165. Bregnard C, Rais O, Voordouw MJ. Masting by beech trees predicts the risk of Lyme disease. *Parasit Vectors.* 2021;14(1):1-22.

166. Haemig PD, Sjöstedt De Luna S, Grafström A, *et al.* Forecasting risk of tick-borne encephalitis (TBE): Using data from wildlife and climate to predict next year's number of human victims. *Scand J Infect Dis.* 2011;43(5):366-72.

167. Palo RT. Tick-borne encephalitis transmission risk: Its dependence on host population dynamics and climate effects. *Vector-Borne Zoonotic Dis*. 2014;14(5):346-52.

168. Nah K, Bede-Fazekas Á, Trájer AJ, *et al.* The potential impact of climate change on the transmission risk of tick-borne encephalitis in Hungary. *BMC Infect Dis*. 2020;20(1).

169. Hönig V, Švec P, Marek L, *et al.* Model of risk of exposure to lyme borreliosis and tick-borne encephalitis virus-infected ticks in the border area of the Czech republic (South Bohemia) and Germany (lower bavaria and upper palatinate). *Int J Environ Res Public Health.* 2019;16(7).

170. Brabec M, Daniel M, Malý M, *et al.* Analysis of meteorological effects on the incidence of tick-borne encephalitis in the Czech Republic over a thirty-year period. *Virol Res Rev*. 2017;1(1):1-8.

171. Danielova V, Daniel M. Climate, ticks and tick-borne encephalitis in Central Europe. In: Nuttall P (Ed.). *Climate, ticks and diseases*. Wallingford, UK: CABI; 2021. p. 331-40.

172. Zeman P. Predictability of tick-borne encephalitis fluctuations. *Epidemiol Infect.* 2017;145:2781-6.

TULARAEMIA

Maria Bellenghi (a), Claudia Cataldo (a), Alessandra Ciervo (b), Francesca Dagostin (c), Giovanni Marini (c), Annapaola Rizzoli (c), Valentina Tagliapietra (c), Luca Busani (a)
(a) Centro di Riferimento per la Medicina di Genere, Istituto Superiore di Sanità, Rome
(b) Dipartimento Malattie Infettive, Istituto Superiore di Sanità, Rome
(c) Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige, Trento

Biological, ecological and molecular features of the causative agent

Disease name

Tularaemia.

Disease agent

Common, scientific and Latin name

Francisella tularensis is the causative microbial agent of tularaemia, a rare zoonotic disease that affects many animal species and humans. *F. tularensis subsp. tularensis* is extremely virulent, whereas *F. philomiragia* and *F. tularensis subsp. novicida* are opportunistic pathogens, causing disease in immunocompromised patients.

Taxonomy

The taxonomic tree of *F. tularensis* is:
- Domain: *Bacteria*
- Phylum: *Proteobacteria*
- Class: *Alphaproteobacteria*
- Order: *Thiotrichales*
- Family: *Francisellaceae*
- Genus: *Francisella*

F. tularensis includes three subspecies of significant clinical relevance for humans i.e., subspecies *tularensis* (type A), subspecies *holarctica* (type B), and subspecies *mediasiatica*. The genus *Francisella* also involves opportunistic pathogens that can cause disease in immunocompromised patients (i.e., *F. novicida*, *F. hispaniensis*, and *F. philomiragia*). In addition, the genus *Francisella* includes *Francisella*-like endosymbionts (FLE) found in ticks and ciliates (i.e., *F. persica, F. endociliophora*, and *F. adeliensis*).

Disease agent characteristics

F. tularensis is a small (0.7-1.7 μm), Gram-negative coccobacillus, strict aerobes, non-motile, non-spore-forming, heterotrophic, oxidase negative and requires cysteine for growing. This microorganism is ease to spread by aerosol and due to its low infectious dose and its virulence, is included in the Centers for Disease Control and prevention (CDC) category A of the bioterrorism agent (https://emergency.cdc.gov/agent/agentlist-category.asp).

Physiochemical properties

Francisella is associated with different clinical and environmental sources, in particular water environment. Experimental studies suggest a long-term survival from 1 to 70 days in various water environments. *F. tularensis* survival seems to be influenced by both water temperature and salinity. In water milieu *F. tularensis subsp. holarctica* remains cultivable up to 28 days at 8°C. Both type A and type B strains remain cultivable up to 8-10 days in fresh-water, or 30-42 days in brackish-water at 21°C. *F. tularensis* can be metabolically active in water but not cultivable *in vitro* on agar plates. This microbial ability was observed for up to 140 days in water. This condition is defined as 'Viable But Non-Culturable' (VBNC) and could be responsible for long-term survival of bacteria in the water environment. The VBNC state has been defined as a state from which bacterial cells cannot be cultured but maintain a metabolic activity and cellular integrity. In addition, the VBNC state may be reversible, as bacteria may become cultivable under favorable conditions. This reversion in the ability to grow on agar plates is called 'resuscitation' of VBNC bacteria. *F. tularensis subsp. holarctica* possesses a mechanosensitive channel that protects this bacterium from hypoosmotic shock when it is released from an infected animal to water. *In vitro* studies have demonstrated that both *F. tularensis subsp. holarctica* and *F. tularensis subsp. tularensis* can form biofilms which is compatible with its natural aquatic reservoir (1-7). Multiple experimental studies have focused on the interaction between *Francisella* species and several amoebae particular in *Acanthamoeba castellanii*. *Francisella* was localized within vacuoles in amoeba trophozoites but were also able to survive in amoebal cysts for several weeks. This latter finding suggested that amoeba could be a long-term reservoir of *Francisella* spp. in water environments. Mosquito larvae may also represent a long-term *F. tularensis* reservoir in the aquatic environment. It has been shown that these larvae present in water can ingest *Francisellae* and ingested bacteria can survive throughout the different maturation stages of these arthropods up to adult mosquitoes (8-9).

Priority level for EU

Tularaemia is a communicable disease subjected to the Commission Implementing Decision (EU) 2018/945. Since 2008, data on human cases have been collected by the European Surveillance System (TESSy) and maintained by European Centre for Disease Control and Prevention (ECDC). In Europe, the surveillance of tularaemia is mandatory and surveillance systems are comprehensive with full national coverage in all EU Member States. In 2021, a total of 876 confirmed cases of tularaemia were reported among 26 Member States (10). In general Sweden is the country that reports the highest number of cases, followed by Finland. Many European countries have reported sporadic cases, imported cases or outbreaks in the past 30 years. The disease has been identified in new areas, with an increasing tendency to emerge and/or re-emerge locally in Europe. The range of known hosts has also expanded to include animal not previously linked to *Francisella*, such as red fox (*Vulpes vulpes*), wild boar (*Sus scrofa*), and raccoon dog (*Nyctereutes procyonoides*). However, surveillance systems in the European countries are not the same, consequently data should be interpreted with caution.

Distribution of the pathogen

Tularaemia is widely distributed in humans, wildlife and arthropod vectors, it is endemic throughout most of European countries, Northern and Central Asia, and North America. The maps

in Figures 1 and 2 represent the distribution of reported cases of *F. tularensis* from human, animal or arthropod vectors. Compared to previously known maps, the pathogen has been found in some new countries in Africa and Asia.

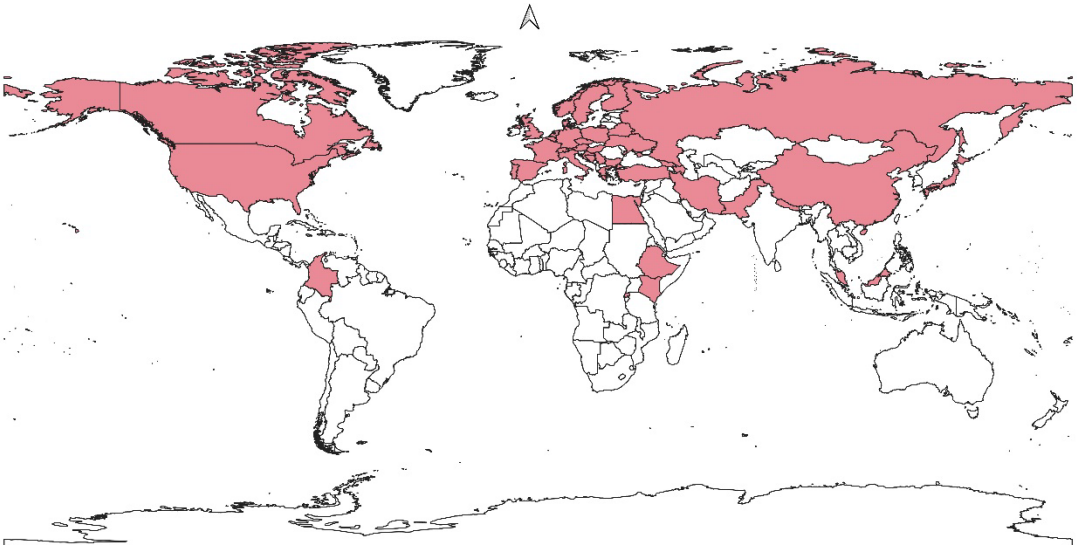

Figure 1. Global distribution of *F. tularensis* detected in human, animal and the environment (from 2000 to 2020)

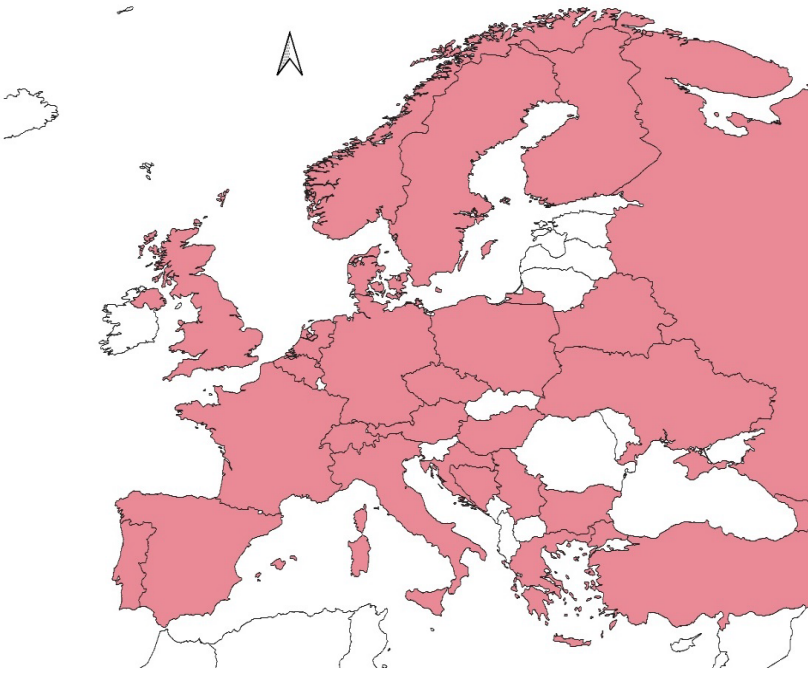

Figure 2. European distribution of *F. tularensis* detected in human, animal and the environment (from 2000 to 2020)

Ecology and transmission routes

The distribution and maintenance of *F. tularensis* may include a system of small natural foci associated to a physical (climate, geology) and biological (flora, fauna) element and to a different mode of transmission.

In this scenario, the pathogen is maintained and can be diffused for long time. The pathogen distribution and its ability to persist in many different ecological niches is related to the high adaptability to different animal reservoirs, vectors and environmental conditions.

In nature, *F. tularensis* is characterized in a terrestrial and aquatic lifecycle. The terrestrial stage involves different animal reservoir, while the aquatic cycle includes mainly mosquitoes, mosquito larvae, and aquatic rodents. In particular, environmental conditions, the aquatic lifecycle may be prevalent over the terrestrial lifecycle, amplifying the persistence, the spreading and the maintenance of the microbial agent (11).

F. tularensis has been detected in a high number of wild species including lagomorphs, rodents, insectivores, carnivores, ungulates, marsupials, birds, amphibians, fish, and invertebrates.

Vectors

F. tularensis natural infections have been documented in different arthropods, although only a subset of these have been identified as important in *F. tularensis* transmission to humans. Arthropods found infected in nature include ticks of the genera *Amblyomma*, *Dermacentor*, *Ixodes* and *Ornithodoros*, mosquitoes of the genera *Aedes*, *Culex*, *Anopheles* and *Ochlerotatus excrucians*, and flies from the *Tabanidae* family (*Tabanus* spp., *Chrisozona* spp. and *Chrisops* spp.). Ticks appear to play an important key role in the ecology of tularaemia among arthropods, as they may carry pathogens over several years and life stages, thus maintaining enzootic tularaemia foci between epizootic periods. The genus *Dermacentor reticulatus* seems to be the most frequent carrier of *F. tularensis* in Central Europe. In Sweden, mosquito-borne infections, which are related to the aquatic life cycle, are a common route for transmission of tularaemia in humans and can lead to major outbreaks. Studies have also provided evidence that mosquito larvae may be infected with *F. tularensis* via water, possibly by ingesting predatory protozoa. In Table 1 is reported the list of arthropod vectors for this pathogen. Specific bibliography for vectors (8, 9, 12-27, 29-37).

Reservoirs

The ecology of tularaemia is not sufficiently clarified because of the different potential host species involved, and may depend on geographical factors, as well as on the susceptibility and sensitivity of respective organisms to the pathogen. Recent observations provide evidence that most of the animals do not serve as amplifying hosts, but are “incidental” dead-end hosts, and therefore may not play a role in the persistence and spread of the pathogen.

The European hare (*Lepus europaeus*), also known as the brown or field hare, is considered an important host of *F. tularensis* and a common vector for the pathogen’s transmission to humans in Europe. In case of chronic infection, European hares may act as long-term reservoir for *F. tularensis*, persistent risk of transmission to humans, either directly or via vectors. Experimental studies showed that infected voles may also show a protracted course of disease representing a prolonged source of environmental contamination. Specific bibliography for reservoirs (1, 14, 16, 21, 22, 34, 35, 38-45).

Table 1. List of arthropod vectors (%) for *F. tularensis* data analysis from literature

Vector	% positive	Method	Tested
Aedes cinereus	4.2-42.9	Pools	<50
Aedes intrudens	0.0	Pools	<50
Aedes punctor	20.0	Pools	<50
Aedes spp.	20.0	Pools	<50
Aedes sticticus	50.0	Pools	<50
Aedes vexans	50.0	Pools	<50
Amblyomma americanum	0.0	Pools	>300
Amblyomma maculatum	0.0	Pools	<50
Amblyomma spp.	0.0	Pools	<50
Culex pipiens/torrentium	0-100.0	Pools	<50
Culiseta alaskaensis	0.0	Pools	<50
Culiseta annulata	0.0	Pools	<50
Dermacentor marginatus	0-33.3	Pools	<50
Dermacentor reticulatus	0-0.3	Pools	<50
Dermacentor reticulatus	0.7-0.9	EXP INF	>300
Dermacentor variabilis	0.0	Pools	50-300
Haemaphysalis concinna	0.3	Pools	>300
Haemaphysalis inermis	0.0	Pools	50-300
Haemaphysalis parva	0.0	Pools	<50
Haemaphysalis punctata	0.0	Pools	<50
Haemaphysalis sulcata	0.0	Pools	<50
Hyalomma aegyptium	0.0	Pools	<50
Hyalomma excavatum	0.0	Pools	<50
Hyalomma marginatum	0.0	Pools	50-300
Hyalomma marginatum marginatum	0.0	Pools	<50
Hyalomma marginatum rufipes	0.0	Pools	50-300
Hyalomma spp.	0.0	Pools	<50
Ixodes ricinus	0-2.2	Pools	50-300
Ixodes scapularis	0.0	Pools	<50
Ixodes spp.	0.0	Pools	<50
Rhipicephalus bursa	0.0	Pools	<50
Rhipicephalus turanicus	0.0	Pools	<50

Pools: results from the testing of pools of arthropods; EXP INF: experimental infection

Drivers of disease emergence and spread

Ecological drivers

Water

Long-term survival of *F. tularensis* in various water environments has been confirmed by several studies. Consumption or contact with surface water from natural springs, ponds or rivulets, or from wells have been identified as significant risk factors for tularaemia infection. Water salinity and levels of macro- and micronutrients are considered important factors that can contribute to the survival of *F. tularensis* in water environments. Experimental studies demonstrated that survival was enhanced in brackish water compared to freshwater, probably because higher concentration of sulphur residues and salinity, that are usually required for the cultivation of *F. tularensis*. Because brackish-water and normal saline support the culturability of

F. tularensis compared to what observed in freshwater, salinity may be an important factor for stability; however, it is likely that the presence of sulphur would enhance the effects of salinity.

Presence of animals

Indirect evidence of the role of animals was the association between mouse and hare faeces and risk of infection during two outbreaks in Kosovo. During the outbreaks, faeces of small rodents were regularly found by the investigation teams in products stored in food stores linked to affected households. It is of interest that presence or proximity of domestic animals is protective towards the risk of *F. tularensis* detection (5, 23).

In Table 2, 3 and 4 the main *F. tularensis* vector environmental and animal drivers are reported.

Table 2. List of *F. tularensis* vector covariates identified from the selected studies in decreasing order according to the number references

Vector drivers	N. of papers# (n. 45)	% of impact* (n. 51)
Tick	40	78.4%
Mosquito	8	15.7%
Other	3	5.9%

number of papers extracted, the number of reference per covariates is higher because from one paper more covariates could have been extracted *% calculated on the total number of references.

Table 3. List of *F. tularensis* environmental covariates identified from the selected studies in decreasing order according to the number references

Environmental drivers	N. of papers# (n. 31)	% of impact* (n. 36)
Water (brackish/saline or fresh water)	16	44.44%
Land use (distance from animal market or main road; percentage grassland; sediment)	7	19.44%
Location (city or rural areas)	4	11.11%
Time	2	5.5%
Altitude	2	5.5%
Air	1	2.77%
Chemical characteristic	1	2.77%
Humidity	1	2.77%
Precipitation (total rainfall)	1	2.77%
Soil	1	2.77%

number of papers extracted, the number of reference per covariates is higher because from one paper more covariates could have been extracted
* % calculated on the total number of references.

Table 4. List of *F. tularensis* animal covariates identified from the selected studies in decreasing order according to the number references

Animal drivers	N. of papers# (n. 69)	% of impact* (n. 115)
Rodent	28	24.3%
Hare	19	16.3%
Canidae	10	8.7%
Mustelidae	6	5.2%
Swine	6	5.2%
Deer	5	4.35%
Host	5	4.35%
Ursidae	5	4.35%
Bird	4	3.48%

Animal drivers	N. of papers# (n. 69)	% of impact* (n. 115)
Bovidae	4	3.48%
Rabbit	4	3.48%
Felid	3	2.6%
Sheep	3	2.6%
Bat	2	1.74%
Mammals	2	1.74%
Monkeys	2	1.74%
Anatidae	1	0.87%
Badger	1	0.87%
Camelidae	1	0.87%
Lynx	1	0.87%
Opossum	1	0.87%
Skunk	1	0.87%
Viverrid	1	0.87%

number of papers extracted, the number of reference per covariates is higher because from one paper more covariates could have been extracted *% calculated on the total number of references.

Land cover

Land cover is an important risk factor for arthropod vectors of *F. tularensis* as far as it can ensure the suitability is to live in newly urbanized/suburban areas, residences surrounded by areas dominated by grassland vegetation. A serosurvey in Rural Azerbaijan identified possible associations between village-level tularaemia seroprevalence and suitable tick habitats, annual rainfall, precipitation in the driest quarter, and altitude (25, 46, 47).

Economical and socio-demographic drivers

Any difference was observed in the risk for females and males, but there is a gender issue related to the risk of professional exposure. Some activities, like hunting, wood cutting or other outdoor activities are associated to higher risk of infection. On the contrary, contact with farming animals or agriculture practices are considered at lower risk. The infection is more frequent in adults and young adults over 14 years of age, but in case of waterborne outbreaks, age ranged from 2 to 81 years can be included. The disease is more common in people residing in rural area compared to those who live in cities, and among risk factor associated to the infection, arthropod bites, drinking water from springs, handling dead animals, or having rodents in the house and surroundings are the most frequently reported. Specific bibliography for economical and socio-demographic drivers (5, 32, 47-56).

Natural history of disease in humans and animals, including symptoms, morbidity and mortality

Brief history of the pathogen and disease

F. tularensis has been recognized as a human pathogen since the beginning of the 20th century. Reports of diseases strongly resembling to tularaemia disease preceded to first confirmed report in 1911. For example, there were cases reported from Utah in 1908, from Norway of a disease called lemming fever during the 1890s, and of Yato-Byo (hare disease) from Japan as early as 1818. There is also a description from Norway from 1653 of a tularaemia-like disease of

lemmings. The first human cases were reported in 1914 in Ohio. As suggested by Edward Francis, the etiological agent became known as *Bacterium tularense,* named after Tulare County where the disease was endemic among rodents. A similar disease was reported under different names from various regions of the United States during the next decade. Francis published a report in 1919 entitled "Deer-fly fever – a disease of man of hitherto unknown etiology." The same author wrote a comprehensive review in 1921, in which he suggested that the disease was transmitted to man from rodents by bites of blood-sucking insects. Moreover, he proposed the disease be named tularaemia. In the commemoration of the pioneering work of Francis, the bacterium eventually received its present-day name, *F. tularensis*. For many years, *F. tularensis* has been considered as a potential biological weapon and, in the 1940s and 1950s, it was one of the agents that was given the highest priority in the offensive programs of the United States and the Soviet Union, and it was stockpiled by the U.S. military even in the late 1960s. The high costs and high morbidity justify the inclusion of *F. tularensis* as an agent of the highest priority together with, for example, anthrax, smallpox, and pandemic influenza. Accordingly, it is one of six agents designated as a category A Select Agent by CDC. These traits are also reflected by the fact that *F. tularensis* is highly contagious for laboratory workers (57-59).

Disease in humans

F. tularensis can be transmitted to humans via different routes, such as direct contact with infected animals (e.g., during animal processing, through ingestion of uncooked meat, and animal bites), arthropods (ticks, horseflies, and mosquitos) and through the consumption of contaminated water or inhalation of contaminated soil, occurring in farming works. The human to human contagious was not reported until now, and the tularaemia disease is transmitted to humans by direct contact with infected animals, tissues or fluids from infectious animals, by ingestion of contaminated water and/or food, or by inhalation of infective aerosols. Early diagnosis and treatment may be difficult because of the aspecific clinical manifestations and its similarity to a wide variety of acute infectious diseases. The acute phase of the disease is characterized by rapidly bacteria proliferation; the severity of the disease depends on the patient's immunological response. This response depends on cell-mediated immunity rather than antibodies, which are good indicators of exposure to the bacterium but do not play a crucial role in protection. The mean incubation period of tularaemia is 3-5 days, but may range from 1-21 days. Onset of tularaemia is sudden, occurring 1 to 10 (usually 2 to 4) days after exposure, with headache, chills, nausea, vomiting, fever of 39.5 to 40° C, and severe prostration. Extreme weakness, recurring chills, and drenching sweats develop. Illness ranges from mild to life-threatening.

Main forms of this disease are listed below:

– *Ulceroglandular*
  This is the most common form of tularaemia and usually occurs following a tick or deer fly bite or after handing of an infected animal. A skin ulcer, accompanied by swelling of regional lymph nodes, appears at the site where the bacteria entered the body.
– *Glandular*
  It is similar to ulceroglandular tularaemia but without the presence of ulcer. Similarly, is acquired through the bite of an infected tick or deer fly or from handling sick or dead animals.
– *Oculoglandular*
  This form occurs when the bacteria enter through the eye. This can occur when a person is butchering an infected animal and touches his or her eyes. Symptoms include irritation and inflammation of the eye and swelling of lymph glands.

- *Oropharyngeal*
  This form results from eating or drinking contaminated food or water. Patients with orophyangeal tularaemia may have sore throat, mouth ulcers, tonsillitis, and swelling of lymph glands in the neck.
- *Typhoidal*
  This form is characterized by general symptoms without the localizing ones of other syndromes.
- *Pulmonary*
  This is the most severe or aerosolization of contaminated milieu form of tularaemia, occurring after inhalation. Symptoms include cough, chest pain, and difficulty breathing and occasional rales may be the only symptom in pneumonia. A dry, nonproductive cough is associated with a retrosternal burning sensation. In untreated cases, temperature remains elevated for 3 to 4 weeks and resolves gradually. Mediastinitis, lung abscess, and meningitis are rare complications.

Mortality is generally related to untreated cases and about 6% in untreated cases of ulceroglandular tularaemia; it is higher for type A infection and for typhoidal, septicemic, and pulmonary form. Death usually results from overwhelming infection, pneumonia, meningitis, or peritonitis. Relapses can occur in inadequately treated cases. One infection confers immunity.

Disease in animals

Signs and symptoms of tularaemia in wild animals are not well documented and are mostly based on post-mortem examinations. The clinical presentation of tularaemia depends on the host species, subspecies of the bacteria, and route of infection. The incubation period is 1–10 days. Type A tularaemia is particularly pathogenic for lagomorphs, with fatal infections also reported in cats and nonhuman primates.

The most common finding upon necropsy is an enlarged spleen and pinpoint white necrotic lesions in the spleen and liver. The best-documented clinical cases are in domestic cats and dogs, captive monkeys, prairie dogs and laboratory animals. Sheep and cats may be subclinically infected or develop bacteraemia, fever, and respiratory infection. Cats may also develop ulceroglandular or oropharyngeal disease, presumably through exposure to infected prey items. Outbreaks in untreated lambs may have up to 15% mortality.

Clinical signs include:

- increased pulse and respiratory rates;
- coughing;
- diarrhea;
- oral ulceration;
- pollakiuria with lymphadenopathy and hepatosplenomegaly.

In particular, the European hare (*Lepus europaeus*), also known as the brown or field hare, is characterized by variable clinical courses ranging from acute deadly septicaemia to protracted courses with only subacute lesions in various organs. In case of chronic infection, histopathological examinations demonstrated differences in the pathogenicity of clade B.FTNF002-00 (subgroup of B.6 and specific for Western Europe) and clade B.13 (subgroup of B.12 and specific for Central and Eastern Europe): while infections with strains of clade B.13 were reported to be associated with polyserositis, affecting the kidneys, pleura, and pericardium, histopathological findings in hares infected with B.FTNF002-00 have been almost invariably characterized by splenitis and hepatitis. These results are in accordance with further observations

in experimentally infected rats, showing significant differences in weight loss, mortality rate, and time to recovery between the two genotypes.

Rodents are very susceptible to *F. tularensis*, and commonly present with severe infection, leading to early death. Nevertheless, experimental studies showed that infected voles may also show a protracted course of disease with chronic nephritis and bacteriuria and could therefore also serve as a prolonged source of environmental contamination.

Availability of preventive, therapeutic and control measures, including licensed or pipelines vaccines

Therapy in humans

Three situations are to be considered:

1. Accidental exposure: antibiotic treatment should be initiated within 24 h and a treatment period of 14 days is recommended with either ciprofloxacin and doxycycline;
2. Exposure most likely did not occur: an increased vigilance may be sufficient, including daily measurement of body temperature for 14 days and a readiness to treat if symptoms appear;
3. Incidental spread of *F. tularensis* by aerosol: potentially exposed persons should be instructed to be alert to the development of fever within 14 days of exposure, and treatment initiated if necessary.

The preferred drugs are Gentamicin (for moderate to severe disease), Doxycycline (for mild disease), Chloramphenicol (used only for meningitis because there are more effective and safer alternatives), and Ciprofloxacin (for mild disease). Continuous wet saline dressings are beneficial for primary skin lesions and may diminish the severity of lymphangitis and lymphadenitis. Surgical drainage of large abscesses is rarely necessary unless therapy is delayed. Intense headache usually responds to oral analgesics. No natural resistance in *F. tularensis* to antibiotics used for clinical therapy has been demonstrated. This is true for aminoglycosides, tetracyclines, chloramphenicol, and quinolones. Due to the potential use of *F. tularensis* for biological weapon, antibiotic resistance remains of concern. Consequently, methods for rapid determination of the susceptibility to various antibiotics, including aminoglycosides, tetracyclines, chloramphenicol, quinolones, and rifampicin are needed.

Therapy in animals

Early treatment is important to minimize risk of fatality. Streptomycin, gentamicin, and tetracyclines are effective at recommended dosage levels. Gentamicin should be continued for 10 days. Because tetracycline and chloramphenicol are bacteriostatic, they should be continued for 14 days to minimize the risk of relapse. Because of the substantial sylvatic (wildlife and tick) component of the *Francisella* life cycle, control involves reducing arthropod infestations and limiting exposure to wildlife, for example by keeping cats and dogs indoors. In some jurisdictions, tularaemia in animals is reportable to public health authorities.

Licensed or pipelined vaccines

There is currently no effective and safe vaccine available against *F. tularensis*.

Other prevention measures

Prevention measures consist in avoiding ingestion, breathing and inoculation of the bacteria. This includes: avoiding drinking untreated surface water; using insect repellent and clothes covering legs and arms to avoid tick and mosquito bites; avoiding contact with dead animals, using gloves when handling wild animals especially skinning of diseased hares, wild rabbits and rodents; not mowing over sick or dead animals, cooking thoroughly game meat before eating; handling biological samples potentially contaminated with *F. tularensis* in biosafety level-3 (BSL-3) laboratories.

Disease specific recommendations

No specific recommendations are reported for tularaemia.

Epidemiological situation at different spatial scales: past and current trends

Tularaemia usually causes epizootics (local amplification with clusters of human cases), but in some areas there is an endemic trend, with regular occurrence of human infections. Sweden has consistent annual numbers of mosquito-borne infections, and Sweden, Finland and Norway reported about 66 percent of the entire number of cases notified in Europe between 2019 and 2020 (Figure 3).

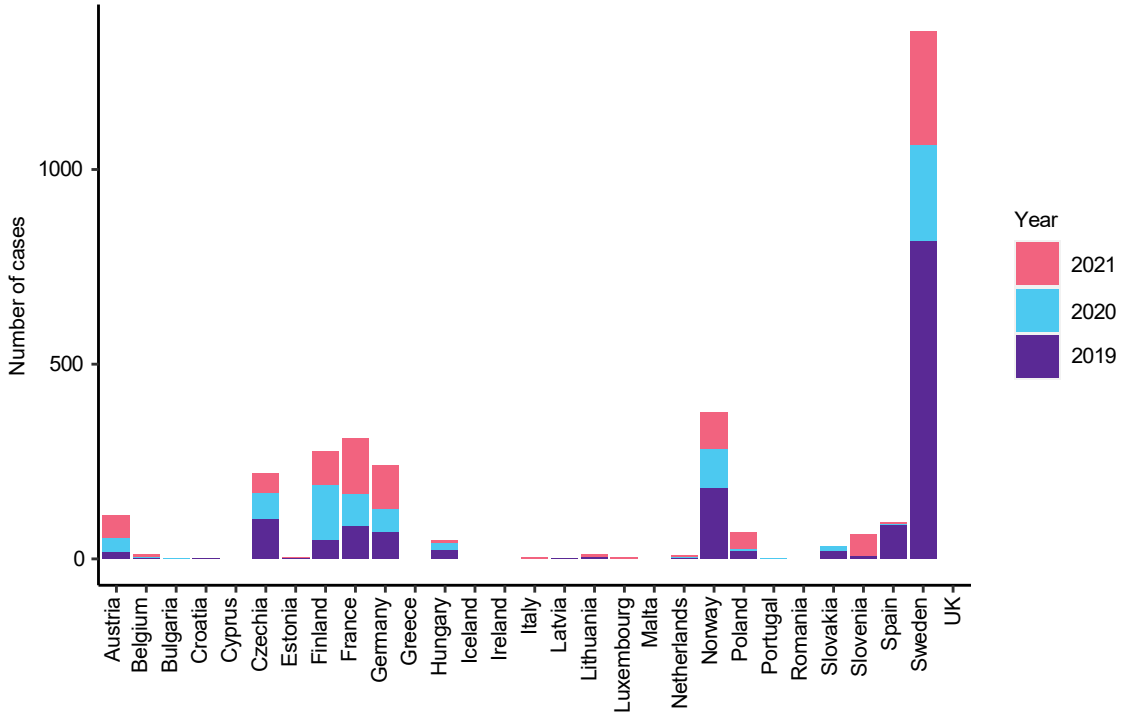

Figure 3. Number of reported cases of tularaemia reported in 29 countries of the European Union and European Economic Area, 2019-2021 (data from http://atlas.ecdc.europa.eu/public/index.aspx)

On the other hand, in Europe there are countries that did not notified cases in the last time period (UK, Ireland, and Iceland). Other European countries that consistently notified human cases were France, Hungary, the Czech Republic, and Germany, almost all with an increasing trend, probably due in part to better surveillance and increased public awareness. Outbreaks in humans are often sporadic and likely to be spatially and temporally variable, in addition to specific sites with potential for clusters of infection. Based on the reporting of human cases the major hotspots for tularaemia at the European level are located in Scandinavia and Central Europe. Data reporting in the period 2012-2020 (Figure 4) has been quite consistent through the years, and considering all the EU/EEA (European Union and European Economic Area) countries together, a slight increasing trend can be observed. Trends were more apparent in individual countries: Norway, France, Czech Republic and Germany showed a trend towards an increasing number of cases, while a trend of decreasing case numbers was apparent in Finland, Hungary and Slovakia, and no trends appeared in data from Sweden.

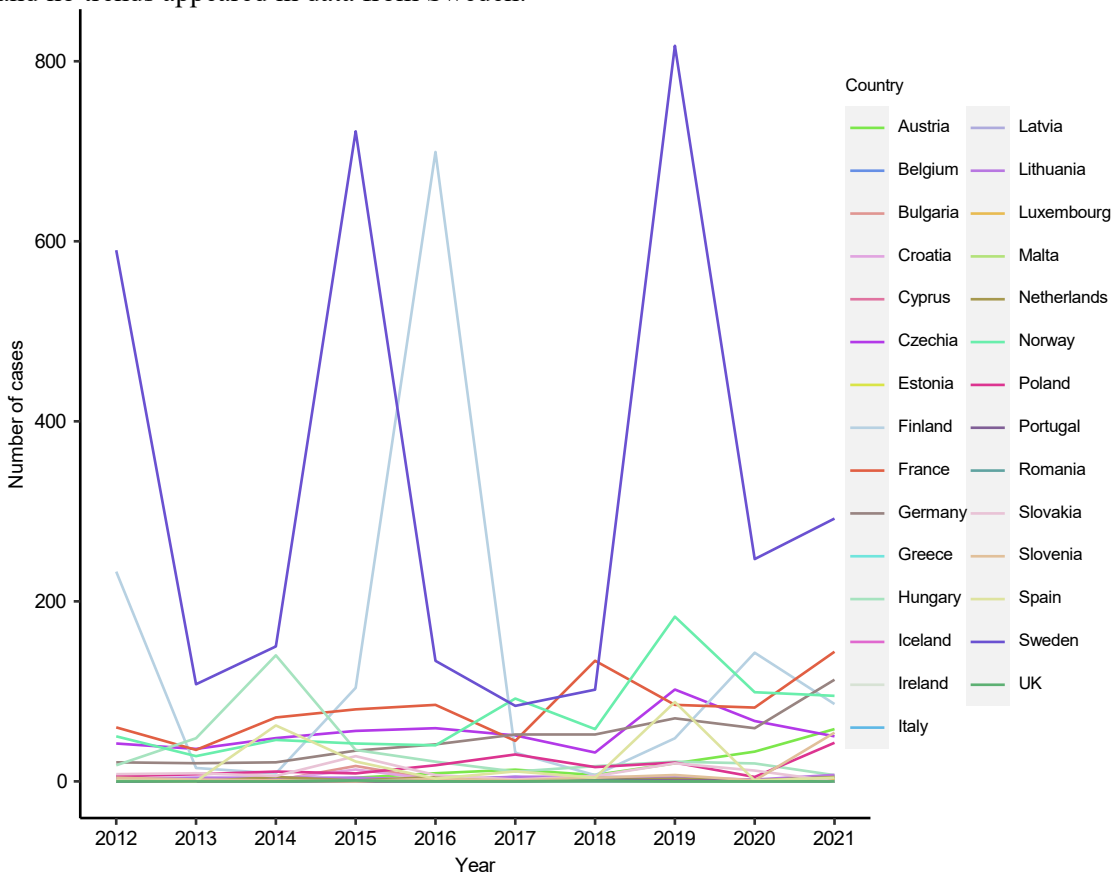

Figure 4. Reported cases of tularaemia reported in 29 countries of the European Union and European Economic Area, 2012-2021 (data from http://atlas.ecdc.europa.eu/public/index.aspx)

Animal cases of tularaemia are voluntarily notified to the World Organization for Animal Health (WOAH), and in the period 2012-2020, 13 EU/EEA countries notified 1475 wild and domestic animal cases to the World Animal Health Information System (WAHIS). Germany notified more than 50 percent of cases and, together with Spain and Finland, accounted for 84 percent of total cases during the period (Figure 5).

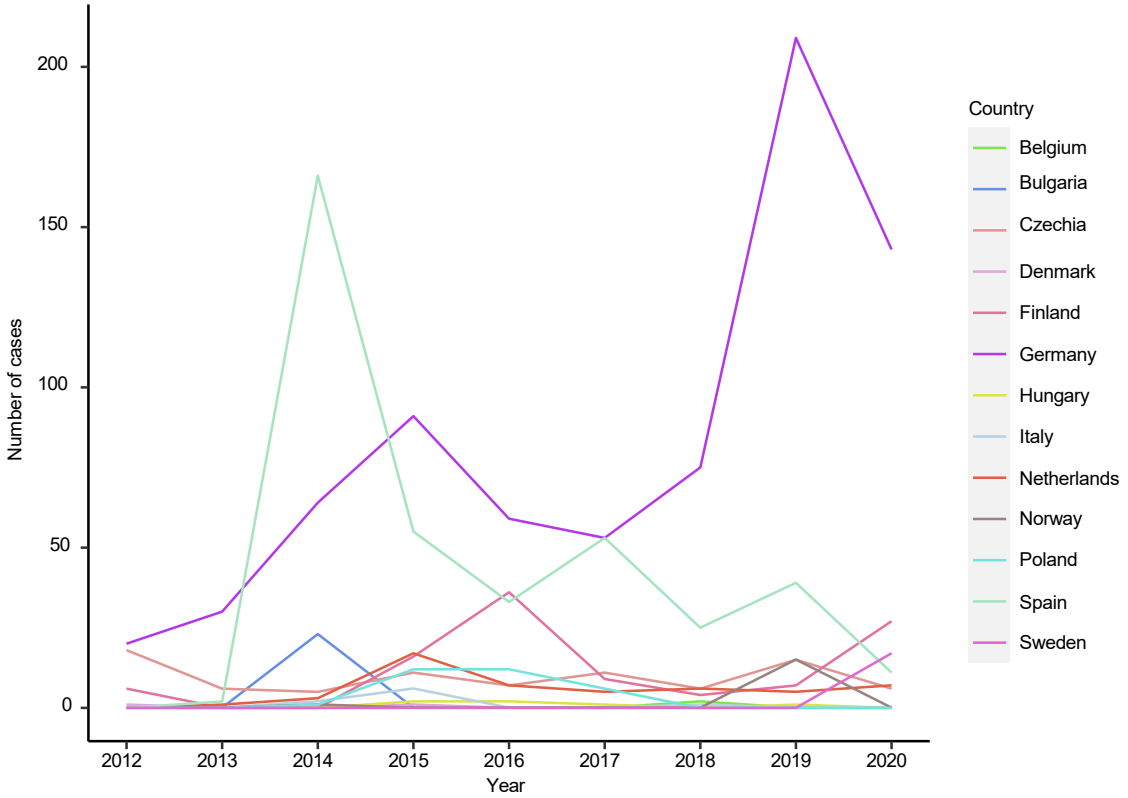

Figure 5. Number of cases of tularaemia reported in animals in 13 countries of the European Union and European Economic Area, 2012–2020 (data from WOAH-WAHIS)

Among the infected animal species, brown hare accounted for 64 percent and bank vole 10 percent. The other 26 species contributed less than 5% each. Most cases occurred in wildlife, but domestic animals, particularly dogs (4%), sheep (3%) and cattle (1%) also tested positive.

Sociological and demographic dimension affecting susceptibility and exposure, including gender

Although the age-related incidence rate of tularaemia is unknown, the disease is known to occur at all ages. Males have a higher incidence in all age categories. In Sweden, an over representation among males has been attributed to their more frequent outdoor professional and leisure activities (60). In most countries where tularaemia is endemic, the disease is seasonal; its incidence seems to be highest during late spring, the summer months and early autumn (61-63). Often, the number of cases shows wide variations from one year to another and this is probably related to climatic factors such as temperature and precipitation.

However, there are virtually no data linking specific climatic conditions and outbreaks of tularaemia. This is an important area for future research that may yield important tools for predicting and possibly preventing outbreaks. Table 5 reports the most *F. tularensis* human covariates which impact on disease spreading, while in Figure 6 and 7 the gender and age distribution is shown.

Table 5. List of *F.tularensis* human covariates identified from the selected studies in decreasing order according to the number references.

Human drivers	n. of papers# (n. 23)	% of impact*(n. 77)
Infection (rate of infections)	40	51.9%
Age (median age)	15	19.5%
Sex (male or female)	11	11%
Profession (healthcare workers, hunters, butchers, rangers, woodcutters)	7	9.1%
Outcomes (hospitalization rate or duration; fatality rate)	2	2.6%
Activity (schooling)	1	1.3%
Human density (n. of households)	1	1.3%

number of papers extracted, the number of reference per covariates is higher because from one paper more covariates could have been extracted *% calculated on the total number of references

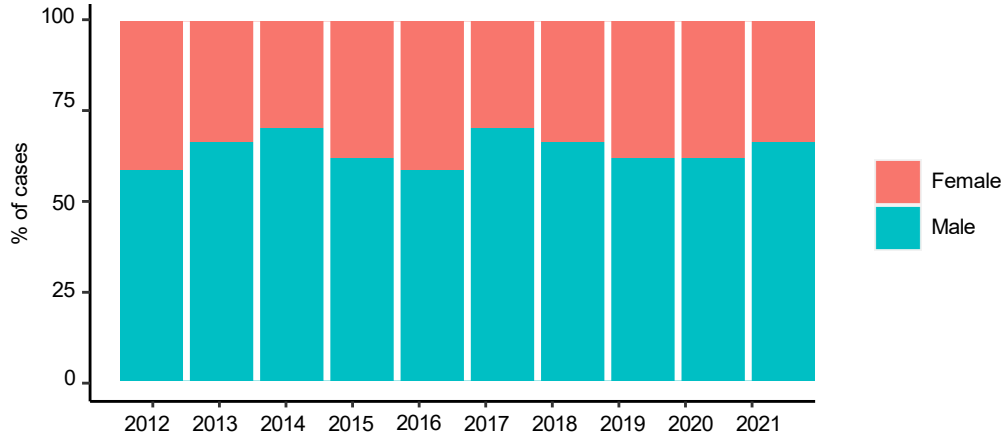

Figure 6. Distribution of *F. tularensis* % of cases by gender (data from TESSy-ECDC)

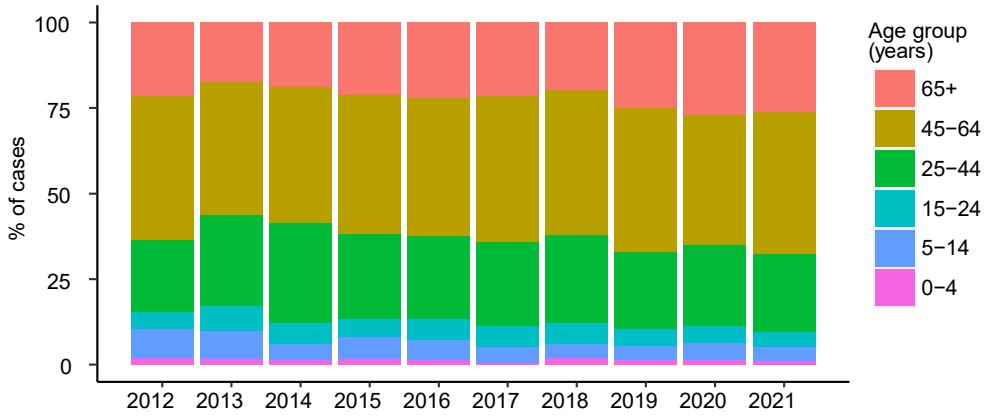

Figure 7. Distribution of *F. tularensis* % of cases by age (data from TESSy-ECDC)

Diagnostic procedures and notification systems used at local, national and European scale

Diagnosis

Human: diagnosis of suspected tularaemia is based on a history of contact with rabbits, hares, rodents or exposure to arthropod vectors, the sudden onset of symptoms, and the characteristic primary lesion. Because this organism is highly infectious, samples and culture media from patients suspected of having tularaemia should be handled with extreme caution and, if possible, processed by a high-level biosafety containment-equipped laboratory with a level 3 rating.

Tularaemia can be difficult to diagnose. It is a rare disease, and the symptoms are aspecific and common to other illnesses. For this reason, it is important to share an epidemiology report and any likely exposures, such as tick and deer fly bites, or contact with sick or dead animals. The case definitions that describe the criteria for diagnosis of tularaemia are: suspect, presumptive and confirmed. The criteria for each are:

– *Suspect*: an exposure history consistent with risks known to be associated with tularaemia together with clinical symptoms compatible with tularaemia.

– *Presumptive*: suggestive clinical symptoms and a clinical sample that tests positive for tularaemia by antigen or DNA detection. A single positive serum is also considered presumptive.

– *Confirmed*: isolation and identification of *F. tularensis* from biological specimen, specific antigen or DNA detection.

The choice of specimen for diagnostic testing is dependent on the form of clinical illness; ulceroglandular, glandular, oculoglandular, oropharyngeal, respiratory, or typhoidal. The following specimens are acceptable for the various forms of illness as specified:

– *Blood*: Whole blood (for all clinical forms of illness);
  – *Serum*: A first specimen should be collected in early course of infection, followed by a second specimen taken in the convalescent period (at least 14 days later and preferably
  3-4 weeks after onset of symptoms). Serum is acceptable for all clinical forms of illness;
  – *Respiratory secretions*: Pharyngeal swabs, bronchial/tracheal washes or aspirates, sputum, transthoracic lung aspirates, or pleural fluid collection (for respiratory, typhoidal, oropharyngeal forms of illness);
  – *Swabs*: Swabs of visible lesions or affected areas should be collected (for ulceroglandular and oculoglandular forms of illness);
  – *Aspirates*: Aspirates from lymph nodes or lesions (for ulceroglandular, glandular, and oropharyngeal forms of illness);
  – *Tissue biopsies*: Tissue samples from lymph nodes (for ulceroglandular, glandular, and oropharyngeal forms of illness). Invasive sampling, such as incision of an affected lymph node, should be avoided during the acute stage of disease;
  – *Autopsy materials*: Samples from visible abscesses and from lymph node, lung, liver, spleen, cerebrospinal fluid, and bone marrow.

Serology is commonly used for confirmation of tularaemia. Antibody responses against *F. tularensis* are generally detectable in patients 10–20 days post-infection. Agglutination, either microagglutination or tube agglutination, is the standard serological test used for determining the

presence of antibody to *F. tularensis*. More recently an Enzyme-Linked Immunosorbent Assay (ELISA) (directed against LipoPolySaccharide-LPS) combined with Western blot (against antigen extracted from whole killed cells) showed very good sensitivity and specificity for diagnosis of infection. Culture provides a conclusive diagnosis of infection and an invaluable resource for molecular epidemiology, subtyping and discovery of novel species and subspecies. Whenever possible, culture should be attempted. *F. tularensis* grows well on several types of cysteine/cystine-supplemented agar including enriched Chocolate Agar (CA), Cystine Heart Agar with 9% chocolatized Blood (CHAB), Buffered Charcoal Yeast Extract (BCYE), Thioglycollate-GlucoseBlood Agar (TGBA), and GC Agar II with 1% haemoglobin and 1% IsoVitaleX. A variety of molecular methods have been described for the molecular detection of *F. tularensis*. PCR can be a valuable diagnostic tool when organisms are non-cultivable or when culture is not recommended due to biosafety concerns. Consist conventional PCR assays targeted at the genes *fopA* or *tul4* encoding the outer membrane proteins. A multiplex real-time TaqMan PCR assay for *F. tularensis* has also been developed.

Animal: confirmation is by culture, serology, or PCR. When individual animals present with consistent clinical signs of septicemic disease, generalized or acute lymphadenopathy, or pneumonia, tularaemia must be considered a possible cause. Tularaemia should also be ruled out when large numbers of sheep show typical signs during periods of heavy tick infestation or when large numbers of rodents or lagomorphs are found dead. Affected sheep should be evaluated for tularaemia and tick paralysis, whereas the etiologic agents to consider in cats and small mammals should also include agents of plague and pseudotuberculosis. When tularaemia is suspected, laboratory personnel should be alerted as a precaution to reduce the risk of laboratory-acquired infection. The infective dose required to transmit this pathogen is extremely low; thus, risk of infection during necropsy or to laboratory personnel is significant, and special procedures and facilities are essential. Diagnosis of acute infection of tularaemia is confirmed by bacterial culture and identification of the bacterium, serology, or PCR. Organisms can be readily isolated from necropsy specimens by use of special media using stringent personal protection protocols. A direct or indirect fluorescent antibody test, or tube agglutination test with a single titer of ≥1:80, is presumptive evidence of exposure, whereas a 4-fold increase in antibody titer between acute and convalescent serum specimens confirms acute infection. PCR can be used to confirm infection rapidly.

The Commission Implementing Decision (EU) 2018/945 of 22 June 2018 on the communicable diseases and related special health issues to be covered by epidemiological surveillance as well as relevant case definitions.

Infrastructure capacity to identify pathogens for each Member State

Tularaemia is among the communicable diseases that according to the Commission Implementing Decision (EU) 2018/945 are covered by epidemiological surveillance. It means that EU Member States are required to establish national capacity of detection and reporting of human cases. The decision provides a case definition and laboratory criteria for case confirmation that are at least one of the following three cases:

1) Isolation of *Francisella tularensis* from a clinical specimen.
2) Detection of *F. tularensis* nucleic acid in a clinical specimen
3) Detection of *F. tularensis* specific antibody response. Diagnosis is routinely made by clinical microbiology laboratories, and there is no specific European-wide reference

laboratory network or national laboratories in most EU countries. Due to the potential biological weapon role, in many countries there is the involvement of Defence Research Agencies or laboratories. In animals, the disease is not reportable at the EU level (Regulation (EU) 2016/429 and Commission Implementing Regulation (EU) 2020/2002) but is reportable at the international level to the World Organization for Animal Health (WOAH). Human surveillance is reported by each EU Member State to ECDC through the surveillance TESSy System.

Estimated influence of environmental change on the disease future trends

Tularaemia is widely distributed in Europe and has local emergence and re-emergence trends in humans and wildlife. The disease has a clear seasonality in humans, but this pattern has not been conclusively demonstrated in wildlife. Assessment of eco-epidemiological trends and estimation of infection risk in relation to projected environmental and climate change are difficult. Different geographical areas have different ecosystems, which influence the epidemiology and disease presentation. Such factors include temperature and humidity, the presence of different types of arthropod vectors, and the variety of small rodent and other wildlife species present. The important role of vectors in tularaemia transmission in the northern Hemisphere and the seasonality of the disease mean that climate change may have an effect on tularaemia transmission patterns in highly endemic areas. Statistical models used to study climate change include mosquito abundance as the main variable; however, the role of animal reservoirs is not often considered in the estimates. Overall, in northern Europe, along a latitudinal gradient, climate change scenarios respectively indicate a future decrease in disease in some Southern areas and an increase in disease in more northern areas. For the rest of Europe, estimating the impact of climate on disease is more difficult. Among the aspects that need better understanding, knowledge of the specific variables that influence the activity of natural tularaemia foci in Europe is one that needs to be improved for effective monitoring of this disease.

References

1. Keim P, Johansson A, Wagner DM. Molecular epidemiology, evolution, and ecology of Francisella. *Ann N Y Acad Sci.* 2007;1105:30-66.
2. Colquhoun DJ, Duodu S. Francisella infections in farmed and wild aquatic organisms. *Vet Res.* 2011;42:47.
3. Birkbeck TH, Feist SW, Verner-Jeffreys DW. Francisella infections in fish and shellfish. *J Fish Dis.* 2011;34:173-187.
4. Berrada ZL, Telford Iii SR. Survival of Francisella tularensis Type A in brackish-water. *Arch Microbiol.* 2011;193:223-226.
5. Broman T, Thelaus J, Andersson A-C, *et al.* Molecular detection of persistent Francisella tularensis subspecies holarctica in natural waters. *Int J Microbiol.* 2011;2011:851946.
6. Gilbert SE, Rose LJ. Survival and persistence of nonspore-forming biothreat agents in water. *Lett Appl Microbiol.* 2012;55:189-194.
7. Hennebique A, Boisset S, Maurin M. Tularemia as a waterborne disease: a review. *Emerg Microbes Infect.* 2019;8:1027-1042.

8. Bäckman S, Näslund J, Forsman M, *et al.* Transmission of tularemia from a water source by transstadial maintenance in a mosquito vector. *Sci Rep.* 2015;5.
9. Brugueras S, Fernández-Martínez B, Martínez-de la Puente J, *et al.* Environmental drivers, climate change and emergent diseases transmitted by mosquitoes and their vectors in southern Europe: A systematic review. *Environ Res.* 2020; 191.
10. European Food Safety Authority, European Centre for Disease Prevention and Control. The European Union One Health 2021 Zoonoses Report. *EFSA Journal.* 2022;20(12):7666:273 pp. https://doi.org/10.2903/j.efsa.2022.7666.
11. Hennebique A, Boisset S, Maurin M. Tularemia as a waterborne disease: a review. *Emerg Microbes Infect.* 2019;8:1027-1042.
12. Bonnet S, De La Fuente J, Nicollet P, *et al.* Prevalence of tick-borne pathogens in adult Dermacentor spp. Ticks from nine collection sites in France. *Vector Borne Zoonotic Dis.* 2013;13:226-236.
13. Duzlu O, Yildirim A, Inci A, *et al.* Molecular investigation of Francisella-like endosymbiont in ticks and Francisella tularensis in ixodid ticks and mosquitoes in Turkey. *Vector Borne Zoonotic Dis.* 2016;16:26-32.
14. Ebani VV, Rocchigiani G, Nardoni S, *et al.* Molecular detection of tick-borne pathogens in wild red foxes (Vulpes vulpes) from Central Italy. *Acta Trop.* 2017;172:197-200.
15. Bielawska-Drózd A, Cieślik P, Żakowska D, *et al.* Detection of Coxiella burnetii and Francisella tularensis in tissues of wild-living animals and in ticks of North-west Poland. *Pol J Microbiol.* 2018;67:529-534.
16. Tomaso H, Otto P, Peters M, *et al.* Francisella tularensis and other bacteria in hares and ticks in North Rhine-Westphalia (Germany). *Ticks Tick-Borne Dis.* 2018;9:325-329.
17. Gehringer H, Schacht E, Maylaender N, *et al.* Presence of an emerging subclone of Francisella tularensis holarctica in Ixodes ricinus ticks from South-Western Germany. *Ticks Tick-Borne Dis.* 2013;4:93-100.
18. Pistone D, Pajoro M, Novakova E, *et al.* Ticks and bacterial tick-borne pathogens in Piemonte region, Northwest Italy. *Exp Appl Acarol.* 2017;73:477-491.
19. Richard S, Oppliger A. Zoonotic occupational diseases in forestry workers - Lyme borreliosis, tularemia and leptospirosis in Europe. *Ann Agric Environ Med.* 2015;22:43-50.
20. Guardone L, Ebani VV, Verin R, *et al.* Molecular detection of arthropod-borne pathogens in Eurasian badgers (Meles meles) from the United Kingdom. *Animals.* 2020;10.
21. Gyuranecz M, Rigó K, Dán A, *et al.* Investigation of the ecology of francisella tularensis during an inter-epizootic period. *Vector Borne Zoonotic Dis.* 2011;11:1031-1035.
22. Lopes de Carvalho I, Toledo A, Carvalho CL, *et al.* Francisella species in ticks and animals, Iberian Peninsula. *Ticks Tick-Borne Dis.* 2016;7:159-165.
23. Janse I, van der Plaats RQJ, Husman AM de R, *et al.* Environmental Surveillance of Zoonotic Francisella tularensis in the Netherlands. *Front Cell Infect Microbiol.* 2018;8:2235-2988.
24. Toma L, Mancini F, Di Luca M, *et al.* Detection of microbial agents in ticks collected from migratory birds in central Italy. *Vector Borne Zoonotic Dis* 2014;14:199-205.
25. Egyed L, Elő P, Sréter-Lancz Z, *et al.* Seasonal activity and tick-borne pathogen infection rates of Ixodes ricinus ticks in Hungary. *Ticks Tick-Borne Dis.* 2012;3:90-94.
26. Wójcik-Fatla A, Zając V, Sawczyn A, *et al.* Occurrence of Francisella spp. in Dermacentor reticulatus and Ixodes ricinus ticks collected in eastern Poland. *Ticks Tick Borne Di.s* 2015;6:253-257.
27. Lundström JO, Andersson A-C, Bäckman S, *et al.* Transstadial transmission of *Francisella tularensis holarctica* in mosquitoes, Sweden. *Emerg Infect Dis.* 2011;17:794-799.

28. Hubalek Z, Treml F, Juricova Z, *et al.* Serological survey of the wild boar (Sus scrofa) for tularaemia and brucellosis in South Moravia, Czech Republic. *Vet Med (Praha).* 2002;47:2-3.
29. Hubálek Z, Rudolf I. *Francisella tularensis* prevalence and load in *Dermacentor reticulatus* ticks in an endemic area in Central Europe: *Francisella tularensis* in *D. reticulatus*. *Med Vet Entomol.* 2017;31:234-239.
30. Hodžić A, Fuehrer H-P, Duscher GG. First molecular evidence of zoonotic bacteria in ticks in Bosnia and Herzegovina. *Transbound Emerg Dis.* 2017;64:1313-1316.
31. Paul REL, Cote M, Le Naour E, *et al.* Environmental factors influencing tick densities over seven years in a French suburban forest. *Parasit Vectors.* 2016;9.
32. Dryselius R, Hjertqvist M, Mäkitalo S, *et al.* Large outbreak of tularaemia, central Sweden, July to September 2019. *Euro Surveill.* 2019;24(42):1900603. doi:10.2807/1560-7917.ES.2019.24.42.1900603.
33. Thelaus J, Andersson A, Broman T, *et al.* Francisella tularensis Subspecies holarctica occurs in Swedish mosquitoes, persists through the developmental stages of laboratory-infected mosquitoes and is transmissible during blood feeding. *Microb Ecol.* 2014;67:96-107.
34. Regier Y, Komma K, Weigel M, *et al.* Combination of microbiome analysis and serodiagnostics to assess the risk of pathogen transmission by ticks to humans and animals in central Germany. *Parasit Vectors*. 2019;12(1):11.
35. Pajoro M, Pistone D, Boccazzi IV, *et al.* Molecular screening for bacterial pathogens in ticks (Ixodes ricinus) collected on migratory birds captured in northern Italy. *Folia Parasitol (Praha).* 2018;65.
36. Franke J, Fritzsch J, Tomaso H, Straube E, Dorn W, Hildebrandt A. Coexistence of pathogens in host-seeking and feeding ticks within a single natural habitat in Central Germany. *Appl Environ Microbiol.* 2010;76(20):6829-6836. doi:10.1128/AEM.01630-10.
37. Pascucci I, Di Domenico M, Dall'Acqua F, *et al.* Detection of lyme disease and Q fever agents in wild rodents in Central Italy. *Vector Borne Zoonotic Dis* 2015;15:404-411.
38. Hestvik G, Uhlhorn H, Westergren E, *et al.* Tularaemia in European brown hares (Lepus europaeus) and mountain hares (Lepus timidus) characterized by histopathology and immunohistochemistry: organ lesions and suggestions of routes of infection and shedding. *J Comp Pathol.* 2017;157:2-3.
39. Desvars A, Furberg M, Hjertqvist M, *et al.* Epidemiology and ecology of Tularemia in Sweden, 1984-2012. *Emerg Infect Dis.* 2015;21:32-39.
40. Otto P, Chaignat V, Klimpel D, *et al.* Serological Investigation of Wild Boars (Sus scrofa) and Red Foxes (Vulpes vulpes) As Indicator Animals for Circulation of Francisella tularensis in Germany. *Vector Borne Zoonotic Dis.* 2014;1:46-51.
41. Bártová E, Kučerová HL, Žákovská A, *et al.* Coxiella burnetii and Francisella tularensis in wild small mammals from the Czech Republic. *Ticks Tick Borne Dis.* 2020;11:101350.
42. Rossow H, Sissonen S, Koskela KA, *et al.* Detection of Francisella tularensis in voles in Finland. *Vector Borne Zoonotic Dis.* 2014;14:193-198.
43. Moinet M, Decors A, Mendy C, *et al.* Spatio-temporal dynamics of tularemia in French wildlife: 2002-2013. *Prev Vet Med* 2016;130:33-40.
44. Tadin A, Tokarz R, Markotic A, *et al.* Molecular survey of zoonotic agents in rodents and other small mammals in Croatia. *Am J Trop Med Hyg.* 2016;94:466-473.
45. Mihelčić M, Habuš J, Vucelja M, *et al.* Prevalence of *Francisella tularensis* in the population of small mammals species in continental forests of Croatia. *Šumar List.* 2018;142:481-485.
46. Richter WR, Sunderman MM, Wendling MQS, *et al.* Evaluation of altered environmental conditions as a decontamination approach for nonspore-forming biological agents. *J Appl Microbiol.* 2020;128:1050-1059.

47. Grunow R, Kalaveshi A, Kühn A, Mulliqi-Osmani G, Ramadani N. Surveillance of tularaemia in Kosovo, 2001 to 2010. *Euro Surveill.* 2012;17(28):20217. doi:10.2807/ese.17.28.20217-en.

48. Desvars A, Furberg M, Hjertqvist M, *et al.* Epidemiology and ecology of tularemia in Sweden, 1984-2012. *Emerg Infect Dis.* 2015;21(1):32-39. doi:10.3201/eid2101.140916.

49. Rossow H, Ollgren J, Hytonen J, *et al.* Incidence and seroprevalence of tularaemia in Finland, 1995 to 2013: regional epidemics with cyclic pattern. *Euro Surveill.* 2015;20(33):21209.

50. Jurke A, Bannert N, Brehm K, *et al.* Serological survey of Bartonella spp., Borrelia burgdorferi, Brucella spp., Coxiella burnetii, Francisella tularensis, Leptospira spp., Echinococcus, Hanta-, TBE- and XMR-virus infection in employees of two forestry enterprises in North Rhine-Westphalia, Germany, 2011-2013. *Int J Med Microbiol.* 2015;305(7):652-662. doi:10.1016/j.ijmm.2015.08.015.

51. Larssen KW, Afset JE, Heier BT, *et al.* Outbreak of tularaemia in central Norway, January to March 2011. *Euro Surveill.* 2011;16:19828.

52. De Keukeleire M, Vanwambeke SO, Cochez C, *et al.* Seroprevalence of borrelia burgdorferi, anaplasma phagocytophilum, and francisella tularensis infections in Belgium: Results of three population-based samples. *Vector Borne Zoonotic Dis.* 2017;17:108-115.

53. Jenzora A, Jansen A, Ranisch H, *et al.* Seroprevalence study of Francisella tularensis among hunters in Germany. *FEMS Immunol Med Microbiol.* 2008;53:183-9.

54. Zukiewicz-Sobczak W, Zwolinski J, Chmielewska-Badora J, *et al.* Prevalence of antibodies against selected zoonotic agents in forestry workers from eastern and southern Poland. *Ann Agric Environ Med.* 2014;21:767-770.

55. Tokarska-Rodak M, Weiner M, Szymanska-Czerwinska M, *et al.* Seroprevalence of selected zoonotic agents among hunters from Eastern Poland. *Pol J Microbiol.* 2018;67:233-236.

56. Rigaud E, Jaulhac B, Garcia-Bonnet N, *et al.* Seroprevalence of seven pathogens transmitted by the Ixodes ricinus tick in forestry workers in France. *Clin Microbiol Infect.* 2016;22:735.e1-735.e9.

57. Hirschmann JV. From squirrels to biological weapons: the early history of Tularemia. *Am J Med Sci.* 2018;356:319-328.

58. Maurin M, Gyuranecz M. Tularaemia: clinical aspects in Europe. *Lancet Infect Dis.* 2016;16:113-124.

59. Sjöstedt A. Tularemia: history, epidemiology, pathogen physiology, and clinical manifestations. *Ann N Y Acad Sci*. 2007;1105:1-29.

60. Eliasson H, Lindbäck J, Nuorti JP, *et al.* The 2000 Tularemia outbreak: a case-control study of risk factors in disease-endemic and emergent areas, Sweden. *Emerg Infect Dis.* 2002;8:956-960.

61. Olsufév NG. (Results and perspectives of the study of natural foci of tularemia in USSR). *Med Parazitol (Mosk).* 1977;46:273-282.

62. Centers for Disease Control and Prevention (CDC). Tularemia--United States, 1990-2000. *MMWR Morb Mortal Wkly Rep*. 2002;51(9):181-184.

63. Tärnvik A, Priebe H, Grunow R. Tularaemia in Europe: an epidemiological overview. *Scand J Infect Dis.* 2004;36:350-355.

USUTU VIRUS INFECTION

Soushieta Jagadesh (a), Claudia Cataldo (b), Francesca Dagostin (c), Di Luca Marco (d), Annapaola Rizzoli (c), William Wint (e), Luca Busani (b)
(a) International Society of Infectious Diseases, Boston (MA)
(b) Centro di Riferimento per la Medicina di Genere, Istituto Superiore di Sanità, Rome
(c) Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige, Trento
(d) Dipartimento Malattie Infettive, Istituto Superiore di Sanità, Rome
(e) Department of Biology, Environmental Research Group Oxford Ltd, Oxford

Biological, ecological, and molecular features of the causative agent

Disease name

Usutu virus infection/ USUV infection.

Disease agent

Common, scientific and Latin name

Usutu virus (USUV).

Taxonomy

USUV, an RNA arbovirus, is member of the Japanese encephalitis serocomplex of the *Flaviviridae* family and of the *Flavivirus* genus (1-3), and is phylogenetically close to Japanese encephalitis virus (JEV) and West Nile virus (WNV) (4, 5).

Phylogenetic studies based on the nucleic acid sequence of the NS5 gene divided USUV strains isolated into eight lineages: three African and five European (6), and that the level of genetic relatedness was found dependent on their geographical origin and the host from which they have been isolated.

Disease agent characteristics

USUV is an enveloped virus of approximately 40-60 nm in diameter, with a single-stranded positive sense RNA comprised of 11064 base pairs with a 5′ N7-methylguanosine-triphosphate cap but lacking a polyA tail at the 3′ end (7, 8).

The genome of USUV comprises a single open reading frame coding for a polyprotein of 3434 amino acids that, which following cleavage, generates three structural proteins (capsid C, pre membrane prM and envelope E) and eight non-structural (NS) proteins (NS1/NS1', NS2a, NS2b, NS3, NS4a, 2K, NS4b and NS5) (4). The capsid protein (C) forms the central body of the virion and is associated with the viral RNA. The prM protein is required for virion assembly and maturation of virions through the folding of the envelope glycoprotein (E) (9). The NS5 of the virus ensures viral RNA replication by its RNA-dependent RNA polymerase domain and a methyltransferase (MTase) domain, which catalyses the capping of new viral RNA molecules (7, 10).

Priority level for EU

Usutu virus infection in humans is not a mandatory notifiable disease at the EU/EEA (European Union and European Economic Area) level.

The sequences of USUV strains obtained in Italy in 2009 from mosquitoes, birds and humans, demonstrated that the sequences obtained from human hosts clustered with the sequences obtained from birds, indicating an endemic distribution of USUV in Europe (11). In Europe, USUV was isolated for the first time in 2001 from dead blackbirds (*Turdus merula*) during an epizootic near Vienna, Austria (12-14). A retrospective analysis of the high mortality of blackbirds in Tuscany, Italy in 1996 was attributed to USUV (15). The virus has since been detected in epizootics in Western, Southern, and Central European countries in birds and mosquitoes (3, 16). Although USUV infections in humans remain asymptomatic, sporadic neuroinvasive disease cases have been reported in Europe, predominantly among immunocompromised and elderly patients (17, 18).

Distribution of the pathogen

In 2015, USUV was reported from mosquitoes, birds or horses in 12 European countries (Germany, Austria, Belgium, Croatia, Spain, France, Greece, Hungary, Italy, the Czech Republic, Serbia and Switzerland) (19-23). USUV infection has also been serologically identified in Slovakia and in Poland in equine and avian populations (24, 25). In the summer of 2016, Belgium, France, Germany and for the first time, the Netherlands reported widespread USUV activity based on live and dead bird surveillance (6, 26, 27). In 2018, USUV spread rapidly across Western Europe suggesting a continuous geographical spread of the virus, along with the colonization of new ecological niches. As of September 2023, 15 out of 30 EU/EEA countries reported USUV circulation among avian and equine animals – Austria, Belgium (28, 29), Croatia, Czechia, France, Germany, Greece (30), Hungary, Italy, Luxembourg, the Netherlands, Poland, Slovakia, Slovenia and Spain (31, 32) (Figure 1) (33). USUV has been predominantly detected in ornithophilic mosquito species of the *Culex* genus – like *Cx. modestus*, *Cx. neavei, Cx. perexiguus, Cx. perfuscus, Cx. pipiens, Cx. quinquefasciatus, Cx. univittatus* – but also to other genera such as *Aedes albopictus, Ae. japonicus, Ae. minutus, Anopheles maculipennis, Culiseta annulata, Mansonia africana, Coquilletidia aurites, Ochlerotatus caspius* and *Oc. detritus. Cx. pipiens*, an ornithophilic species, which can also feed on mammals, including humans, is considered to be the main vector in Europe (34). Europe encompasses highly suitable areas for *Cx. pipiens,* ranging from Mediterranean regions to Northern countries (Figure 2). Previous studies have detected USUV in nine mosquito species across Europe (Table 1).

Table 1. Mosquito species found infected by USUV across Europe

Species	Country (ref.)
Aedes albopictus	Italy (35-37), Croatia (38)
Aedes japonicus	Austria (39)
Anopheles maculipennis	Italy (37)
Culex modestus	Czech Republic (40), Italy (41), Belgium (42)
Culex perexiguus	Spain (43)
Culex pipiens	Austria (39), France (22), Germany (21, 44, 45), Italy (35-37, 46-51), Serbia (20), Spain (52), Switzerland (53)
Culiseta annulata	Italy (37)
Ochlerotatus caspius	Italy (35, 37)
Ochlerotatus detritus	Italy (37)

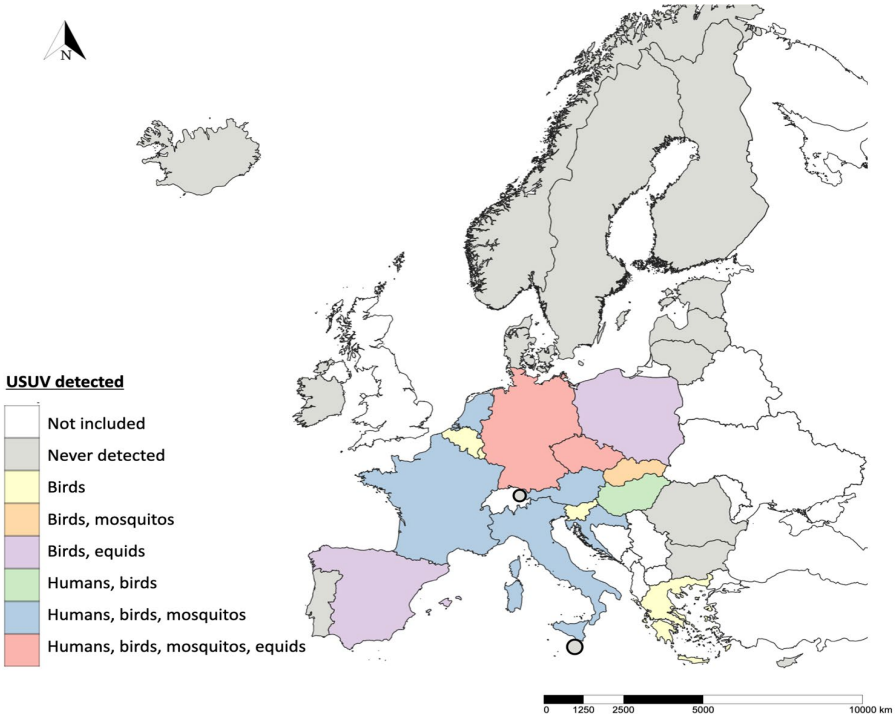

Figure 1. Geographical distribution of Usutu virus infections in humans, animals (birds-equids) and mosquitoes in the European Union and European Economic Area

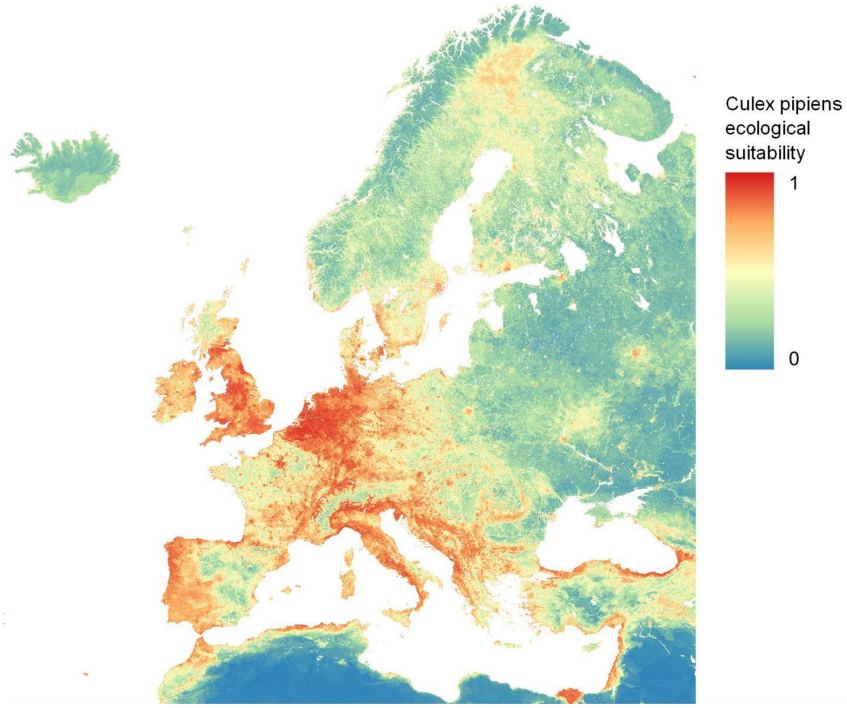

Figure 2. Current 1-km probability of presence of *Cx. pipiens* across Europe, produced using random forest and boosted regression trees analyses (source ERGO group)

Ecology and transmission routes

As with a majority of arboviruses, the transmission dynamics of USUV is influenced by biological and environmental factors, such as distribution and population density of vector and reservoir species, the extrinsic incubation period, humidity, temperature, host immunity, etc. (8). USUV and WNV are genetically, antigenically, and epidemiologically closely related. There is a significant interest on the possibility of overlapping in transmission cycles influencing the spatiotemporal trends of circulation of the two viruses in Europe (1) as both viruses are mainly transmitted by *Culex* mosquitoes, with migratory birds acting as the major amplifying host. Viral RNA was detected simultaneously for both viruses (54, 55), confirming that co-infection does occur. However, the co-circulation and co-infection remains uncertain at a population level.

The transmission cycle of USUV involves the vectors, ornithophilic mosquitos and the amplifying hosts and reservoirs, birds (Figure 3).

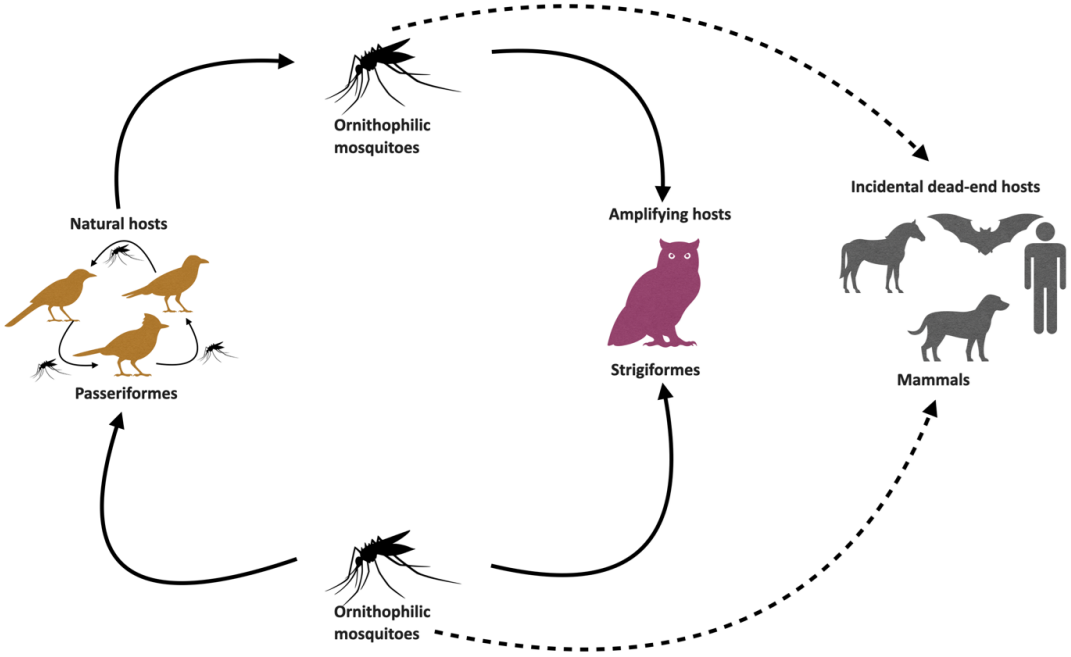

Figure 3. Biological cycle of USUV infection

Infected ornithophilic mosquitos feed on birds, often species belonging to *Passeriformes* and *Strigiformes* (56). The infected birds develop sufficient viremia to allow transmission of the virus to a new mosquito during a subsequent bite. Some mosquitos of the *Culex* species may also take their blood meal from a mammalian host including humans, equids dogs, etc.

Reservoirs

USUV is maintained through an enzootic cycle, similar to WNV, between passerine birds mainly Eurasian blackbirds (*Turdus merula*) or magpies (*Pica pica*) and Strigiformes, such as the Great Gray Owl (*Strix nebulosa*) as amplifying hosts and ornithophilic mosquitoes as vectors. Thus, the main USUV natural hosts are birds, with infection being reported in 93 different species

belonging to 35 families (57). The most affected species is the Eurasian blackbirds (*Turdus merula*) followed by gray owls (*Strix nebulosa*), and house sparrows (*Passer domesticus*) (12, 58). Occasionally, USUV was detected in birds in captivity (59) in Germany (60) (i.e. marabou stock, ruddy shellducks, red-breasted geese, Humboldt penguins, laughing kookaburras, steamer ducks, greater flamingos, snowy owls, Ural owls, white storks, Egyptian vultures, and Eurasian eagle owls), France (61) (i.e. Abyssinian ground hornbills, common peafowls, emus, scarlet ibis, and greater rheas) and Slovenia (62) (i.e. pelicans, Eurasian eagle-owls, barn and snowy owls). The list of birds detected with USUV infections in Europe is summarized in Table 2 (1).

Table 2. Updated list of birds with USUV clinical infections – RT-PCR (+)

Order	Common name	Scientific name	Countries with USUV
Passeriformes	Blackbird	*Turdus merula*	Austria (13, 63, 64), Czech Republic (65), France (23), Germany (34, 66), Hungary (63), Italy (11, 41,67), Netherlands (26), Belgium (68), Switzerland (69)
	Common starling	*Sturnus vulgaris*	Germany (34,66), Italy (11,41,67)
	Song trush	*Turdus philomelos*	Austria (64), Germany (70) Spain (31)
	Canary	*Serinus canaria domestica*	Germany (34)
	House sparrow	*Passer domesticus*	Austria (13,64), Germany (66), Switzerland (69)
	Blue (great) tit	*Parus caeruleus (major)*	Austria (13), Switzerland (69)
	European greenfinch	*Chloris chloris*	Switzerland (69)
	European robin	*Erithacus rubecula*	Austria (13), Switzerland (69)
	Bullfinch	*Pyrrhula pyrrhula*	Belgium (71)
	Nuthatch	*Sitta europaea*	Austria (13)
	Eurasian jay	*Garrulus glandarius*	Hungary (63), Italy (67)
	Magpie	*Pica pica*	Italy (11,72)
	Barn swallows	*Hirundo rustica*	Austria (14)
Strigiformes	Great grey owl	*Strix nebulosa*	Austria (13), Germany (60,70,73), Switzerland (69), France (74), Italy (75), Netherlands (26)
	Long-eared owl	*Asio otus*	Germany (60), Italy (76)
	Snowy owl	*Bubo scandiacus*	Germany (60), Switzerland (69)
	Tengmaml's owl	*Aegolius funereus*	Switzerland (69), IT
	Hawk owl	*Surnia ulala*	Switzerland (69), DE
	Pygmy owl	*Glaucidium passerinum*	Switzerland (69)
	Tawny owl	*Strix aluco*	Germany (60), Italy (76)
	Eurasian scops owl	*Otus scops*	Italy (76)
	Little owl	*Athene noctua*	Italy (76)
Coraciiformes	Common kingfisher	*Alcedo atthis*	Germany (77)
	European bee-eater	*Merops apiaster*	Italy (78)
Piciformes	Great spotted woodpecker	*Dendrocopos major*	Belgium (71)
	European green woodpecker	*Picus viridis*	Germany (66,79)
Charadriiformes	Inca tern	*Larosterna inca*	Germany (66,79)
	Yellow-legged gull	*Larus michahellis*	Italy (41)
Accipitriformes	Greater spotted eagle	*Aquila clanga*	Italy (41)
Caprimulgiformes	Nightjar	*Caprimulgus europaeus*	Italy (41,80)
Pelecaniformes	Grey heron	*Ardea cinerea*	Italy (41)
Columbiformes	Collared dove	*Streptopelia decaocto*	Italy (41,80)
Galliformes	Red-legged partridge	*Alectoris rufa*	Italy (76)

Vectors

Ornithophilic mosquito species of the *Culex* genus are the predominant vectors of USUV with *Cx. pipiens* being the main vector in Europe (*see* Table 1). Mosquito vectors are responsible for virus transmission between birds and to susceptible mammals, such as humans and horses, which are incidental dead-end hosts, with short-lasting and low-level viremia. The transmission rate of USUV not only varies between different mosquito species and virus strains but also their geographical location (40) (Table 3).

Table 3. Infection, dissemination and transmission rates for mosquitoes following oral exposure to USUV

Species	Country	Infection rate[a]	Dissemination rate[b]	Transmission rate[c]
Cx. pipiens	Netherlands (81)	80%		69%
Cx. pipiens	Belgium (40)	16.2%	0	0
Aedes albopictus	Italy (46)	0		
Cx. modestus	Belgium (40)	60%	66.7%	50%
Cx. pipiens	France (82)	4.3%	100%	100%
Ae. rusticus	France (82)	0	0	0
An. plumbeus	France (82)	0	0	0
Ae. albopictus	France (82)	-	-	4.2% at 10 dpi 29.2% at 14 dpi 12.5% at 17 dpi 16.7% at 21 dpi 16.7% at 28 dpi
Cx. pipiens biotype *molestus*	Germany (83)	80%	37.5%	100% at 10 dpi
		66.7%	100%	75% at 21 dpi
Cx. pipiens biotype *molestus*	Serbia (83)	81.3%	100%	15.4% 16 dpi
		80%	100%	50% at 21 dpi
Cx. torrentium	Germany (83)	12.5%	100%	100%
Cx. pipiens biotype *pipiens*	Netherlands (84)	-	-	18%
Cx. pipiens biotype *molestus*	Netherlands (84)	-	-	30%

[a] No. infected mosquito bodies/no. mosquitoes tested
[b] No. infected mosquitoes with virus in organs/no. mosquitoes tested.
[c] No. infected mosquitoes with virus in saliva/no. mosquitoes tested.

dpi: day-post-infection

Cx. pipiens that were exposed to USUV and WNV simultaneously via infectious blood meal displayed significantly reduced USUV transmission compared to mosquitoes that were only exposed to USUV (from 15% to 3%), while the infection and transmission of WNV was unaffected (85). In contrast, when mosquitoes were pre-infected with USUV via infectious blood meal, WNV transmission was significantly reduced (from 44% to 17%).

Incidental hosts

Humans and non-human mammals are incidental dead-end hosts.

These hosts are not involved in the transmission cycle as they correspond to epidemiological dead ends for viral propagation because the viremia in the infected mammals is not high enough to ensure transmission through mosquito bites.

Apart from humans, USUV (or antibodies against it) has been detected in several mammals, including:

- horses: Poland (25), Italy, Croatia, Serbia and Spain (86);
- dogs: Italy (87) and Slovenia (88);
- squirrels: Italy (89);
- bats: Belgium (28), Germany (90);
- wild boars: Serbia (91);
- roe deers: Serbia (91);
- various deer species: Spain (92);
- zoo mammals (i.e. Asian lions, maned wolves, Iberian wolves, grey and northwestern wolves, African wild dogs, chimpanzees, common elands, giant pandas, Malayan tapirs, white rhinoceros, guinea pigs, rabbits, and red foxes): France (61, 93), Spain (92, 94), Slovenia (62) and other Central European country (59).

USUV was detected for the first time in reptiles, green lizards, in Slovakia (95).

Drivers of the disease emergence and spread

Ecological drivers

Studies report an increase in mosquito-borne disease outbreaks including USUV in Europe (6, 96) in the last decade due to changes in the temperature and precipitation attributable to climate change (97, 98) and anthropogenic landscape modifications (99-101).

There is a lack of evidence on the impact of climate change on the epidemiology of USUV infection, particularly considering that the weather can influence bird movements, vector abundance and virus replication in the vectors.

Cx pipens, the main vector of USUV in humans, was observed to have a strong relation with temperature (102). The study demonstrated that higher temperatures lead to higher mosquito abundances regardless of soil type. Larval stages of *Cx. pipiens* and related sibling species can inhabit a wide range of habitats, including artificial habitats (103). Land cover in urban areas consists mostly of artificial surfaces, which offer plenty of breeding sites filled with a small volume of water without the risk of predation (104-106). Studies also observed that artificial and clay surfaces, frequent in urban landscapes, increased mosquito abundance due to water stagnation in comparison to sandy soils (102).

Climate, land use, and biodiversity loss impact the distribution of bird populations which has an indirect effect on USUV emergence and spread. European breeding birds have shifted their range by 2.4 km per year, on average in the recent three decades (107). Studies also demonstrate that *Strigiformes* (owl, amplifying hosts of USUV) will shift to higher altitudes due to anthropogenic climate change (108).

Further ecological studies are required to get an understanding of the relationship between climate variables and human case incidence and to refine models or predictions of outbreak occurrences. Modelling the epidemiology of WNV throughout Europe under different climate change scenarios (IPCC scenarios) could help mapping the potential distribution of USUV, as co-circulation of both viruses is frequent.

Natural history of disease in humans and animals, including symptoms, morbidity and mortality

Brief history of the pathogen and disease

Following its first identification in South Africa in 1959, USUV has been detected in avian, equid and mosquito species in several African countries: Central African Republic, Senegal, Ivory Coast, Nigeria, Uganda, Burkina Faso, Tunisia, and Morocco (109-112). The first case of human infection by USUV was reported in the Central African Republic in the 1980s and a second case was diagnosed in Burkina Faso in 2004 (109). For these two cases, mild clinical signs were reported: fever and skin rash.

Phylogenetic studies suggest that at least three USUV introductions have occurred in Europe along the migratory routes from Africa. The virus has been introduced in Spain on two occasions in the 1950s and then in the 1990s along an eastern Atlantic migratory route (113). A unique introduction in Italy and Austria has been reported in the 1980s along a Black Sea/Mediterranean migratory route (15, 113). USUV has been classified into eight lineages: African (Africa 1/2/3) or European (Europe 1/2/3/4/5). Most strains currently circulating in Europe belong to the European lineages of USUV. However, African 2 lineage of USUV has also been identified in France in a human patient (74) and in owls in Berlin Zoo in 2015 (114). Furthermore, African lineages continue to be introduced into the continent, like the African 2 and 3 lineages reported in 2018 in *Cx. pipiens* in the South of France (22). The Europe 1 lineage is thought to derive from a Senegalese strain that reached Spain and was implicated in the first avian epizootic in blackbirds Austria in 2001(14). Europe 2 lineage is an Austrian strain from 1993, reappearing during the autochthonous Italian cases of 2009-2010 and the Austrian and Hungarian cases in 2016 (20). European 3 lineage is from an Italian strain that circulated in 2007 that was responsible for the massive bird die-off observed in Germany in 2011/2013 (114), in France in 2015 (23), and in Belgium in 2016 (29). European 4 lineage comprises strains circulating in Italy in 2010 and 2015 (16). Finally, the Europe 5 lineage was isolated from infected birds in Germany in 2016 (16).

Disease in humans

Humans are infected with USUV following a bite from an infected mosquito. No transfusion-associated USUV infection has been reported (115). Due to limited clinical data available on USUV infection, the incubation period of 3 to 12 days was estimated based on WNV infection as reference (116). Following incubation, a brief viraemic phase is triggered and the patient may develop symptoms (117). Most USUV infections in humans remain largely asymptomatic with sporadic cases with neurological manifestations due to neuroinvasive disease. Severe disease is often associated with compromised immune systems and advanced age (17, 18, 118). Impaired neurological functions were observed in neuroinvasive diseases such as meningitis, encephalitis, and polyneuritis (17, 18, 74, 118-120). Symptoms of neuroinvasive disease are nuchal rigidity, hand tremor, and hyperreflexia (54).

Complications of USUV fulminant hepatitis have been reported following USUV neuroinvasive disease (18) in immunosuppressed patients. One of the samples during the retrospective screening of CSF in Montpellier, France tested positive for USUV (74). The patient was diagnosed with idiopathic facial paralysis which was putatively associated with an acute USUV infection. Rarely, in benign cases, the symptoms of an acute USUV infection resemble that of a febrile illness (109, 118) with fever, arthralgia, myalgia, headache, and rash. WNV and

other arboviral diseases such as Japanese encephalitis (with history of travel to South-East Asia) to be considered as differential diseases.

Disease in animals

Although USUV has been detected in equids and other mammals, clinical features and pathological lesions of USUV infection have been described only in birds (133). Infected birds present as non-specific clinical features like immobility, apathy, ruffled feathers and neurological signs such as ataxia, paresis, tremors, torticollis, inability to fly and seizures (134).

Availability of preventive, therapeutic and control measures, including licensed or pipelines vaccines

Therapy in humans

Treatment in humans is symptomatic. A recent study demonstrated interferon therapy was a potential therapeutic intervention in human and veterinary medicine (135). Favipiravir, a broad-spectrum viral RNA polymerase inhibitor, was shown to reduce USUV load in a mice model (136).

Therapy in animals

Ivermectin has been suggested as a candidate for treatment of USUV infection in captive birds (137).

Licensed or pipelined vaccines

To date, there are no authorized vaccines against USUV infections. A study described the protective effect of a recombinant DNA vaccine against lethal challenge with USUV in alpha/beta interferon receptor deficient mouse model (138).

Another study reported the protective effect of an attenuated WNV-dengue virus 2 chimeric vaccine against USUV in the same mouse model.

However, the efficacy and safety of these vaccine candidates in birds has not been evaluated. Considering the rarity of USUV associated disease in mammals, the use of immunoprophylaxis in humans is not considered to be justified.

Other prevention measures

Blood safety

In EU/EEA, 45 USUV positive blood donations have been reported in Austria (29 with a peak of 20 in 2018), Italy (6 in 2018), and Netherlands (10, with 8 in 2018). None of the EU/EEA countries implemented specific Substances of Human origin (SoHo) safety measures for USUV infection, except the deferral of proven flavivirus positive donations.

However, measures applied to secure the safety of blood and other SoHO donations in relation to the risk of WNV infection including WNV NAT testing could mitigate the likelihood of donor-derived USUV transmission, the areas of co-circulation of the two viruses (33).

Mosquito control and other prevention strategies

Control measures against mosquitoes can be applied preventively (e.g., larval and adult control) before and during the transmission season as the principal vectors of USUV are *Culex* spp. mosquitoes. However, there is little evidence on the direct effect of preventive mosquito control on the intensity of USUV outbreaks (33).

Reactive mosquito control measures (e.g., insecticide treatments in affected areas during outbreaks) is a widely applied method, although the implementation (geographic coverage, timing, repetitions) greatly vary in depending on the legislation of the use of biocides, available resources, and the public and animal health impacts of the outbreaks in different countries.

The use of individual protective measures to prevent mosquito bites (e.g., repellents, nets) and the reduction of mosquito breeding sites (e.g., stagnant water around households) is facilitated by public awareness campaigns at the start of and during the transmission season.

Eleven EU/EEA countries performed mosquito control actions and/or citizen education programmes on mosquito control (33). Among these, Croatia, Czechia, France, Germany, Greece, Italy, Slovakia, and Spain performed both. Malta focused only on citizen education programmes, and Cyprus, Hungary, and Romania solely on control actions.

Citizen education programmes provided information on mosquitoes, bite prevention, and breeding. In certain countries such as Germany, Italy, and Spain, tools or apps have been provided to citizens for reporting mosquito presence and biting incidents.

In some countries the control measures are intensified during detected WNV circulation in avian and mammalian hosts. This also benefits the control of USUV in circulation.

Epidemiological situation at different spatial scales: past and current trends

USUV is endemic in several EU/EEA countries. As of September 2023, 15 out of 30 EU/EEA countries reported USUV circulation among avian and equine animals.

However, human cases in Europe remains sporadic.

The first autochthonous human cases of USUV infection in Europe were reported in 2009 in Italy.

Since then, eight countries including Austria, Croatia, Czechia (131), France, Germany (127, 139), Hungary (132, 140), Italy, and the Netherlands have reported autochthonous cases of USUV infections in humans (33). These eight countries reported a total of 109 cases of acute USUV infection, most of which were reported by Italy (n=56, 54%), Austria (n=26, 25%) and the Netherlands (n=11, 11%) (Table 4).

Table 4. Description of human Usutu cases in EU/EEA countries

Country	Year (ref.)	Cases	Age	Sex	c/o	Symptomatology	Population	Lineage
Italy	2009 (17)	1	60+	F	+	Meningo-encephalitis	Clinical case	EU1
	2009 (18)	1	40+	F	+	Encephalitis	Clinical case	EU2
	2008-9 (121)	3	40	M	+	Meningo-encephalitis	Meningoencephalitis patients	-
			73	M	+			
			54	F	+			
	2008-11 (120)	10	60+	M	+	Meningo-encephalitis /asymptomatic	Meningoencephalitis patients + various healthy and sick subjects	-
	2016-18 (115)	25				Asymptomatic	Blood donors	-
	2017-18 (122)	9				Asymptomatic	Blood donors	EU2/3/4
	2017-18 (118)	8	67	F	+	1 encephalitis, 6 fever, 1 viremia	Suspected infection and +ve donors	EU2
	2018 (123)	2				Asymptomatic	Blood donors	EU2
	2018 (124)	1				Asymptomatic	Blood donors	EU1
	2022 (125)	6				2 fever, 1 asymptomatic	Clinical case	-
Croatia	2013 (119)	3	29	F	none	Meningo-encephalitis	Meningoencephalitis patients	-
			61	M	none			
			56	M	+			
	2018 (126)	3	25	-	none	Neuroinvasive disease	Neuroinvasive cohort	EU2
			84	-	none			
			60	-	+			
Germany	2016 (127)	1				Healthy	Blood donors	EU3
France	2016 (74)	1	39	M	none	Idiopathic facial paralysis	Patients with infectious and/or neurological signs	AF2
	2022 (128)	1				Fever	Clinical case	-
Austria	2017 (129)	6				Asymptomatic	Blood donors	EU2
	2018 (55)	18				Asymptomatic	Blood donors	EU2/ AF3
	2021 (130)	1	81	M	+	Meningitis	Clinical case	EU2
Czech Republic	2018 (131)	1	46	W	none	Meningo-encephalitis	Clinical case	-
Hungary	2018 (132)	1	40+	M	none	Aseptic meningitis	Clinical case	EU2
Nether-lands	2018	7				Asymptomatic	Blood donors	EU3

Sociological and demographical dimension affecting susceptibility and exposure, including gender

Severe neuroinvasive disease following USUV infection is associated with compromised immune systems and advanced age (17, 18, 118). However, no gender predilection has been reported.

Diagnostic procedures and notification systems used at local, national and European scale

Diagnosis

Diagnosing USUV infection in humans relies on the following diagnostic techniques (1): i) the detection of viral RNA in blood and in cerebrospinal fluid (CSF); ii) the isolation of the virus in cell culture and/or; iii) indirect assay detecting anti-USUV antibodies (IgM and G) in the serum and the CSF of patients.

The diagnosis of USUV in humans is challenged by the limited availability of validated commercial tests. USUV serological assays are based on Enzyme-Linked Immunosorbent Assay (ELISA) tests or immunofluorescence tests developed by national reference laboratories, performed with viral antigens or virus isolates (141). However, these tests lack specificity due to the risk of serological cross-reactions with infections by closely related flaviviruses, such as WNV (123, 142). These tests need further confirmation by sero neutralization assays (123, 143). However, WNV NATs validated for donor screening can detect USUV with high sensitivity (144). Direct diagnosis of USUV by isolating the virus in cell cultures such as Vero, BHK-21, and C6/36 cultures and visualizing cytopathic effects. The presence of USUV RNA has been demonstrated in blood, urine, and CSF of patients with acute infection (17, 18, 74, 118-120). Molecular methods include USUV-specific real-time RT-PCR assays, either as a single-target or multiplex test, and broad-range flavivirus RT-PCR followed by amplicon sequencing (121, 144, 145).

Infrastructure capacity to identify pathogens for each Member State

Twenty EU/EEA countries including Austria, Belgium, Croatia, Czechia, Estonia, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Liechtenstein, Lithuania, Malta, the Netherlands, Romania, Slovakia, Slovenia, and Spain have the laboratory capability to detect USUV infection, mostly by the national reference laboratories (146). Diagnosis of USUV infection include USUV specific PCR or broad-range pan-flavivirus PCR and sequencing from blood, tissue and/or CSF. Serological diagnosis detects USUV specific antibody response or the flavivirus antibody response using other cross-reactive serological methods, followed by a neutralization assay.

Four countries (Greece, Germany, Italy, and Norway) developed case definitions for USUV infection in humans based on clinical (encephalitis, meningitis with clear CSF,

polyradiculoneuritis (similar to Guillain-Barre´), acute flaccid paralysis) and laboratory criteria (detection of specific antibodies in serum of CSF, isolation or detection of virus's nucleic acid).

There is need for a standardized case definition for USUV infection in humans in EU/EEA countries.

Currently, USUV is not a major public health threat in the EU/EEA but monitoring virus circulation and its pathogenicity is important to early detect any change in the epidemiology of the disease (146). Thus, not a notifiable disease. Improvements in monitoring might be achieved with a standard case definition of USUV infection in the EU/EEA, and with an integrated approach, including humans, animals, and vectors in USUV surveillance for early detection. Such integrated surveillance systems exist for WNV surveillance in Austria, Croatia, Czechia, France, Germany, Greece, Italy, the Netherlands, and Spain (33). Amongst these nine countries, Croatia, Germany, and Italy have integrated USUV in their WNV surveillance.

Estimated influence of environmental change on the disease future trends

It was unknown how climate change, land use modifications, and biodiversity loss impacts the epidemiology of USUV infection in humans, particularly considering that the weather can influence bird movements, vector abundance and virus replication in the vectors.

References

1. Clé M, Beck C, Salinas S, Lecollinet S, Gutierrez S, Van de Perre P, *et al.* Usutu virus: A new threat? *Epidemiol Infec*t. 2019;147:e232.
2. McIntosh B. Usutu (SA Ar 1776), nouvel arbovirus du groupe B. *Int Cat Arboviruses*. 1985;3:1059-60.
3. Bakran-Lebl K, Camp JV, Kolodziejek J, Weidinger P, Hufnagl P, Cabal Rosel A, *et al.* Diversity of West Nile and Usutu virus strains in mosquitoes at an international airport in Austria. *Transbound Emerg Dis*. 2022 Jul;69(4):2096-109.
4. Calisher CH, Gould EA. Taxonomy of the virus family Flaviviridae. *Adv Virus Res*. 2003;59:1-19.
5. Beck C, Jimenez-Clavero MA, Leblond A, Durand B, Nowotny N, Leparc-Goffart I, *et al.* Flaviviruses in Europe: complex circulation patterns and their consequences for the diagnosis and control of West Nile disease. *Int J Environ Res Public Health*. 2013 Nov 12;10(11):6049-83.
6. Cadar D, Lühken R, van der Jeugd H, Garigliany M, Ziegler U, Keller M, *et al.* Widespread activity of multiple lineages of Usutu virus, western Europe, 2016. *Euro Surveill.* 2017 Jan 26;22(4):30452.
7. Bakonyi T, Gould EA, Kolodziejek J, Weissenböck H, Nowotny N. Complete genome analysis and molecular characterization of Usutu virus that emerged in Austria in 2001: comparison with the South African strain SAAR-1776 and other flaviviruses. *Virology.* 2004 Oct 25;328(2):301-10.
8. Roesch F, Fajardo A, Moratorio G, Vignuzzi M. Usutu virus: an arbovirus on the rise. *Viruses*. 2019 Jul 12;11(7).
9. Smit JM, Moesker B, Rodenhuis-Zybert I, Wilschut J. Flavivirus cell entry and membrane fusion. *Viruses.* 2011 Feb 22;3(2):160-71.
10. Albentosa-González L, Clemente-Casares P, Sabariegos R, Mas A. Polymerase activity, protein-protein interaction, and cellular localization of the Usutu virus NS5 *Protein. Antimicrob Agents Chemother*. 2019 Dec 20;64(1).

11. Savini G, Monaco F, Terregino C, Di Gennaro A, Bano L, Pinoni C, *et al.* Usutu virus in Italy: an emergence or a silent infection? *Vet Microbiol*. 2011 Aug 5;151(3-4):264-74.
12. Weissenböck H, Kolodziejek J, Fragner K, Kuhn R, Pfeffer M, Nowotny N. Usutu virus activity in Austria, 2001-2002. *Microbes Infect*. 2003 Oct;5(12):1132-6.
13. Chvala S, Bakonyi T, Bukovsky C, Meister T, Brugger K, Rubel F, *et al.* Monitoring of Usutu virus activity and spread by using dead bird surveillance in Austria, 2003-2005. *Vet Microbiol*. 2007 Jun 21;122(3-4):237-45.
14. Weissenböck H, Kolodziejek J, Url A, Lussy H, Rebel-Bauder B, Nowotny N. Emergence of Usutu virus, an African mosquito-borne flavivirus of the Japanese encephalitis virus group, central Europe. *Emerg Infect Dis*. 2002 Jul;8(7):652-6.
15. Weissenböck H, Bakonyi T, Rossi G, Mani P, Nowotny N. Usutu virus, Italy, 1996. *Emerg Infect Dis*. 2013 Feb;19(2):274-7.
16. Vilibic-Cavlek T, Petrovic T, Savic V, Barbic L, Tabain I, Stevanovic V, *et al.* Epidemiology of Usutu Virus: The European Scenario. *Pathog Basel Switz*. 2020 Aug 26;9(9).
17. Pecorari M, Longo G, Gennari W, *et al.* First human case of Usutu virus neuroinvasive infection, Italy, August-September 2009. *Euro Surveill*. 2009;14(50):19446.
18. Cavrini F, Gaibani P, Longo G, Pierro AM, Rossini G, Bonilauri P, *et al.* Usutu virus infection in a patient who underwent orthotropic liver transplantation, Italy, August-September 2009. *Euro Surveill*. 2009 Dec 17;14(50):19448.
19. Ashraf U, Ye J, Ruan X, Wan S, Zhu B, Cao S. Usutu virus: an emerging flavivirus in Europe. *Viruses*. 2015 Jan 19;7(1):219-38.
20. Kemenesi G, Buzás D, Zana B, *et al.* First genetic characterization of Usutu virus from Culex pipiens mosquitoes Serbia, 2014. *Infect Genet Evol.* 2018;63:58-61. doi:10.1016/j.meegid.2018.05.012.
21. Scheuch DE, Schäfer M, Eiden M, *et al.* Detection of Usutu, Sindbis, and Batai viruses in mosquitoes (Diptera: Culicidae) collected in Germany, 2011–2016. *Viruses*. 2018;10(7):389.
22. Eiden M, Gil P, Ziegler U, *et al.* Emergence of two Usutu virus lineages in Culex pipiens mosquitoes in the Camargue, France, 2015. *Infect Genet Evol.* 2018;61:151-154.
23. Lecollinet S, Blanchard Y, Manson C, Lowenski S, Laloy E, Quenault H, *et al.* Dual emergence of Usutu virus in common blackbirds, Eastern France, 2015. *Emerg Infect Dis*. 2016 Dec;22(12):2225.
24. Csank T, Drzewnioková P, Korytár Ľ, *et al.* A serosurvey of Flavivirus Infection in horses and birds in Slovakia. *Vector Borne Zoonotic Dis.* 2018;18(4):206-213. doi:10.1089/vbz.2017.2216
25. Bazanow B, van Vuren P, Szymanski P, Stygar D, Fracka A, Twardon J, *et al.* A survey on West Nile and Usutu viruses in horses and birds in Poland. *Viruses*. 2018 Feb;10(2).
26. Rijks JM, Kik ML, Slaterus R, Foppen R, Stroo A, IJzer J, *et al.* Widespread Usutu virus outbreak in birds in the Netherlands, 2016. *Euro Surveill*. 2016 Nov 10;21(45):30391.
27. Michel F, Fischer D, Eiden M, Fast C, Reuschel M, Müller K, *et al.* West Nile Virus and Usutu virus monitoring of wild birds in Germany. *Int J Environ Res Public Health*. 2018 Jan 22;15(1).
28. Benzarti E, Sarlet M, Franssen M, Cadar D, Schmidt-Chanasit J, Rivas JF, *et al.* Usutu virus epizootic in Belgium in 2017 and 2018: evidence of virus endemization and ongoing introduction events. *Vector Borne Zoonotic Dis*. 2020 Jan;20(1):43-50.
29. Garigliany M, Linden A, Gilliau G, Levy E, Sarlet M, Franssen M, *et al.* Usutu virus, Belgium, 2016. *Infect Genet Evol*. 2017 Mar;48:116-9.
30. Chaintoutis SC, Dovas CI, Papanastassopoulou M, Gewehr S, Danis K, Beck C, *et al.* Evaluation of a West Nile virus surveillance and early warning system in Greece, based on domestic pigeons. *Comp Immunol Microbiol Infect Dis*. 2014 Mar;37(2):131-41.

31. Höfle U, Gamino V, de Mera IGF, Mangold AJ, Ortíz JA, de la Fuente J. Usutu virus in migratory song thrushes, Spain. *Emerg Infect Dis.* 2013 Jul;19(7):1173-5.
32. Llorente F, Pérez-Ramírez E, Fernández-Pinero J, Soriguer R, Figuerola J, Jiménez-Clavero MA. Flaviviruses in game birds, southern Spain, 2011-2012. *Emerg Infect Dis*. 2013 Jun;19(6):1023-5.
33. European Centre for Disease Prevention and Control, European Food Safety Authority, *Surveillance, prevention and control of West Nile virus and Usutu virus infections in the EU/EEA*. Stockholm: 2023.
34. Becker N, Jöst H, Ziegler U, Eiden M, Höper D, Emmerich P, *et al.* Epizootic emergence of Usutu virus in wild and captive birds in Germany. *PloS One*. 2012;7(2):e32604.
35. Calzolari M, Bonilauri P, Bellini R, Albieri A, Defilippo F, Maioli G, *et al.* Evidence of simultaneous circulation of West Nile and Usutu viruses in mosquitoes sampled in Emilia-Romagna region (Italy) in 2009. *PloS One*. 2010 Dec 15;5(12):e14324.
36. Tamba M, Bonilauri P, Bellini R, *et al.* Detection of Usutu virus within a West Nile virus surveillance program in Northern Italy. *Vector Borne Zoonotic Dis.* 2011;11(5):551-557. doi:10.1089/vbz.2010.0055.
37. Mancini G, Montarsi F, Calzolari M, Capelli G, Dottori M, Ravagnan S, *et al.* Mosquito species involved in the circulation of West Nile and Usutu viruses in Italy. *Vet Ital.* 2017 Jun 30;53(2):97-110.
38. Klobucar A, Savic V, Curman Posavec M, Petrinic S, Kuhar U, Toplak I, *et al.* Screening of mosquitoes for West Nile virus and Usutu virus in Croatia, 2015-2020. *Trop Med Infect Dis*. 2021 Apr 2;6(2).
39. Camp JV, Kolodziejek J, Nowotny N. Targeted surveillance reveals native and invasive mosquito species infected with Usutu virus. *Parasit Vectors*. 2019 Jan 21;12(1):46.
40. Soto A, De Coninck L, Devlies AS, Van De Wiele C, Rosales Rosas AL, Wang L, *et al.* Belgian Culex pipiens pipiens are competent vectors for West Nile virus while Culex modestus are competent vectors for Usutu virus. *PLoS Negl Trop Dis*. 2023 Sep 20;17(9):e0011649.
41. Calzolari MB Paolo; Bellini, Romeo; Albieri, Alessandro; Defilippo, Francesco; Tamba, Marco; Tassinari, Massimo; Gelati, Antonio; Cordioli, Paolo; Angelini, Paola; Dottori, Michele. Usutu Virus Persistence and West Nile Virus Inactivity in the Emilia-Romagna Region (Italy) in 2011. *PloS One*. 2013;8(5):e63978-NA.
42. Wang L, Rosales Rosas AL, De Coninck L, Shi C, Bouckaert J, Matthijnssens J, *et al.* Establishment of Culex modestus in Belgium and a glance into the virome of Belgian mosquito species. *mSphere.* 2021 Apr 21;6(2).
43. Vazquez A, Jimenez-Clavero M, Franco L, Donoso-Mantke O, Sambri V, Niedrig M, *et al.* Usutu virus: potential risk of human disease in Europe. *Euro Surveill*. 2011 Aug 4;16(31):19935.
44. Sieg M, Schmidt V, Ziegler U, Keller M, Höper D, Heenemann K, *et al.* Outbreak and cocirculation of three different Usutu Virus strains in Eastern Germany. *Vector Borne Zoonotic Dis.* 2017 Sep;17(9):662-4.
45. Jöst H, Bialonski A, Maus D, Sambri V, Eiden M, Groschup MH, *et al.* Isolation of Usutu virus in Germany. *Am J Trop Med Hyg*. 2011 Sep;85(3):551-3.
46. Puggioli A, Bonilauri P, Calzolari M, Lelli D, Carrieri M, Urbanelli S, *et al.* Does Aedes albopictus (Diptera: Culicidae) play any role in Usutu virus transmission in Northern Italy? Experimental oral infection and field evidences. *Acta Trop*. 2017 Aug;172:192-6.
47. Carletti F, Colavita F, Rovida F, Percivalle E, Baldanti F, Ricci I, *et al.* Expanding Usutu virus circulation in Italy: detection in the Lazio region, central Italy, 2017 to 2018. *Euro Surveill*. 2019 Jan;24(3).

48. Pautasso A, Radaelli MC, Ballardini M, Francese DR, Verna F, Modesto P, *et al.* Detection of West Nile and Usutu Viruses in Italian Free Areas: Entomological Surveillance in Piemonte and Liguria Regions, 2014. *Vector Borne Zoonotic Dis*. 2016 Apr;16(4):292-4.

49. Cerutti F, Giacobini M, Mosca A, Grasso I, Rambozzi L, Rossi L, *et al.* Evidence of mosquito-transmitted flavivirus circulation in Piedmont, north-western Italy. *Parasit Vectors*. 2012 May 22;5:99.

50. Busani L, Capelli G, Cecchinato M, Lorenzetto M, Savini G, Terregino C, *et al.* West Nile virus circulation in Veneto region in 2008-2009. *Epidemiol Infect*. 2011 Jun;139(6):818-25.

51. Grisenti M, Vázquez A, Herrero L, Cuevas L, Perez-Pastrana E, Arnoldi D, *et al.* Wide detection of Aedes flavivirus in north-eastern Italy--a European hotspot of emerging mosquito-borne diseases. *J Gen Virol*. 2015 Feb;96(Pt 2):420-30.

52. Busquets N, Alba A, Allepuz A, Aranda C, Ignacio Nuñez J. Usutu virus sequences in Culex pipiens (Diptera: Culicidae), Spain. *Emerg Infect Dis*. 2008 May;14(5):861-3.

53. Engler O, Savini G, Papa A, Figuerola J, Groschup M, Kampen H, *et al.* European surveillance for West Nile virus in mosquito populations. *Int J Environ Res Public Health*. 2013 Oct;10(10):4869-95.

54. Vilibic-Cavlek T, Kaic B, Barbic L, Pem-Novosel I, Slavic-Vrzic V, Lesnikar V, *et al.* First evidence of simultaneous occurrence of West Nile virus and Usutu virus neuroinvasive disease in humans in Croatia during the 2013 outbreak. *Infection*. 2014 Aug;42(4):689-95.

55. Aberle SW, Kolodziejek J, Jungbauer C, Stiasny K, Aberle JH, Zoufaly A, *et al.* Increase in human West Nile and Usutu virus infections, Austria, 2018. *Euro Surveill*. 2018 Oct;23(43).

56. Farajollahi A, Fonseca DM, Kramer LD, Marm Kilpatrick A. "Bird biting" mosquitoes and human disease: a review of the role of Culex pipiens complex mosquitoes in epidemiology. *Infect Genet Evol.* 2011;11(7):1577-1585. doi:10.1016/j.meegid.2011.08.013.

57. Benzarti E, Linden A, Desmecht D, Garigliany M. Mosquito-borne epornitic flaviviruses: an update and review. *J Gen Virol*. 2019 Feb;100(2):119-32.

58. Bakonyi T, Erdélyi K, Ursu K, Ferenczi E, Csörgo T, Lussy H, *et al.* Emergence of Usutu virus in Hungary. *J Clin Microbiol*. 2007 Dec;45(12):3870-4.

59. Buchebner N, Zenker W, Wenker C, Steinmetz HW, Sós E, Lussy H, *et al.* Low Usutu virus seroprevalence in four zoological gardens in central Europe. *BMC Vet Res*. 2013 Aug 6;9:153.

60. Michel F, Sieg M, Fischer D, Keller M, Eiden M, Reuschel M, *et al.* Evidence for West Nile virus and Usutu virus infections in wild and resident birds in Germany, 2017 and 2018. *Viruses*. 2019 Jul 23;11(7).

61. Constant O, Bollore K, Clé M, *et al.* Evidence of exposure to USUV and WNV in zoo animals in France. *Pathogens*. 2020;9(12):1005..

62. Kvapil P, Račnik J, Kastelic M, Bártová E, Korva M, Jelovšek M, *et al.* A Sentinel serological study in selected zoo animals to assess early detection of West Nile and Usutu virus circulation in Slovenia. *Viruses*. 2021 Apr 6;13(4).

63. Bakonyi T, Erdélyi K, Brunthaler R, Dán Á, Weissenböck H, Nowotny N. Usutu virus, Austria and Hungary, 2010-2016. *Emerg Microbes Infect*. 2017 Oct 11;6(10):e85.

64. Chvala S, Kolodziejek J, Nowotny N, Weissenböck H. Pathology and viral distribution in fatal Usutu virus infections of birds from the 2001 and 2002 outbreaks in Austria. *J Comp Pathol.* 2004 Oct;131(2-3):176-85.

65. Hubálek Z, Rudolf I, Čapek M, Bakonyi T, Betášová L, Nowotny N. Usutu virus in blackbirds (Turdus merula), Czech Republic, 2011-2012. *Transbound Emerg Dis*. 2014 Jun;61(3):273-6.

66. Ziegler U, Jöst H, Müller K, Fischer D, Rinder M, Tietze DT, *et al.* Epidemic spread of Usutu Virus in Southwest Germany in 2011 to 2013 and monitoring of wild birds for Usutu and West Nile viruses. *Vector Borne Zoonotic Dis Larchmt N*. 2015 Aug;15(8):481-8.

67. Calzolari M, Gaibani P, Bellini R, Defilippo F, Pierro A, Albieri A, *et al.* Mosquito, bird and human surveillance of West Nile and Usutu viruses in Emilia-Romagna Region (Italy) in 2010. *PloS One*. 2012;7(5):e38058.

68. Rouffaer LO, Steensels M, Verlinden M, Vervaeke M, Boonyarittichaikij R, Martel A, *et al.* Usutu Virus epizootic and Plasmodium coinfection in Eurasian blackbirds (Turdus merula) in Flanders, Belgium. *J Wildl Dis*. 2018 Oct;54(4):859-62.

69. Steinmetz HW, Bakonyi T, Weissenböck H, Hatt JM, Eulenberger U, Robert N, *et al.* Emergence and establishment of Usutu virus infection in wild and captive avian species in and around Zurich, Switzerland--genomic and pathologic comparison to other central European outbreaks. *Vet Microbiol*. 2011 Mar 24;148(2-4):207-12.

70. Ziegler U, Bergmann F, Fischer D, Müller K, Holicki CM, Sadeghi B, *et al.* Spread of West Nile virus and Usutu virus in the German bird population, 2019-2020. *Microorganisms*. 2022 Apr 12;10(4).

71. Garigliany MM, Marlier D, Tenner-Racz K, Eiden M, Cassart D, Gandar F, *et al.* Detection of Usutu virus in a bullfinch (Pyrrhula pyrrhula) and a great spotted woodpecker (Dendrocopos major) in north-west Europe. *Vet J Lond Engl* 1997. 2014 Jan;199(1):191-3.

72. Escribano-Romero E, Jiménez de Oya N, Camacho MC, Blázquez AB, Martín-Acebes MA, Risalde MA, *et al.* Previous Usutu virus exposure partially protects magpies (Pica pica) against West Nile Virus disease but does not prevent horizontal transmission. *Viruses.* 2021 Jul 20;13(7).

73. Schulze C, Ziegler U, Schlieben P, Fast C, Bock S, Schüle A, *et al.* Fatal systemic Usutu virus infection in two juvenile great grey owls (Strix nebulosa) from a German zoological garden. *J Comp Pathol*. 2017 Jan 31;156:113.

74. Simonin Y, Sillam O, Carles MJ, Gutierrez S, Gil P, Constant O, *et al.* Human Usutu virus infection with atypical neurologic presentation, Montpellier, France, 2016. *Emerg Infect Dis*. 2018 May;24(5):875-8.

75. Manarolla G, Bakonyi T, Gallazzi D, Crosta L, Weissenböck H, Dorrestein GM, *et al.* Usutu virus in wild birds in northern Italy. *Vet Microbiol.* 2010 Feb 24;141(1-2):159-63.

76. Lauriano A, Rossi A, Galletti G, Casadei G, Santi A, Rubini S, *et al.* West Nile and Usutu Viruses' surveillance in birds of the province of Ferrara, Italy, from 2015 to 2019. *Viruses*. 2021 Jul 14;13(7).

77. Störk T, de le Roi M, Haverkamp AK, Jesse ST, Peters M, Fast C, *et al.* Analysis of avian Usutu virus infections in Germany from 2011 to 2018 with focus on dsRNA detection to demonstrate viral infections. *Sci Rep*. 2021 Dec 17;11(1):24191.

78. Mancuso E, Cecere JG, Iapaolo F, Di Gennaro A, Sacchi M, Savini G, *et al.* West Nile and Usutu virus introduction via migratory birds: a retrospective analysis in Italy. *Viruses*. 2022 Feb 17;14(2).

79. Saiz JC, Blazquez AB. Usutu virus: current knowledge and future perspectives. *Virus Adapt Treat*. 2017 Oct 16;9:27-40.

80. Giglia G, Mencattelli G, Lepri E, Agliani G, Gobbi M, Gröne A, *et al.* West Nile Virus and Usutu virus: a post-mortem monitoring study in wild birds from rescue centers, Central Italy. *Viruses*. 2022 Sep 9;14(9).

81. Fros JJ, Miesen P, Vogels CB, Gaibani P, Sambri V, Martina BE, *et al.* Comparative Usutu and West Nile virus transmission potential by local Culex pipiens mosquitoes in north-western Europe. *One Health Amst Neth*. 2015 Dec;1:31-6.

82. Martinet JP, Bohers C, Vazeille M, Ferté H, Mousson L, Mathieu B, *et al.* Assessing vector competence of mosquitoes from northeastern France to West Nile virus and Usutu virus. *PLoS Negl Trop Dis*. 2023 Jun;17(6):e0011144.

83. Holicki CM, Scheuch DE, Ziegler U, Lettow J, Kampen H, Werner D, *et al.* German Culex pipiens biotype molestus and Culex torrentium are vector-competent for Usutu virus. *Parasit Vectors*. 2020 Dec 30;13(1):625.

84. Abbo SR, Visser TM, Koenraadt CJM, Pijlman GP, Wang H. Effect of blood source on vector competence of Culex pipiens biotypes for Usutu virus. *Parasit Vectors*. 2021 Apr 8;14(1):194.

85. Wang H, Abbo SR, Visser TM, Westenberg M, Geertsema C, Fros JJ, *et al.* Competition between Usutu virus and West Nile virus during simultaneous and sequential infection of Culex pipiens mosquitoes. *Emerg Microbes Infect*. 2020 Dec;9(1):2642-52.

86. Gaibani P, Rossini G. An overview of Usutu virus. *Microbes Infect*. 2017 Aug;19(7-8):382-7.

87. Montagnaro S, Piantedosi D, Ciarcia R, Loponte R, Veneziano V, Fusco G, *et al.* Serological evidence of mosquito-borne flaviviruses circulation in hunting dogs in Campania Region, Italy. *Vector-borne zoonotic dis*. 2019 Feb 1;19(2):142-7.

88. Knap N, Korva M, Ivović V, Kalan K, Jelovšek M, Sagadin M, *et al.* West Nile Virus in Slovenia. *Viruses*. 2020 Jul 3;12(7):720.

89. Romeo C, Lecollinet S, Caballero J, Isla J, Luzzago C, Ferrari N, *et al.* Are tree squirrels involved in the circulation of flaviviruses in Italy? *Transbound Emerg Di*s. 2018 Oct;65(5):1372-6.

90. Cadar D, Becker N, Campos R de M, Börstler J, Jöst H, Schmidt-Chanasit J. Usutu virus in bats, Germany, 2013. *Emerg Infect Dis*. 2014 Oct;20(10):1771-3.

91. Escribano-Romero E, Lupulović D, Merino-Ramos T, Blázquez AB, Lazić G, Lazić S, *et al.* West Nile virus serosurveillance in pigs, wild boars, and roe deer in Serbia. *Vet Microbiol*. 2015 Apr 17;176(3-4):365-9.

92. Garcia-Bocanegra I, Paniagua J, Gutierrez-Guzman A, Lecollinet S, Boadella M, Arenas-Montes A, *et al.* Spatio-temporal trends and risk factors affecting West Nile virus and related flavivirus exposure in Spanish wild ruminants. BMC *Vet Res*. 2016 Nov 9;12.

93. Bournez L, Umhang G, Faure E, Boucher JM, Boué F, Jourdain E, *et al.* Exposure of wild ungulates to the Usutu and tick-borne encephalitis viruses in France in 2009-2014: evidence of undetected flavivirus circulation a decade ago. *Viruses*. 2019 Dec 19;12(1).

94. Caballero-Gomez J, Terriza D, Pujols J, Martinez-Nevado E, Carbonell M, Guerra R, *et al.* Monitoring of bluetongue virus in zoo animals in Spain, 2007-2019. *Transbound Emerg Dis*. 2022 Jul;69(4):1739-47.

95. Čabanová V, Šikutová S, Straková P, Šebesta O, Vichová B, Zubríková D, *et al.* Co-circulation of West Nile and Usutu flaviviruses in mosquitoes in Slovakia, 2018. *Viruses*. 2019 Jul 12;11(7).

96. Vilibic-Cavlek T, Savic V, Petrovic T, Toplak I, Barbic L, Petric D, *et al.* Emerging trends in the epidemiology of West Nile and Usutu virus infections in Southern Europe. Front *Vet Sci*. 2019 Dec 6;6.

97. Brugueras S, Fernandez-Martinez B, Martinez-de la Puente J, Figuerola J, Porro T, Rius C, *et al.* Environmental drivers, climate change and emergent diseases transmitted by mosquitoes and their vectors in southern Europe: A systematic review. *Environ Res.* 2020 Dec;191.

98. Reiter P. Climate change and mosquito-borne disease. *Environ Health Perspect*. 2001;109(suppl 1):141-61.

99. Mohlmann T, Wennergren U, Talle M, Favia G, Damiani C, Bracchetti L, *et al.* Community analysis of the abundance and diversity of mosquito species (Diptera: Culicidae) in three European countries at different latitudes. *Parasit Vectors*. 2017 Oct 23;10.

100. Esser HJ, Liefting Y, Ibáñez-Justicia A, *et al.* Spatial risk analysis for the introduction and circulation of six arboviruses in the Netherlands. *Parasit Vectors.* 2020;13(1):464. doi:10.1186/s13071-020-04339-0

101. Gangoso L, Aragonés D, Martínez-de la Puente J, *et al.* Determinants of the current and future distribution of the West Nile virus mosquito vector Culex pipiens in Spain. *Environ Res.* 2020;188:109837. doi:10.1016/j.envres.2020.109837.

102. Krol L, Blom R, Dellar M, van der Beek JG, Stroo ACJ, van Bodegom PM, *et al.* Interactive effects of climate, land use and soil type on Culex pipiens/torrentium abundance. *One Health Amst Neth*. 2023 Dec;17:100589.

103. Becker N, Jöst A, Weitzel T. The Culex pipiens complex in Europe. *J Am Mosq Control Assoc*. 2012 Dec;28(4s):53-67.

104. Stead D. Urban planning, water management and climate change strategies: adaptation, mitigation and resilience narratives in the Netherlands. *Int J Sustain Dev World Ecol.* 2014 Jan 2;21(1):15-27.

105. Washburn JO. Regulatory factors affecting larval mosquito populations in container and pool habitats: implications for biological control. *J Am Mosq Control Assoc*. 1995 Jun;11(2 Pt 2):279-83.

106. Ramasamy R, Surendran S. Mosquito vectors developing in atypical anthropogenic habitats: Global overview of recent observations, mechanisms and impact on disease transmission. *J Vector Borne Dis.* 2016 Jun;53(2):91-8.

107. Howard C, Marjakangas EL, Morán-Ordóñez A, Milanesi P, Abuladze A, Aghababyan K, *et al.* Local colonisations and extinctions of European birds are poorly explained by changes in climate suitability. *Nat Commun*. 2023 Jul 20;14(1):4304.

108. Matadamas RE, Enríquez PL, Guevara L, Navarro-Sigüenza AG. Stairway to extinction? Influence of anthropogenic climate change on distribution patterns of montane Strigiformes in Mesoamerica. *Avian Conserv Ecol.* 2022;17(2):37. https://doi.org/10.5751/ACE-02314-170237

109. Nikolay B, Diallo M, Boye CS, Sall AA. Usutu virus in Africa. *Vector Borne Zoonotic Dis.* 2011;11(11):1417-23. doi:10.1089/vbz.2011.0631.

110. Ben Hassine T, De Massis F, Calistri P, Savini G, BelHaj Mohamed B, Ranen A, *et al.* First detection of co-circulation of West Nile and Usutu viruses in equids in the south-west of Tunisia. *Transbound Emerg Dis*. 2014 Oct;61(5):385-9.

111. Durand B, Haskouri H, Lowenski S, Vachiery N, Beck C, Lecollinet S. Seroprevalence of West Nile and Usutu viruses in military working horses and dogs, Morocco, 2012: dog as an alternative WNV sentinel species? *Epidemiol Infect.* 2016 Jul;144(9):1857-64.

112. Mossel EC, Crabtree MB, Mutebi JP, Lutwama JJ, Borland EM, Powers AM, *et al.* Arboviruses isolated from mosquitoes collected in Uganda, 2008-2012. *J Med Entomol*. 2017 Sep 1;54(5):1403-9.

113. Engel D, Jost H, Wink M, Borstler J, Bosch S, Garigliany M, *et al.* Reconstruction of the evolutionary history and dispersal of Usutu Virus, a neglected emerging arbovirus in Europe and Africa. *MBIO*. 2016 Jan;7(1).

114. Ziegler U, Fast C, Eiden M, Bock S, Schulze C, Hoeper D, *et al.* Evidence for an independent third Usutu virus introduction into Germany. *Vet Microbiol.* 2016 Aug 30;192:60-6.

115. Domanović D, Gossner CM, Lieshout-Krikke R, Mayr W, Baroti-Toth K, Dobrota AM, *et al.* West Nile and Usutu virus infections and challenges to blood safety in the European Union. *Emerg Infect Dis*. 2019 Jun;25(6):1050-7.

116. Lustig Y, Sofer D, Bucris ED, Mendelson E. Surveillance and diagnosis of West Nile Virus in the face of flavivirus cross-reactivity. *Front Microbiol*. 2018;9:2421.

117. Cadar D, Simonin Y. Human Usutu virus infections in Europe: a new risk on horizon? *Viruses*. 2022 Dec 27;15(1).

118. Pacenti M, Sinigaglia A, Martello T, De Rui ME, Franchin E, Pagni S, *et al.* Clinical and virological findings in patients with Usutu virus infection, northern Italy, 2018. *Euro Surveill*. 2019 Nov;24(47).

119. Santini M, Vilibic-Cavlek T, Barsic B, Barbic L, Savic V, Stevanovic V, *et al.* First cases of human Usutu virus neuroinvasive infection in Croatia, August-September 2013: clinical and laboratory features. *J Neurovirol*. 2015 Feb;21(1):92-7.

120. Grottola A, Marcacci M, Tagliazucchi S, *et al.* Usutu virus infections in humans: a retrospective analysis in the municipality of Modena, Italy. *Clin Microbiol Infect.* 2017;23(1):33-7. doi:10.1016/j.cmi.2016.09.019

121. Cavrini F, Della Pepa ME, Gaibani P, Pierro AM, Rossini G, Landini MP, *et al.* A rapid and specific real-time RT-PCR assay to identify Usutu virus in human plasma, serum, and cerebrospinal fluid. *J Clin Virol*. 2011 Mar;50(3):221-3.

122. Faggioni G, De Santis R, Pomponi A, Grottola A, Serpini GF, Meacci M, *et al.* Prevalence of Usutu and West Nile virus antibodies in human sera, Modena, Italy, 2012. *J Med Virol*. 2018 Oct;90(10):1666-8.

123. Percivalle E, Cassaniti I, Sarasini A, Rovida F, Adzasehoun KMG, Colombini I, *et al.* West Nile or Usutu Virus? A Three-Year Follow-Up of Humoral and Cellular Response in a Group of Asymptomatic Blood Donors. *Viruses*. 2020 Jan 29;12(2).

124. Caracciolo I, Mora-Cardenas E, Aloise C, Carletti T, Segat L, Burali MS, *et al.* Comprehensive response to Usutu virus following first isolation in blood donors in the Friuli Venezia Giulia region of Italy: Development of recombinant NS1-based serology and sensitivity to antiviral drugs. *PLoS Negl Trop Dis*. 2020 Mar;14(3):e0008156.

125. Bella A, Venturi G, Riccardo F, Iapaolo F, F. Monaco FF, Calistri P. *Sorveglianza integrata del West Nile e Usutu virus. Risultati nazionali. Bollettino n. 20 del 2 novembre 2022.* Istituto Superiore di Sanità, IZS Teramo; 2022.

126. Vilibic-Cavlek T, Savic V, Sabadi D, Peric L, Barbic L, Klobucar A, *et al.* Prevalence and molecular epidemiology of West Nile and Usutu virus infections in Croatia in the 'One health' context, 2018. *Transbound Emerg Dis*. 2019 Sep;66(5):1946-57.

127. Cadar D, Maier P, Müller S, Kress J, Chudy M, Bialonski A, *et al.* Blood donor screening for West Nile virus (WNV) revealed acute Usutu virus (USUV) infection, Germany, September 2016. *Euro Surveill*. 2017 Apr 6;22(14):30501.

128. Agence Régionale de Santé – Nouvelle Aquitaine. Confirmation d'une infection autochtone à virus Usutu (secteurs des Landes et de Gironde). *Communiqué de presse,* 9 novembre 2022. Available from: https://www.nouvelle-aquitaine.ars.sante.fr/media/102015/download?inline; last visited July 2023.

129. Bakonyi T, Jungbauer C, Aberle SW, Kolodziejek J, Dimmel K, Stiasny K, *et al.* Usutu virus infections among blood donors, Austria, July and August 2017 - Raising awareness for diagnostic challenges. *Euro Surveill*. 2017 Oct;22(41).

130. Graninger M, Hubmer S, Riederer F, Kettner S, Hauk M, Auf T, *et al.* The first case of Usutu virus neuroinvasive disease in Austria, 2021. *Open Forum Infect Dis*. 2022 Jul;9(7):ofac255.

131. Zelená H, Kleinerová J, Šikutová S, *et al.* First autochthonous West Nile lineage 2 and Usutu virus infections in humans, July to October 2018, Czech Republic. *Pathogens*. 2021;10(6):651. doi:10.3390/pathogens10060651.

132. Nagy A, Mezei E, Nagy O, Bakonyi T, Csonka N, Kaposi M, *et al.* Extraordinary increase in West Nile virus cases and first confirmed human Usutu virus infection in Hungary, 2018. *Euro Surveill*. 2019 Jul;24(28).

133. Agliani G, Giglia G, Marshall EM, Gröne A, Rockx BHG, van den Brand JMA. Pathological features of West Nile and Usutu virus natural infections in wild and domestic animals and in humans: A comparative review. *One Health.* 2023;16:100525. doi:10.1016/j.onehlt.2023.100525

134. Giglia G, Agliani G, Munnink BBO, Sikkema RS, Mandara MT, Lepri E, *et al.* Pathology and Pathogenesis of Eurasian Blackbirds (Turdus merula) Naturally Infected with Usutu Virus. *Viruses*. 2021 Jul 28;13(8).

135. Wald ME, Sieg M, Schilling E, Binder M, Vahlenkamp TW, Claus C. The interferon response dampens the Usutu virus infection-associated increase in glycolysis. *Front Cell Infect Microbiol.* 2022;12:823181.

136. Segura Guerrero NA, Sharma S, Neyts J, Kaptein SJF. Favipiravir inhibits *in vitro* Usutu virus replication and delays disease progression in an infection model in mice. *Antiviral Res*. 2018 Dec;160:137-42.

137. Wald ME, Claus C, Konrath A, Nieper H, Muluneh A, Schmidt V, *et al.* Ivermectin inhibits the replication of Usutu virus *in vitro*. *Viruses*. 2022 Jul 27;14(8).

138. Martín-Acebes MA, Blázquez AB, Cañas-Arranz R, Vázquez-Calvo Á, Merino-Ramos T, Escribano-Romero E, *et al.* A recombinant DNA vaccine protects mice deficient in the alpha/beta interferon receptor against lethal challenge with Usutu virus. *Vaccine*. 2016 Apr 19;34(18):2066-73.

139. Allering L, Jöst H, Emmerich P, Günther S, Lattwein E, Schmidt M, *et al.* Detection of Usutu virus infection in a healthy blood donor from south-west Germany, 2012. *Euro Surveill*. 2012 Dec 13;17(50):20341.

140. Nagy A, Csonka N, Takács M, Mezei E, Barabás É. West Nile and Usutu virus seroprevalence in Hungary: A nationwide serosurvey among blood donors in 2019. *PloS One*. 2022;17(4):e0266840.

141. Europe. Directive 2002/98/EC of the European Parliament and of the Council of 27 January 2003 setting standards of quality and safety for the collection, testing, processing, storage and distribution of human blood and blood components and amending Directive 2001/83/EC. *Official Journal of the European Union* L 33, 8.2.2003

142. Berneck BS, Rockstroh A, Barzon L, Sinigaglia A, Vocale C, Landini MP, *et al.* Serological differentiation of West Nile virus- and Usutu virus-induced antibodies by envelope proteins with modified cross-reactive epitopes. *Transbound Emerg Dis*. 2022 Sep;69(5):2779-87.

143. Sinigaglia A, Pacenti M, Martello T, Pagni S, Franchin E, Barzon L. West Nile virus infection in individuals with pre-existing Usutu virus immunity, northern Italy, 2018. *Euro Surveill.* 2019 May;24(21).

144. Zaaijer HL, Slot E, Molier M, Reusken CBEM, Koppelman MHGM. Usutu virus infection in Dutch blood donors. *Transfusion*. 2019 Sep;59(9):2931-7.

145. Del Amo J, Sotelo E, Fernández-Pinero J, Gallardo C, Llorente F, Agüero M, *et al.* A novel quantitative multiplex real-time RT-PCR for the simultaneous detection and differentiation of West Nile virus lineages 1 and 2, and of Usutu virus. *J Virol Methods.* 2013 May;189(2):321-7.

146. Angeloni G, Bertola M, Lazzaro E, Morini M, Masi G, Sinigaglia A, *et al.* Epidemiology, surveillance and diagnosis of Usutu virus infection in the EU/EEA, 2012 to 2021. *Euro Surveill*. 2023 Aug;28(33).

WEST NILE DISEASE

Annapaola Rizzoli (a), Giulia Mencattelli (a, b, c), Daniele Arnoldi (a), Maria Bellenghi (d), Claudia Cataldo (d), Francesca Dagostin (a), Di Luca Marco (e), Mitra Drakulovic (f), Soushieta Jagadesh (g), Giovanni Marini (a), Beatriz Fernandez Martinez (h), Valentina Tagliapietra (a), William Wint (i), Luca Busani (d)
(a) Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige, Trento
(b) Centro Agricultura, Alimenti, Ambiente, Università di Trento, San Michele all'Adige, Trento
(c) Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo,
(d) Centro di Riferimento per la Medicina di Genere, Istituto Superiore di Sanità, Rome
(e) Dipartimento Malattie Infettive, Istituto Superiore di Sanità, Rome
(f) Department for Communicable Diseases Prevention and Control, National Public Health Institute "Dr Milan Jovanovic-Batut", Belgrade
(g) International Society of Infectious Diseases, Boston (MA), United States
(h) Centro Nacional de Epidemiología, Instituto de Salud Carlos III, Consorcio de Investigación Biomédica en Red Epidemiología y Salud Pública, Madrid
(i) Department of Biology, Environmental Research Group Oxford Ltd, Oxford

Biological, ecological and molecular features of the causative agent

Disease name

West Nile virus infection; West Nile virus disease; West Nile fever (WNF); West Nile virus neuroinvasive disease (WNND).

Disease agent

Common, scientific and Latin name

West Nile virus (WNV).

Taxonomy

WNV is a mosquito-borne virus, part of the genus *Flavivirus,* family *Flaviviridae* and member of the Japanese Encephalitis antigenic serocomplex (1).

Disease agent characteristics

WNV is an enveloped virus of about 50 nm in diameter with an icosahedral symmetry. Like for other Flaviviruses, the genome of WNV is a single-stranded, positive-sense RNA, of about 11 kilobases (kb) enclosed in a nucleocapsid containing one long single open reading frame (ORF) confined at each side by one 5' and one 3' non coding-region (UTR), respectively (23). The single ORF encodes for a polyprotein that is processed in three structural proteins (C, preM, and E), and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (2). Each of the viral proteins, either structural or non-structural, plays a different and specific role in the biology and/or the pathogenesis of WNV infections. The non-structural proteins are involved in viral replication, virion assembly, and in mechanisms of host's immune response evasion (2), while

the three structural proteins make up the mature virion. In particular, the proteins M and E are responsible for several virus properties, like host specificity, tissue tropism, replication capacity, and cells T and B response induction (4).

WNV is part of the Japanese encephalitis virus serocomplex, sharing cross-neutralization antibodies with viruses which are able to cause encephalitis in humans, as well as viruses which rarely cause human disease, such as the Usutu virus (5).

Physiochemical properties

WNV is rapidly inactivated in the environment. Low temperatures preserve infectivity, with stability being greatest below -60°C. It is inactivated by heat (50 to 60°C for at least 30 minutes), ultraviolet light, and gamma irradiation.

The virus is also susceptible to disinfectants such as 3 to 8% formaldehyde, 2% glutaraldehyde, 2 to 3% hydrogen peroxide, 500 to 5,000 ppm available chlorine, alcohol, 1% iodine, and phenol iodophors. When added to Enzyme-Linked Immunosorbent Assay (ELISA) wash buffer there is a 10-fold decrease in titre per 24-hour period at 28°C. Ribavirin and interferon can inhibit WNV *in vitro*.

WNV is classified as a risk group level 3, requiring handling with biosafety level (BSL) 3 precautions (6).

Priority level for EU

WNV is considered a re-emerging public health challenge and a future health threat with significant economical implication in the European Union (EU) also in relation to the need to guarantee the quality and safety of substances of human origin intended for humans' application (SoHO) and thus to be monitored by epidemiological surveillance following a One Health integrated approach (7-14).

WNV infections became notifiable in the European Union (EU) since 2008, but surveillance with EU coverage was achieved later. The EU countries report human cases to the European Centre for Disease Prevention and Control (ECDC) which, in turn, produces annual epidemiological summaries and, since 2011, weekly surveillance updates during the WNV transmission season (May to November). EU countries also report animal outbreaks, mainly based on equine and birds' surveillance through the Animal Disease Information System (ADIS https://food.ec.europa.eu/animals/animal-diseases/animal-disease-information-system-adis_en).

Within the framework of the EU enlargement cooperation, EU enlargement countries also report human infections to the ECDC. The main objective of timely WNV surveillance at the EU level is to provide early warning to public health professionals about areas with human WNV infections thereby preventing human-to-human transmission via donation of contaminated SoHO. The EU blood safety directive obliges blood establishments to defer donors for 28 days after leaving an area where human cases were detected unless an individual donation nucleic acid test is negative (8, 12).

Distribution of the pathogen

Currently WNV is the widest distributed arbovirus globally, being found from tropical to north temperate latitudes on all the continents except Antarctica (15, 16). The virus itself has undergone

adaptive genetic changes while expanding its geographic distribution. The epidemic of 1999 in the New York City area and its expansion and establishment throughout the American continent highlighted the ability of this virus to leap from one hemisphere to another (17). WNV circulation in European Member States is known since 1958 and EU enlargement countries after 1972 (18) with an increase in the number of countries reporting local WNV transmission either in humans and animals in recent years (19, 20, 12, 21).

Since December 2022, at European scale, WNV human and animal infection have been reported in at least 15 EU countries including Austria, Bulgaria, Croatia, Cyprus, Czech Republic, France, Germany, Greece, Hungary, Italy, the Netherlands, Portugal, Romania, Slovenia and Spain, other than in 5 neighbouring EU candidate countries including Albania, Montenegro, Serbia (22), Turkey and Kosovo* (Figure 1 and Figure 2).

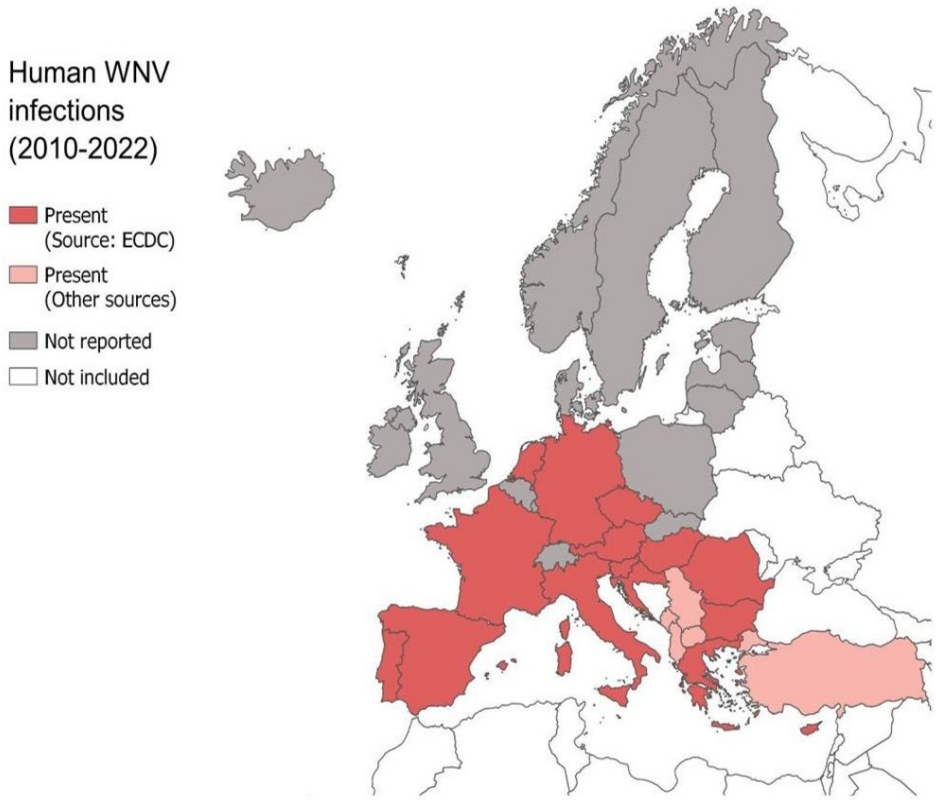

Figure 1. Countries of the European Union and European Economic Area that reported at least one locally acquired WNV human infection in the period 2010-2022. (Data from TESSy-ECDC and from other sources as literature and national reports)

* This designation is without prejudice to positions on status and is in line with United Nations Security Council Resolution 1244/99 and the International Court of Justice Opinion on the Kosovo declaration of independence.

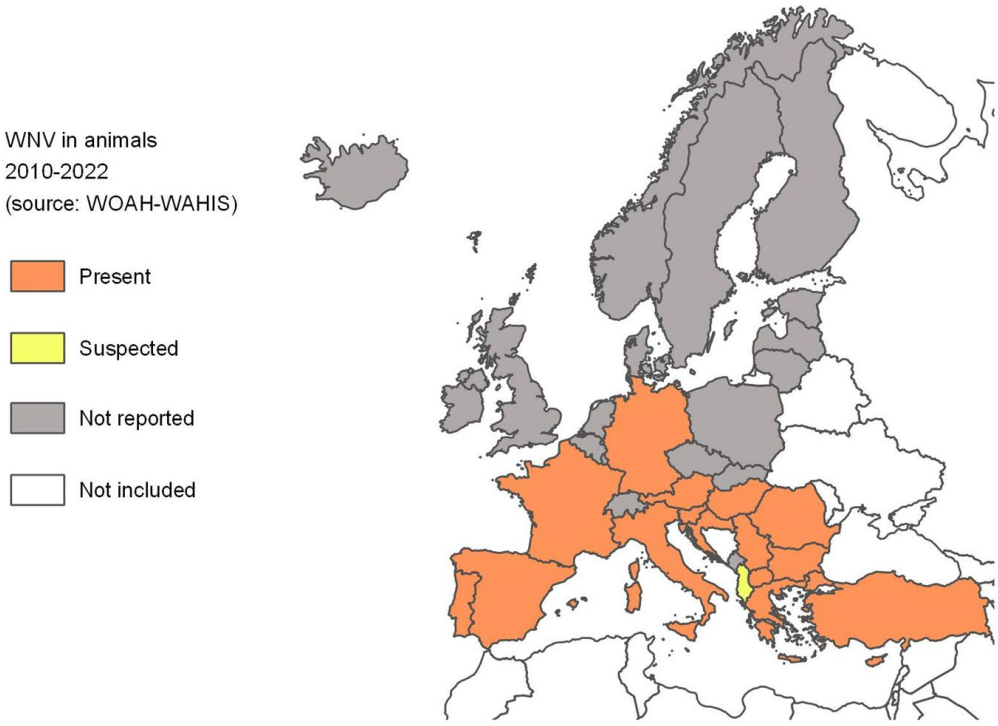

Figure 2. Distribution of WNV infections in animal hosts in the period 2010-2022, based on the data contained on the official reports (immediate notifications and follow-up reports, six-monthly reports and annual reports) submitted by the relevant Veterinary Services through the WAHIS-WOAH

Diversity and distribution of WNV lineages in Europe

WNV is characterized by high genetic diversity. Like other RNA viruses lacking proofreading replication, the WNV genome displays extraordinary adaptability with many variants that have evolved independently in different parts of the world. Phylogenetic analyses identified at least eight evolutionary lineages of which WNV lineages 1 (WNV-1) and 2 (WNV-2) are the most widespread and pathogenic, causing several outbreaks in humans and animals around the world (23-25). Moreover, studies have shown the high virulence of WNV-7, nowadays classified as a distinct flavivirus called Koutango virus (KOUTV), in mice and a potential risk for humans has been highlighted following a severe accidental infection in a Senegalese lab worker (26). The KOUTV is exclusively present in Africa, circulating in Senegal, Gabon, Somalia, and Niger, but its invasion into other Continents could represent a possible future threat worldwide (27).

In Europe, the maximum likelihood tree of all available WNV genome sequences showed that six lineages were detected so far in the Continent (25). Among them, WNV-1 and WNV-2 are the most widespread, responsible for an increasing number of outbreaks and deaths either in humans and animals. As the two lineages move from one area to another, principally through reservoir bird migration, strains of different origins can co-circulate in particular areas as seen in Italy (23,

24, 25). Back-and-forth exchanges between West Africa and Western-Mediterranean European countries have been recently proved for WNV-1 while WNV- 2 seem to be historically characterized by two major independent one-way introductions from South Africa to Central Europe (Hungary), from where the lineage then spread, established, and co-circulated in many European countries (28).

WNV-2 accounts for 82% of all WNV sequences detected in the Continent so far, and the widest distribution since it has been found in 15 European countries (25).

The earliest WNV-2 genome (JX041631.1) was obtained from bird samples of Eastern Europe (Ukraine) in 1980 (25). The sub-lineage 2a (WNV-2a) emerged in 2004 in Hungary becoming in the past ten years the dominant lineage in Europe (29, 30). In addition, the sub-lineage 2b (WNV-2b) was reported and composed of sequences mainly of Romania, Italy, and Russia (2011-2015), Serbia (2013), and Greece (2018) (25, 29). WNV-2a has been involved in the exceptional number of infections occurred in 2018, a year characterized by an early start of the epidemic season and by the largest WNV outbreak observed so far in Central and Southern Europe, with over 2,000 symptomatic human cases, most of them reported in Italy and Serbia (24, 25, 31-33). The same year, WNV-2a emerged also in France and it was associated with the most important human WNV epidemics identified so far (34). In Spain, WNV-2a has only been detected in birds and mosquito in the Northeast (Catalonia) (35, 36).

In comparison, WNV-1 positive samples have been found in seven European countries (Austria, Italy, Spain, France, Hungary, Romania and Portugal) since 1971. Most WNV-1 sequences have been obtained in Italy (72% of total WNV-1 sequences), where the strain first appeared in Europe in 1998 (37). Most European WNV outbreaks were caused by this lineage, up to 2010, when the WNV-2 increased its circulation in the following year, causing serious epidemics in many European countries (34). Recently, WNV-1 is re-emerging in areas characterized by long unnoticed circulation as well as in new European countries and it can be associated with severe neurological forms and deaths in humans and animals (24, 23, 38). However, evidence of differences in WNV-1 and WNV-2 severity is still lacking.

WNV sequences belonging to lineages 3, 4, 8 and 9 were only sporadically reported and all of them were collected from mosquitoes. WNV-3 strains were only found in Czech Republic in 1997 and 2006; WNV-4 in Romania in 2012-13; WNV-8 in Spain in 2006, while WNV-9 genomes were obtained in Hungary in 2011 and in Austria in 2013. In addition, up to 2021, these lineages were only collected from non-human hosts (mainly birds, mosquitoes, and some equines) (25).

Ecology and transmission routes

WNV is an ecological generalist, and it is characterized by a complex eco-epidemiology, being adaptable to different vector and host species, principally mosquitoes of the genus *Culex* and birds (Figure 3). Its circulation in Europe is usually associated with two main different cycles and habitats: the rural/sylvatic and the urban synanthropic cycles. Rural locations, including river deltas and floodplain areas, help creating the sylvatic cycle, with wild, usually nesting wetland, birds and ornithophilic mosquitoes maintaining the viral transmission. Irrigation from agriculture is also heavily linked to a greater incidence of human and veterinary WNV infections (20, 39). In urban synanthropic cycles, mosquitoes feeding preference shift can determine the enhancement of the viral transmission to humans (7). However, these two cycles can overlap, so urban areas located close to wetlands and irrigated croplands can be particularly affected by the virus circulation.

Humans and equids are highly susceptible to the infection but do not develop a sufficient level of viremia to further infect mosquitoes and are therefore considered dead-end non-competent

hosts. A minimum viremia level of 10^4-10^5 PFU/mL has been established as necessary for infecting feeding *Culex* mosquitoes and thus allowing further virus transmission (40).

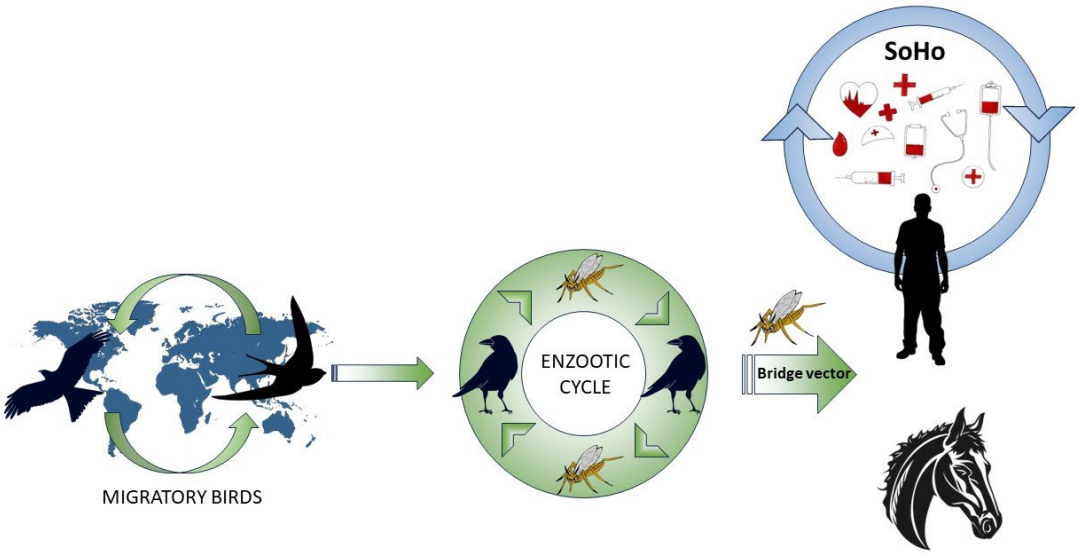

Figure 3. West Nile virus transmission cycle

Mosquitoes mostly acquire infection horizontally, while feeding on an infected host, but vertical transmission (passage of virus from female parent directly to offspring) is also possible, although apparently less efficient (23, 41-43).

Several abiotic and biotic factors, such as climatic condition, habitat structure, anthropization, land cover and management, host communities' composition, host herd immunity, host behaviour, mosquito community composition, and vector host preferences, affect the virus transmission within vectors and hosts (7, 42, 44-49).

Seasonality in WNV transmission is also dependent on vector mosquitoes' annual phenology, which can vary according to the species and the local environmental condition. For example, a recent study provides new evidence that urban warming may inhibit the *Cx. pipiens* diapause initiation during autumn, thereby extending the active biting season of temperate mosquitoes (50).

Because of these features, outbreaks of WNV infection exhibit high variability in their extension across different regions and time (45).

Vectors

Mosquito species of the genus *Culex* have a worldwide distribution and are considered among the species of greater medical importance as they can act as vectors for various zoonotic arboviruses from several virus families (51,52). Among them, *Cx. pipiens* mosquito species are considered the most important WNV vectors in Europe (53). Transmission rates vary between 0 and 60% with no intrinsic difference in vector competence between northern and Southern populations (48). Moreover, the species comprise two behaviourally different biotypes, *Cx. pipiens* (more ornithophilic and abundant in natural habitat) and *Cx. molestus* (preference for

mammals and more opportunistic feeding behaviour) which can form hybrids. Biotype *molestus* and hybrids *pipiens-molestus* are thought to play a more important role in WNV spillover from birds to humans especially in urban and periurban areas (7, 54), other than in overwintering mechanisms (23). No difference has been observed in the vector competence among the two biotypes. However, higher temperatures increase the transmission rates of biotype *pipiens* and hybrids, but not of biotype *molestus*, the latter mainly distributed in Southern and Central European countries, and considered an efficient WNV "bridge" vector, being able to transmit the pathogen between birds as well as from birds to mammals, including humans with high transmission rates of 40-55% (52).

This shows the importance of identifying *Cx. pipiens* at the biotype level and suggests that closely related mosquitoes may have different vector competence under variable climatic conditions (48, 54-56).

Among other *Culex* species, *Cx. torrentium* also exhibit high WNV transmission values observed in the laboratory, with rates of 17% (24°C) and 24% (27°C) although its role needs to be confirmed with further field studies (51). In Europe, *Cx. torrentium* usually occurs together with *Cx. pipiens*, with the first being the dominant species in northern Europe and the second prevailing in regions south of the Alps. In Central Europe such as Austria or Germany, both sister species can be found in sympatry (51, 57). It is therefore necessary to distinguish among the two species using molecular techniques (53).

Cx. modestus, which is distributed mainly in Southern and Central European countries, is also considered an efficient WNV "bridge" vector, being able to transmit pathogens between birds as well as from birds to mammals, including humans with high transmission rates of 40-55% (52).

Finally, *Cx. perexiguus* is established in Southwest Spain, related to rice fields. In some areas where the disease is endemic, it has been found to play an important role as the main human vector (58). In Figures 4 and 5, maps with the probability of occurrence of *Cx. torrentium, Cx modestus* and *Cx. pipiens* in Europe are reported.

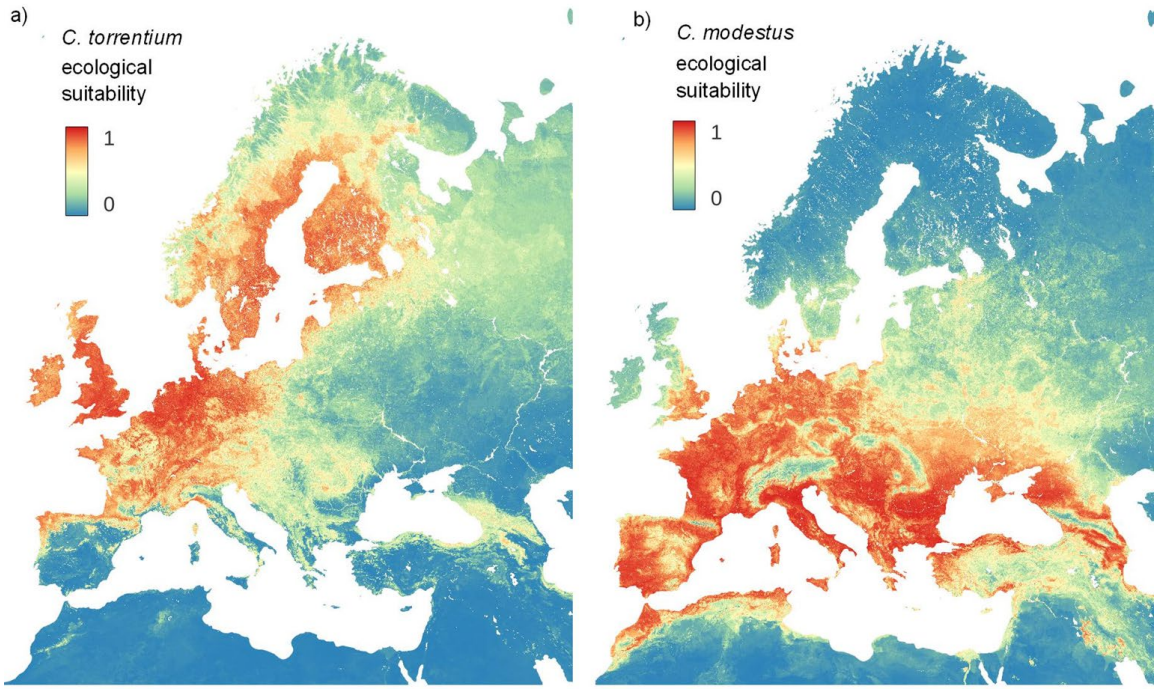

Figure 4. Current 1-km probability of presence of *Cx. torrentium and Cx modestus* across Europe, produced using random forest and boosted regression trees analyses (source ERGO group)

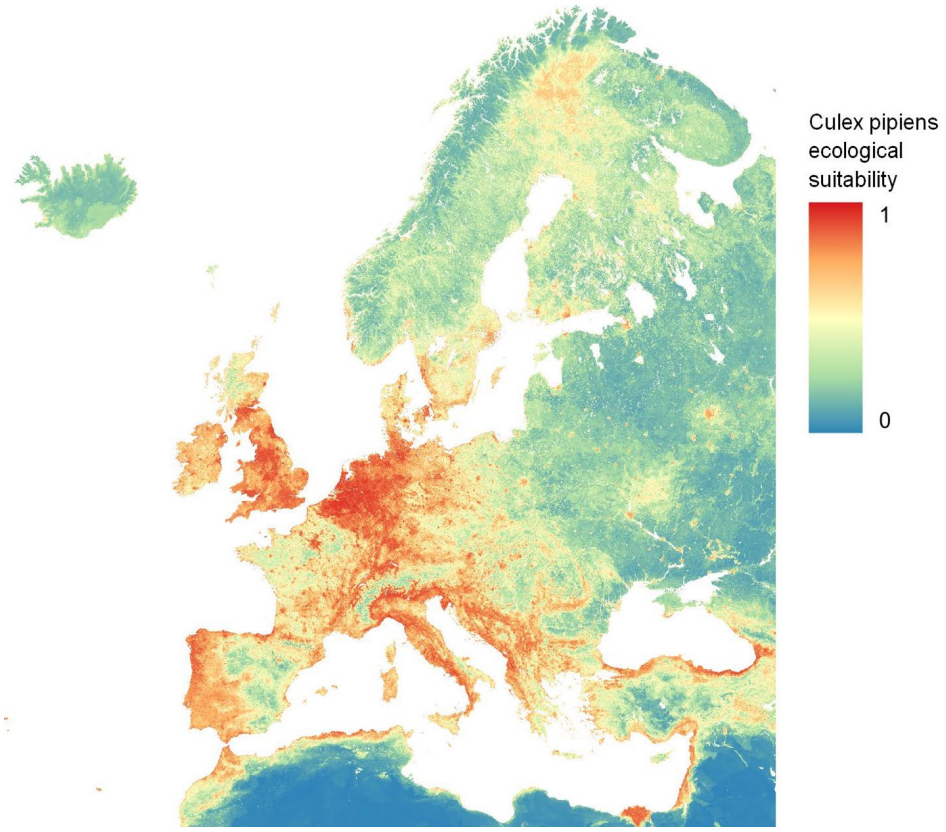

Figure 5. Current 1-km probability of presence of *Cx. pipiens* across Europe, produced using random forest and boosted regression trees analyses (source ERGO group)

WNV vector competence and transmission rates have also been compared for various European mosquito species other than *Culex* species as in the case of *Aedes* (*Ae. albopictus, Ae. detritus, Ae. japonicus japonicus, Ae. vexans)* and *Ochlerotatus caspius* (43, 48, 51, 53, 57). In some experimental studies, *Ae. japonicus*, which has recently spread over considerable parts of Central Europe, has proven to be more efficient than *Cx. pipiens* (63, 76). *Ae. vexans* may also be epidemiologically important due to its generally high abundance despite its moderate competence proved in laboratory (43).

Although vector competence has been determined for a limited number of species and populations of the same mosquito species in different areas, WNV has been isolated from, or detected in, field-collected specimens of numerous mosquito species native to Europe, as shown in Table 1.

WNV has also been isolated from other arthropods other than mosquitoes including hard ticks (*Hyalomma marginatum* and *Rhipicephalus sanguineus*), soft ticks (*Ornithodoros maritimus* and *Argas hermanni*), swallow bug (*Cimex hirundinis*), and chicken mites (*Ornithonyssus sylviarum*). However, the contribution of ticks in the natural WNV transmission cycles and dispersal has not been clarified yet (16, 23).

Table 1. A list of mosquito species confirmed as competent vectors for West Nile virus in Europe

Genus	Species	Reference
Culex	*pipiens*	(59-63)
Culex	*pipiens pipiens*	(48, 64)
Culex	*pipiens molestus*	(64)
Culex	*pipiens hybrid (pip x mol)*	(64)
Culex	*modestus*	(62, 63, 65)
Culex	*perexiguus*	(66, 67)
Culex	*quinquefasciatus*	(68)
Culex	*torrentium*	(51, 69)
Culex	*theileri*	(63)
Culex	*univittatus*	(67)
Culex	*europaeus*	(70)
Culex	*tritaeniorhynchus*	(71)
Aedes	*vexans*	(55, 61, 63, 68)
Aedes	*caspius*	(62, 63)
Aedes	*albopictus*	(61, 63, 72)
Aedes	*detritus*	(63, 73)
Aedes	*dorsalis*	(63, 74)
Aedes	*geniculatus*	(63, 75)
Aedes	*japonicus*	(63, 76)
Aedes	*punctor*	(75)
Aedes	*cinereus*	(77)
Uranotaenia	*unguiculata*	(78)
Anopheles	*plumbeus*	(63, 75)
Anopheles	*maculipennis*	(77, 79, 56)
Coquillettidia	*richiardii*	(20, 79)
Ochlerotatus	*cantans*	(20, 77)
Ochlerotatus	*sticticus*	(70)
Culiseta	*longiareolata*	(20)
Culiseta	*morsitans*	(20)

Hosts

WNV is able to infect a wide range of animal species, including birds, mammals, reptiles and amphibians, which can be infected, develop antibodies and, in some cases, clinical signs. However, several infected hosts are considered dead-end hosts since they are unable to further transmit the virus to mosquitoes (80). Competent reservoir hosts are found especially among bird species and it is generally acknowledged that *Passeriformes* (especially *Corvidae*, *Fringillidae,* and *Passeridae* families) *Charadriiformes* (*Laridae*), *Falconiformes,* and *Strigiformes* include highly competent species, although viraemic levels vary depending on species, viral lineage and strain (40, 80, 81). Domestic birds are generally less susceptible to infection; domestic chickens and turkeys do not develop clinical infection, while domestic geese, ducks and companion birds (e.g. *Psittaciformes*) can suffer encephalitis. Among mammals, high and long-lasting viremia sufficient to potentially infect vectors has been experimentally and/or naturally demonstrated in lemurs, small rodents, lagomorphs, amphibians, and reptiles (reviewed in 40, 80, 2, 82). However, most of experimental infections to assess reservoir competence have been conducted on bird species and rarely on wild mammals in Europe (40, 82-86). As such, the role of wild mammals as potential WNV amplifying hosts is uncertain, with most experimental infections resulting in detectable antibodies but no to low-level of viremia. Certain mammalian species, such as tree

squirrels, mesopredators, pigs, wild boars, and roe deer have been suggested as species of interest for WNV serological surveillance (87).

In Table 2 a list of European vertebrate species susceptible to WNV infection and with a viremia level above 4 log10 plaque-forming unit (PFU/mL) is reported. According to (40), species with viremia level ≤ 4 log10 PFU/mL (low viremia) are considered non-competent hosts. Species with medium viremia (mean peak viremia 4-6 log10 PFU/mL) and high viremia (mean peak viremia > 6 log10 PFU/mL) are considered competent hosts.

Table 2. List of vertebrate species with reported WNV medium and high viremia level

Genera	Species	Common name	References	Note
Alectoris	*rufa*	Red-legged partridge	(40, 89)	
Anas	*platyrhynchos*	Mallard	(40, 90)	
Anser	*anser*	Common goose	(40)	
Anser	*anser domesticus*	Domestic geese	(100)	in 1- to 11-day old chicks
Branta	*canadensis*	Canada goose	(40, 90)	
Charadrius	*vociferus*	Killdeer	(90)	migratory through Europe
Columba	*livia*	Rock pigeon	(40, 92)	
Columba	*livia*	Rock dove	(90, 102)	
Corvus	*monedula*	European jackdaws	(83)	
Corvus	*cornix*	Hooded crow	(40)	
Corvus	*corone*	Carrion crows	(104)	
Coturnix	*coturnix*	Quails	(95)	
Falco	*rusticolus*	Gyrfalcons	(38, 40)	
Falco	*rusticolus, cherrug*	Hybrid falcon	(38)	
Falco	*rusticolus, peregrinus*	Hybrid falcon	(38)	
Felis	*catus*	Domestic cat	(99)	10^3 to 10^4 PFU/mL
Gallus	*gallus*	Chickens	(101)	young chickens - 7-week old
Larus	*delawarensis*	Ring-billed gull	(90)	occasionally migrate through Europe
Mesocricetus	*auratus*	Hamster	(96, 97)	10^5 TCID 50/mL
Mus	*musculus*	Lab mouse	(105)	
Passer	*domesticus*	House sparrow	(40, 84, 90)	
Pica	*pica*	Magpie	(103)	
Procyon	*lotor*	Racoon	(98)	
Rana	*ridibunda*	Lake frog	(94, 95)	
Sciurus	*carolinensis*	Grey squirrel	(88)	
Streptopelia	*decaocto*	Eurasian collared-dove	(40, 93)	
Sturnus	vulgaris	European starling	(40, 90)	

TCID 50: Tissue Culture Infectious Dose (TCID 50)

In Europe, several eco-epidemiological investigations have been carried out on wild birds. In Italy, one of the most affected EU countries, the infection has been reported in 19 bird orders and 44 species, as reported by the National Reference Centre for Foreign Animal Diseases (CESME) at the Istituto Zooprofilattico Sperimentale of Abruzzo and Molise in Teramo (IZSAM-Teramo) (https://westnile.izs.it). For instance, in the Emilia Romagna region, one of the most affected areas in Europe, surveillance on wild birds, carried out from 2015 to 2019 revealed that *Galliformes* and *Strigiformes* scored the highest prevalence of infection detected by PCR (13%). They were

followed by *Columbiformes* (10%) and *Charadriformes* (7%). The order *Passeriformes* showed an average prevalence of 5%, with European blackbird (*Turdus merula)* accounting for a prevalence of 4.3%. Notable infected species were greenfinch (*Carduelis chloris*), scops owl (*Otus scops*), house martin (*Delichon urbica*), house sparrow (*Passer domesticus*), and pheasant (*Phasianus colchicus*) (106).

In Spain, sporadic WNV outbreaks observed in horses and wild birds from Extremadura (western Spain) during 2016 and 2017 seasons prompted a survey in wild birds, focused on specimens coming from two wildlife rehabilitation centres. Between October 2017 and December 2019, samples from 391 wild birds, belonging to 56 different species, were collected and analysed in search of evidence of WNV infection. The analysis of serum samples for WNV-specific antibodies by ELISA, whose specificity was subsequently confirmed by virus-neutralisation test (VNT) showed positive results in 18.23% birds belonging to 18 different species. *Pelecaniformes* (33.33%), *Accipitriformes* (25.77%) and *Strigiformes* (22.92%) orders had the higher seroprevalence. Remarkably, for the first time in Europe, WNV-specific antibodies were found in a black stork (*Ciconia nigra*). Analysis by real time RT-PCR in symptomatic birds confirmed the presence of WNV-1 RNA in griffon vulture (*Gyps fulvus*) and little owls (*Athene noctua*) (107). Moreover, following the largest epidemic outbreak ever reported in Spain, another cross-sectional study was conducted to assess the circulation and risk factors associated with WNV exposure in wild bird populations. Results showed that group species (raptors), age (>1-year old), and size (large) were the main risk factors related to WNV seropositivity in wild birds (108). Seropositivity was found in 37.8% of the 37 species analysed.

In northern Serbia, of 92 wild bird sera tested, seven (8%) were IgG ELISA positive. They belonged to three species: four Mute Swans (*Cygnus olor*); two White-tailed Eagles (*Haliaeetus albicilla*) and one Common Pheasant (*Phasianus colchicus*) (109).

Migratory birds possibly favor the WNV long-distance dispersal. Getting infected prior to or during migration, birds may carry the virus in their blood (and other tissues) over long distances and eventually infect mosquitoes and/or their predators in destination territories (110). As well as migratory birds considered to have an essential role in long distance spread, resident birds seem to have an essential role in long distance spread, resident birds have a paramount importance in local dispersal and endemization of flaviviruses at a smaller and medium spatial scale. In particular, resident bird species such as waterfowls, corvids, or birds of prey have been shown to be highly sensible to WNV (110). For instance, raptors, considered WNV reservoir and amplifying hosts of infection, are usually found to be seriously affected during WNV outbreaks, demonstrating their potential role in the virus seasonal introduction and circulation (81, 111, 108). Moreover, higher prevalence and susceptibility to WNV might point out the existence of infection through predation of infected prey in these birds. Consequently, they are considered important target species when designing cost-effective surveillance for monitoring both seasonal WNV circulation in endemic countries and its emergence into new areas (81, 112).

Corvids, such as the Eurasian magpie (*Pica pica*), the hooded crow (*Corvus corone cornix*), and the Eurasian jay (*Garrulus glandarius*), are also known to be susceptible to WNV infection. These birds are periodically shot or trapped to contain their abundance and they are screened as sentinel for WNV circulation in Italy since 2012 (113). However, the trend of infection observed in these species is indicative, but not exhaustively, of the intensity of WNV circulation. Other species, such as the small passerine birds, might be involved in WNV transmission in Europe, as previously observed in other continents such as the USA (110). For instance, the extensive capturing and sampling of wild birds in the Netherlands has allowed the recent report of the first locally acquired WNV detection in passerines, thus revealing their potential role in enzootic transmission (114).

Knowledge gaps related to bird role in circulation of WNV in Europe remain; the reservoir competence and immunological response to the infection for many European avian species is still not known (40, 7, 110, 82) and further studies in this direction are needed.

Drivers of disease emergence and spread

Ecological drivers

WNV displays high ecological plasticity, being able to adapt to different vector-hosts assemblages. In a recent systematic review carried out to identify the most relevant environmental factors affecting the WNV spread in Europe those which appear to exert the major effect were changes observed in temperature and precipitation patterns and the expansion of anthropized habitats (49). Environmental factors modulate the vector presence and abundance, and their Extrinsic Incubation Period (EIP) while human-derived changes in landscape may provide favorable conditions for mosquito breeding and susceptible host assemblage, especially in urban and peri-urban areas, other than in agricultural landscapes (44, 49, 115, 116).

In particular, temperature is one of the most important variables being able to affect the whole WNV transmission system. Firstly, it affects the mosquito population cycle and abundance, since it determines both the start-point and the duration of the vector's season and it is also associated with mosquitoes' population density and geographical distribution (117).

There is an optimal known range of temperatures (25-35°C) for the mosquitoes to develop and *Cx. pipiens* embryonic development cannot be completed below 7°C. Both larval development and adult survival are shorter at higher temperatures being the highest survival at 25°C. Vector abundance is a good predictor of WNV risk, also in case of *Cx. modestus* other than of *Cx. pipiens* (116).

The effect of environmental temperatures on WNV establishment in Europe via *Cx. pipiens* populations through use of a basic reproduction number (R0) model was assessed (118). WNV establishment was determined to be possible between 14°C and 34.3°C, with the optimal temperature at 23.7°C. The widespread thermal suitability for WNV establishment highlights the importance of European surveillance and the need for increased research into mosquito and bird distribution (118).

Furthermore, temperature plays a significant role in the vector competence, extrinsic incubation period, and intensity of infection of WNV within mosquito vectors. This mechanism then allows the vectors to transmit WNV earlier by shortening the gonotrophic cycle, resulting also in an increased biting rate although the effect can differ among *Cx. pipiens* biotypes (64). The average northern European summer temperatures of 18°C appear to be an important limiting factor for WNV transmission (48).

The temperature exerts an indirect effect on the intensity and duration of the outbreaks and it appears to be a reliable early warning predictor of its occurrence (44, 119, 120). Also, the mean temperature of the warmest quarter of the preceding year is considered one of the most important drivers of WNV outbreak European-wide (116). Likewise, a higher than usual spring temperature in this range (22-26°C) early in a year could also be a precursor of WNV-outbreak during the latter half of the same year (116). Similarly, temperature can be used as a predictor of vector abundance and its infection rate, as observed in Serbia where the number of collected *Cx. pipiens* mosquitoes and the monthly distribution of WNV-positive pools followed a similar profile over time as the mean temperature, between April and October 2013 in Vojvodina province (56).

In a recent study aimed at modelling the WNV Force Of Infection (FOI), spring temperatures were positively associated with FOI (121). Similarly, the timing of the peak of WNV incidence

appears to be influenced only by summer temperatures while the infection peak tends to be demonstrated to be earlier when summer temperature is higher. Furthermore, higher temperature is correlated with high WNV genetic diversity during the entire history of WNV-2a spread in Europe (25). Moreover, studies carried out in France, Greece, Italy and Serbia revealed that average temperature was a consistently good predictor across sites. In South Banat district (NUTS level 3) in Serbia the maximum temperature of warmest part of the year and the annual temperature range, as, well as, the presence of rivers (Danube and Tamis rivers) and its changes in water streams were the best environmental predictors of WNV infection outbreaks in time period 2017-2019 (122).

Regarding precipitation, ecological studies suggest that drought events can lead to outbreaks in the following year due to changes in the mosquito food web structure. Lower precipitation in winter implies less water availability in the region and such conditions are a likely indicator of the aggregation of host birds and the vectors at available water bodies, which could amplify the virus transmission rates (44, 116).

Human driven activities which determine land use and urbanization have also major effects on the vector and host abundance and community composition (49, 115, 123). The differences of WNF incidence rates among European Mediterranean countries with similar weather conditions could be explained by differences in the human interaction with suitable habitats such as wetlands or flooded fields. Areas with high levels of agricultural activities may accelerate WNV dispersal velocity as well as attract the spread direction of WNV in Europe. Meanwhile, WNV is likely to spread in urbanized areas, in line with high abundance of *Cx. pipens* which is a species able to maintain significant populations in cities (49). However, the WNV FOI is expected to be higher in anthropized semi-natural areas such as populated forests, wetlands and river basins (44, 121).

There are other local factors which could affect WNV circulation. For instance, regional differences in mosquito species distribution, including the occurrence and rate of hybrid population of *Cx. pipiens*, and vector competence might be crucial (25, 46). Furthermore, the different dynamic histories of WNV variants (WNV-2a and WNV-2b) might also be associated with the presence of different mosquito species assemblages, which needs further investigation (25).

Among biotic factors, also bird community composition affects WNV intensity of circulation and based on available data (123), developed a structural bird borne WND risk map. However, data on presence, abundance, phenology, migration at municipal and provincial scale of the majority of the bird species of relevance for WNV ecology are usually lacking, therefore representing a significant limitation in risk modelling (123).

Bird species vary in their susceptibility to WNV infection and role in the ecology of the disease. Migratory birds are important not only by the fact of being a reservoir but also for the possibility of introducing new virus lineages or strains. However, resident and short-range migratory birds play an important role in virus dispersal. For example, WNV-2a in Europe spread at a high spread rate (88 km/year to 218 km/year) and, therefore, is more likely correlated with short migrant bird movement than the flight range of *Culex* mosquitoes (approx. 500 m to 2 km/year) (25). Therefore, although the risk of WNV depends on both the presence of infected birds and the presence of competent mosquitoes transmitting the disease, bird movements seed the infections into mosquito populations occurring far away and introduce WNV into new regions. A better understanding of bird migration pattern and phenology at European scale under a global change scenario is essential for a better understanding of WNV temporal and spatial dynamics (124).

Natural history of disease in humans and animals, including symptoms, morbidity and mortality

Brief history of the pathogen and disease

WNV was isolated for the first time in Africa in 1937 in the West Nile district of the northern province of Uganda, following a campaign aimed at monitoring the circulation of Yellow Fever virus. A new virus was isolated in a febrile 37-years-old woman and named WNV in relation to the district where it was found.

After these first investigations, cases were reported in Israel, Egypt, France, and South Africa throughout the 1950s to 1970s, followed by large outbreaks in Romania and Russia in the 1990s. WNV infections were reported for the first time in Algeria and Morocco in 1994 and 1996, respectively. In 1999, the first WNV cases in the Western Hemisphere were identified in New York City. WNV is currently considered as the most widespread flavivirus on the globe (15, 17, 112, 125-127).

Disease in humans

In humans, the WNV incubation period varies between 2 and 15 days after infection. WNV infection is often asymptomatic in humans (80%), however some patients (20%) may present febrile illness WNF, characterized by fevers and maculopapular rash (50% of cases), headache, myalgia, arthralgia, malaise, anorexia, nausea, vomiting, diarrhea, abdominal pain, retro-orbital pain, pharyngitis, and lymphadenopathy (128- 132). Acute form of WNV may persists for up to 60 days and can be characterized by a biphasic condition (2, 132).

In less than 1% of cases, mainly in elderly or immunocompromised people, it can lead to WNND characterized by encephalitis, meningitis and/or acute flaccid paralysis and sometimes to fatal outcomes. Pancreatitis, orchitis, myocarditis, haemorrhagic fever, nephritis, hepatitis, and rhabdomyolysis may be rare non-neurological presentations associated with WNV infection. A biphasic fever, usually helpful for WNV diagnosis, is an additional characteristic feature. After recovery, patients may present long-term sequelae, such as persistent movement disorders, functional disabilities, weakness, cognitive impairments, depression and, in some cases, early death (133).

In Europe, the case fatality ratio among patients with WNV disease reported by the ECDC from 2017 to 2022 was 9% in 2017 (26 deaths out of 288 confirmed cases in EU and neighbouring countries), 8.6% in 2018 (180/2083), 10.8% in 2019 (50/463), 11.3% in 2020 (38/336), 8.2% in 2021 (13/159) and 8% in 2022 (92/1113) (Figure 6).

Age and gender are the main intrinsic predisposing factors for the disease and its relative severity. In general, older individuals are more susceptible. In fact, the risk of acquiring the disease increases by 1.5 folds for every 10 years of age. It has also been demonstrated that patients older than 75 years might succumb to the infection, and this could be explained by aging related innate immunosenescence. Thus, aging affects several antiviral pathways, including cellular pathways (macrophage related defence) and cytokines such as type I Interferon (IFN) and Toll-like receptor 3 (TLR 3)-mediated antiviral pathways, leading to increased susceptibility to viral infection including WNV (134). In addition, males have been shown to be more at risk than females. Pre-existing conditions and diseases such as cancers, cardiovascular, renal and cerebrovascular diseases, and diabetes are also other major risk factors. Immunosuppressed individuals have 40 times higher risk of contracting the disease and dying from WNV infection (reviewed in 2, 132, 135-137).

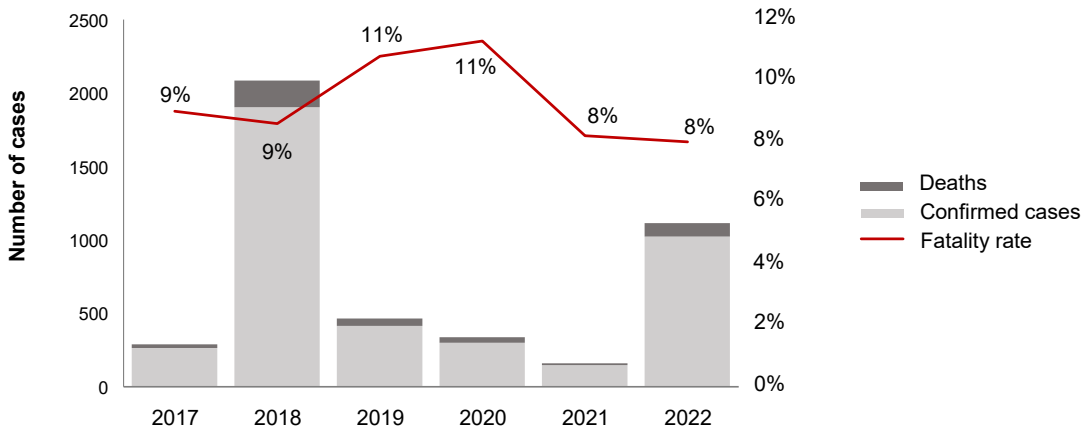

Figure 6. Number of reported WNV human cases, deaths and fatality rate, European Union and European Economic Area countries, 2017-2022 (data extracted from ECDC Epidemiological update reports)

In Europe, based on the analyses of TESSy data on infection for the period 2012-2022, males resulted more affected than females (Figure 7). WNF was more common in males (61.6%) than in females (38.4%) with a male-to-female ratio of 1.6:1. Most infections occurred in people over the age of 50 (77.6% of the total, of which 48.6% occurred in males and 29% in females over 50 years old). Notification rates increased with age in both sexes, peaking at 0.13 cases per 100,000 population in males aged 70-79 years.

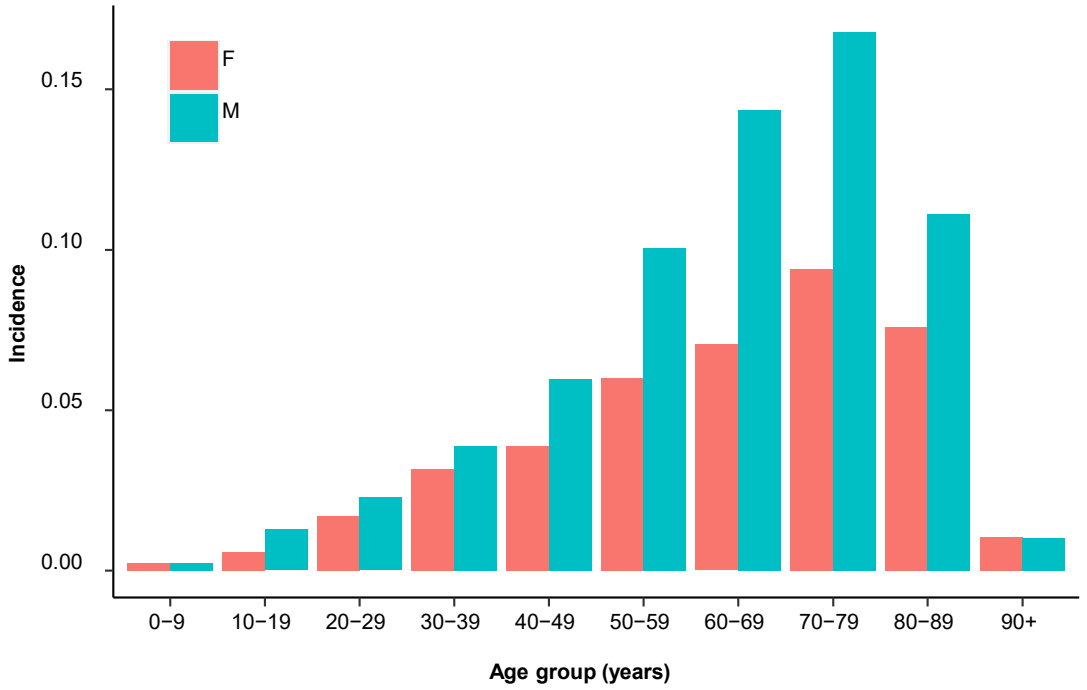

Figure 7. Incidence of WNV infections per 100,000 population, by sex and age group and male-to-female ratio by age group, European Union and European Economic Area countries, 2012-2022 (data from TESSy-ECDC)

Common human exposure routes

WNV is mostly transmitted to humans through mosquito bites. The transmission occurs especially when mosquitoes are active (i.e. between spring and autumn) and most infections in humans and equids are observed between July and September in temperate and subtropical areas. Human behaviour, occupation and leisure activities can influence the likelihood of contracting WNV disease. In fact, the exposure to mosquito bites, when coupled with the presence of infected mosquitoes and birds, can result in an increased probability of getting infected (132).

Other transmission routes include the possibility of infection through SoHO as blood transfusion, organ, tissues or cells transplantation from an infected donor, intrauterine transmission and through breastfeeding. Technician and laboratory workers may also be infected during manipulations of infected material through percutaneous injections or inhalation of droplets in the air (132).

Disease in animals

From a veterinary standpoint, WNV is the causative agent of WNF, which might develop into asymptomatic forms, benign forms (flu-like syndrome) and neuro-invasive forms. In Europe, most of the clinical signs are reported in equids and birds.

Horses

Among many flaviviruses causing disease in mammals, WNV is probably the one with a major impact on equid health. Horses are sporadically infected by the virus and, although in most cases they remain asymptomatic, around 20% can develop clinical signs that use to be more severe than in humans and have important health and economic consequences (138, 139).

Overall, the combination, severity, and duration of clinical signs can be highly variable. Low grade fever, obtunded mentation, inappetence, colic, or lameness can be among the first recognized signs in diseased animals. In different outbreaks the most frequent clinical signs observed are ataxia (57%-100%), weakness (30%-100%), and muscle fasciculations (42%-100%). Ataxia can be symmetrical or asymmetrical, such as weakness affecting either fore/hindlimbs or all. Fewer horses present with hyper-responsiveness and cranial nerve deficits such as facial paralysis, vestibular ataxia, drooping lip and/or inability to swallow, photophobia and central blindness. Abnormal behaviour such as obtunded mentation, somnolence, disorientation, hyperexcitability and aggressive behaviour as well as changes in personality have also been associated with the infection.

Birds

Birds are the natural hosts of WNV and play a key role on the virus epidemiology, with many species susceptible to the infection (40). The disease shows up due to the virus invasion of different organs, liver, spleen, kidney, heart, and mainly the central nervous system, and can lead to death within 24-48 h later (138). Clinical signs in susceptible infected avian species include ruffled feathers, lethargy, ataxia, unusual posture, inability to fly or to hold head upright, head tremors, seizures, leg paralysis, nystagmus, and weight loss (40). Corvids and raptors appear particularly susceptible to WNV infection, with the latter often exhibiting neurological signs, in some cases leading to the death (40, 81, 82).

Although abundant information exists regarding experimental infections of birds with WNV, especially with the WNV/NY99 strain (89-91), studies about the pathogenicity of Euro-Mediterranean strains in different avian species are still scarce (82, 83, 92, 103, 104, 140, 141).

In fact, the pathogenicity of new strains is worth to be studied in order to obtain a better understanding of WNV eco-epidemiology (82).

Common animal exposure routes

Vector borne transmission is the main transmission route for WNV in animals (91). However, birds can also get infected through direct contact, since they are able to shed high-virus titres through oral and cloacal secretions. Particularly, direct transmission in contact birds might occur through faecal-oral or oral-oral routes, as demonstrated in a few species, mainly in *Corvidae* and *Laridae* families, or by skin or feather picking. Moreover, in laboratory experiments, transmission through direct contact has also been described in common goose, chicken and only in one occasion in red-legged partridge (91). Direct contact transmission could play a role in WNV epidemiology in those situations in which wild birds aggregate in high densities, as in breeding colonies, roosting and feeding areas, or stopovers during migration (142) Another means of WNV transmission is through the ingestion of infected mosquitoes, infected prey or infected tissues and organs (91). Persistent infection, defined as the detection of the virus in host tissues after viremia has subsided, might increase the probability of the viral transmission through the oral route. In fact, predation of infected birds characterized by a persistent infection associated to high viral load in their organs might likely result in the WNV transmission, months after the end of the mosquito season (23, 143).

Availability of preventive, therapeutic and control measures, including licensed or pipelined vaccines

To date, no WNV-specific therapy is available and vaccines are only licensed for use in horses but not in humans. While several methodologies for the vaccine development have been successfully applied and have contributed to significantly reducing the WNV incidence in horses in the US, none have progressed to phase III clinical trials in humans (139).

Therapy in humans

Despite the great efforts invested in recent years in the development of prophylactic measures against this pathogen, there is currently no specific drug or therapy licensed for its treatment (139). Some therapies that have shown variable effectiveness against WNV include IVIG, interferon, and ribavirin (128). Patients with severe meningeal symptoms often require pain control for headaches and antiemetic therapy other than rehydration for associated nausea and vomiting. Patients with encephalitis require close monitoring for the development of high intracranial pressure and seizures. Furthermore, they should be monitored for the inability to protect their airway, also in the case of poliomyelitis. In fact, acute neuromuscular respiratory failure may develop rapidly, and prolonged ventilatory support may be required. Various drugs have been evaluated or empirically used for WNV disease; however, none have shown specific benefit to date.

Therapy in animals

In horses, survival rate for WNV encephalitis is high compared with other infectious encephalitis (55-70%). Treatment of WNND is mainly supportive (138).

Licensed or pipelined vaccines

Humans

There is no vaccine authorized for humans, nor candidates close to being licensed. Although several veterinary vaccines have been licensed, WNV vaccines for humans have not progressed beyond phase 1 or 2 clinical trials (139, 144).

Animals

Horses

Four of the six licensed vaccines are currently on the market. The WN-Innovator, with a classic inactivated whole virion-based approach, was the first to be developed and was licensed by the United States Department of Agriculture (USDA) in 2003. Live attenuated recombinant viruses have also been used (either based on canary poxvirus or yellow fever virus), as well as a plasmid DNA vaccine, which was the first licensed by the USDA, although it was subsequently withdrawn from the market by the manufacturers. However, despite their proven efficacy, these vaccines still exhibit some limitations, such as the need for repeated administrations to get a solid initial immunization, and the relatively short duration of the induced immunity, which makes necessary annual boosters (139).

Birds

Several commercial and experimental vaccine candidates have been assayed in wild and domestic birds, although no one has yet been authorized. Overall, they induced humoral and, although less analysed, cellular responses, and reduced disease, injury, viremia, viral shedding, and mortality associated with WNV. Furthermore, if they induce herd immunity, they could help prevent outbreaks and the spread of the virus. For example, a prospective vaccination of the entire population of California condors (*Gymnogyps californianus*), an endangered species, performed before the WNV arrival would have helped preventing its infection and possible extinction. Likewise, vaccination also greatly reduced virus incidence in domestic geese in Israel. However, the implementation of bird vaccines faces several drawbacks, such as the feasibility of access to the target host, mainly for wild species, and the administration route. In any case, its availability could benefit domestic populations (farm birds, including those for hunting and restocking activities), as well as wild ones (as those housed in rehabilitation centers and wildlife reserves, and in recreational facilities, like zoos (139).

Other prevention measures

In the absence of a vaccine, one prevention measure to reduce the risk of infection in humans consists in raising awareness of personal and community behaviours. In particular, it would be important to increase protection against mosquito bites by avoiding human exposure, through i) the reduction of outdoor activities at peak biting times and sleeping outside, ii) the wearing of light coloured, long-sleeved shirts and trousers, iii) the use of mosquito nets and repellents (131). In addition, it is necessary to implement mosquitoes surveillance and control measures such as removing or treating mosquitoes breeding sites located near cities and villages, reduce the risk of animal-to-human transmission wearing protective equipment when handling animals and during slaughtering and culling operations and, prevent infectious disease transmission associated with organ and tissue transplantation and blood transfusion, implementing donors epidemiological screening and laboratory testing tools, specifically during WNV outbreak (2).

In countries with endemic circulation of WNV along with other arbovirosis such as Italy and Spain, national integrated One Health surveillance and control plan are established (32, 35) For example, in Italy, a specific surveillance plan (National Plan for Arbovirosis), coordinated by the Istituto Superiore di Sanità (ISS) and by the Istituto Zooprofilattico Sperimentale di Abruzzo e Molise (IZSAM), in collaboration with the Ministry of Health, is in place to ensure the early detection of potential cases of WNV and minimize any spread of the disease. The plan also includes guidelines and recommendations for mosquito surveillance and control. In certain countries such as Germany, Italy, and Spain, tools or apps have been provided to citizens for reporting mosquito presence and biting incidents.

Disease specific recommendations

Regulation on substances of human origin

In July 2022, the European Commission adopted the proposal for a Regulation on standards of quality and safety for Substances Of Human Origin (SoHO) intended for human application. By repealing the Blood Directive (2002/98/EC) and the Tissues and cells Directive (2004/23/EC) both evaluated in 2019), the proposed Regulation concludes the revision of the legal framework for blood, tissues and cells, which was included in the REFIT Annex (#37 p.15) of the Commission's Work Programme for 2021.

Currently, as recommended by ECDC (https://www.ecdc.europa.eu/en/west-nile-fever/facts) to prevent transfusion-transmitted WNV infections, European Union/European Economic Area (EU/EEA) countries should implement 28-day blood donor deferral or individual donation nucleic acid testing (ID-NAT) of prospective donors who have visited or live in an affected area.

In affected areas, blood establishments* should follow recommendations provided in the EU preparedness plan for blood safety. Donors of organs, tissues and cells living in or returning from an affected area should be tested for WNV infection.

Systematic collection of epidemiological information on WNV infection among donors and recipients of SoHO is an important tool for national authorities to better assess the risk of transmission and impact of preventive measures on the availability of SoHO.

According to the preparedness plan for WNV blood safety in the EU, blood establishments in affected areas should:

- temporarily interrupt blood collection or implement NAT screening for blood donations from WNV affected areas
- quarantine, retest and discard positive blood components in storage at the time of implementation of measures and retrieve and quarantine blood components derived from whole blood donated 120 days prior the date of collection of the ID-NAT-positive donation
- enhance donor post-donation information, especially about fever, influenza-like illness or other acute symptoms within 15 days after donation
- strengthen post-transfusion haemovigilance and perform look-back analysis in any case of transfusion-transmitted WNV infection for a period dating 120 days prior to the donation of implicated blood components; and
- consider the use of pathogen inactivation procedures.

* According to Directive 2002/98/EC, 'blood establishments' are structures or bodies that are responsible for any aspect of the collection and testing of human blood or blood components, whatever their intended purpose, and their processing, storage, and distribution when intended for transfusion

Epidemiological situation at different spatial scales: past and current trends in Europe

Past trends

WNV circulation in Europe has been reported since 1958 (20). Before 2010, major WNV outbreaks occurred throughout Europe, notably in Romania and Russia in the 1990s, Hungary in 2008, Greece in 2010 (17). Since 2010, WNV has been reported in an increasing number of EU countries including Austria, Bulgaria, Croatia, Cyprus, France, Greece, Hungary, Italy, the Netherlands, Portugal, Romania, Slovenia and Spain, other than northerly regions that had not previously reported cases, like Germany and the Czech Republic. Moreover, it has been described in five EU neighbouring countries including Albania, Montenegro, Serbia, Turkey, and Kosovo. The epidemiological situation of WNF in Europe is heterogeneous: some European countries report outbreaks in humans and animals every year while others have never reported any autochthonous cases (8).

Outbreaks have occurred annually in multiple regions, with a peak in 2018 that affected more regions than had been recorded in previous years (39).

In Europe, there were, on average, 18 newly affected areas annually between 2011 and 2017, and 45 additional areas reported in 2018, with most infections occurring from early summer to early autumn and peaking in August (145).

Until 2017, the countries with the highest number of cases in the EU were Greece, Italy, Romania, and Hungary and one of the neighbouring countries, Serbia. ECDC reported a 7.2-fold increase in cases from 2017 to 2018, especially in Bulgaria (15-fold), France (13.5-fold), and Italy (10.9-fold), which is partially attributed to the unusually hot spring and summers. The same was true for Serbia with notification numbers 8.5 times higher in 2018 compared to 2017. In 2020, a major outbreak was reported in Spain with 77 confirmed cases, of which 72 developed neuroinvasive disease and seven died, a strong impact that could be, among others, partly attributed to reduced vector control activities during that season (145, 35, 17).

Current trends

For the period 2008-2022 a total number of 5358 (4142 confirmed) human cases have been reported to the ECDC (TESSy data), with the highest numbers observed in 2018 and 2022 (Figure 8).

Over the past years, there has been an increase in the number of countries reporting surveillance data in animals (146). In 2021, the number of human cases in the EU was 158, being the lowest figure of the period 2017-2021. 54 infections were reported on October: 7 in Greece, 52 in Italy, 7 in Hungary, 7 in Romania, 6 in Spain, 3 in Austria, 3 in Germany, and 17 in Serbia, with cases reported for the first time in Spree-Neiße in Germany and La Spezia in Italy.

While the number of tested equids appears to correlate with WNV circulation, increasing in years when a greater number of cases are reported, there has been an overall increase in the number of tested birds over time, reflecting an intensification of the surveillance system in place.

Since the beginning of the 2022 transmission season and as of 23 November 2022, EU/EEA countries have reported 965 human cases of WNV with higher infection observed in Italy (586), Greece (284), Romania (46), Hungary (14), Germany (11), Croatia (8), Austria (6), Spain (5), France (4) and Slovakia (1).

EU/EEA countries have reported 92 deaths in Italy (37), Greece (31) and Romania (5). EU-neighbouring countries have reported 246 human cases of WNV infection in Serbia (226) and 12 deaths in Serbia (25, 147).

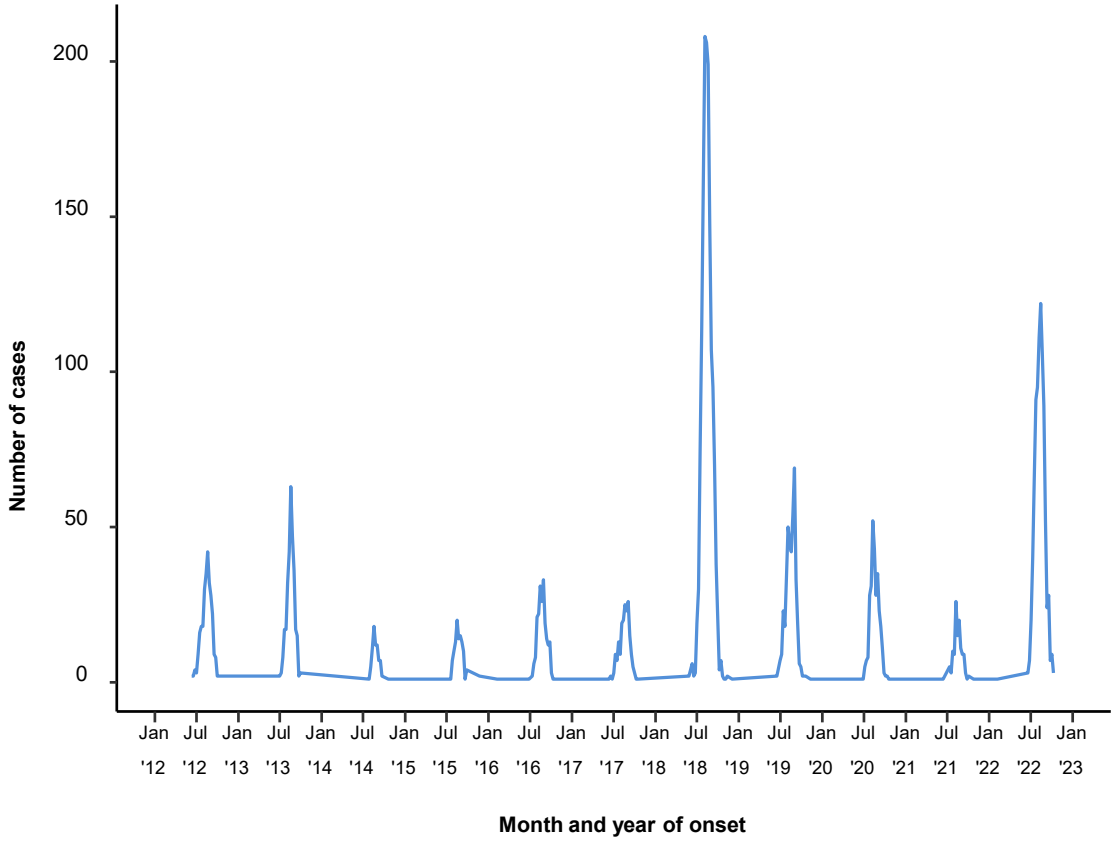

Figure 8. Number of reported WNV human infections by week of onset, European Union and European Economic Area countries, 2012-2022 (data from TESSy-ECDC)

During the 2022 transmission season, within the reporting countries, human cases of WNV infection were reported from 107 different NUTS 3 or GAUL 1 regions, of which the following regions reported human cases of WNV infection for the first time ever: Bouches-du-Rhône in France, Harz, Vogtlandkreis and Salzlandkreis in Germany, Pistoia, Lucca, Monza e della Brianza, Biella, Cagliari and Catania in Italy, Brasov in Romania, Moravicki in Serbia and Tarragona and Córdoba in Spain.

Moreover, 93 outbreaks among equids and 314 outbreaks among birds have been reported by EU/EEA countries.

Outbreaks among equids have been reported by Italy (44), Germany (15), Greece (9), Croatia (8), Spain (6), France (5), Hungary (3), Portugal (2) and Austria (1).

Outbreaks among birds have been reported by Italy (249), Germany (51), Spain (9), Austria (2), Croatia (2) and Hungary (1).

Current trend in some European countries

Italy

WNV human infections in Italy are mostly located along the Po valley (northern part of the Country), with a mean of 167 cases reported annually and an average notification rate of 0.28 per 100 000 inhabitants over the period 2012-2022. The total number of 1841 cases were reported between 2012 and 2022 (Figures 9 and 10).

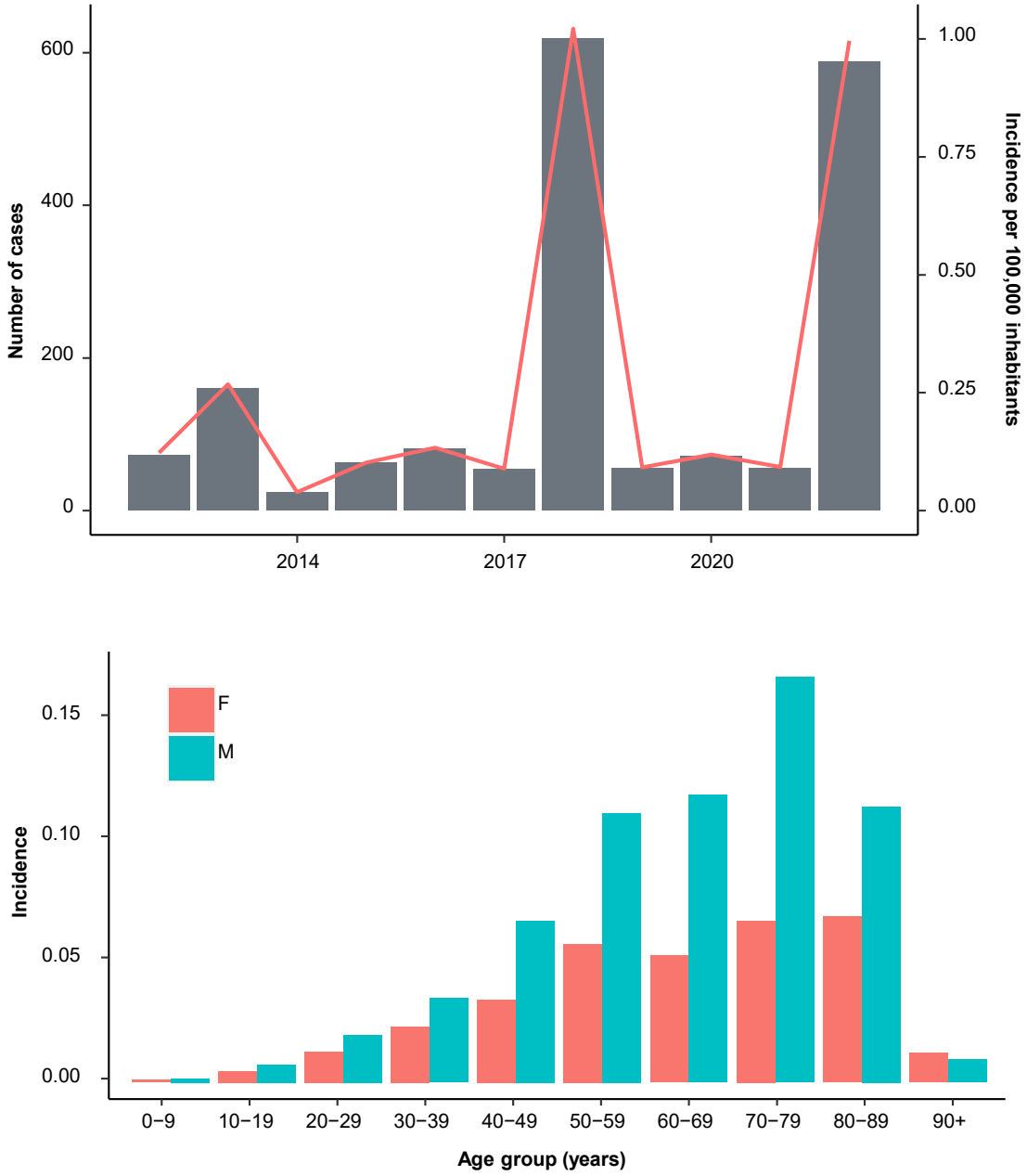

Figure 9. ITALY - WNV: cases and incidence per 100,000 inhabitants by year and incidence by gender and age group (n = 1841) (data fromTESSy-ECDC)

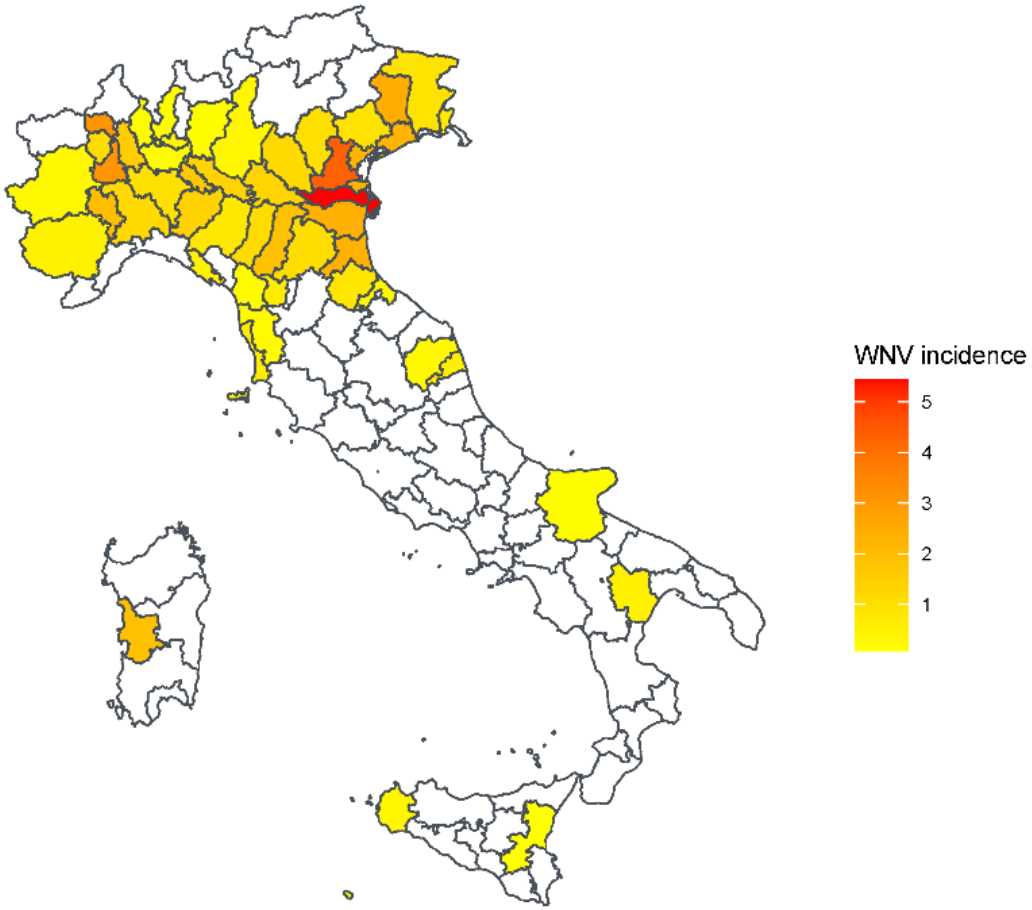

Figure 10. ITALY - WNV: average annual incidence rate per 100,000 inhabitants by NUTS3 (n=1703, period 2012–2022) (data TESSy-ECDC)

France

Few human infections of WNV are reported annually in France, with a mean of about 5 cases and an average notification rate of 0.009 per 100 000 inhabitants over the period 2012-2022. Particularly, between 2012 and 2022, 56 cases were reported (Figure 11).

Although the enzootic WNV circulation is often observed in the South of France (34), most of the human infections from France seems to be acquired abroad, as only seven locally acquired cases were reported from the Var (n=5) and Bouches-du-Rhône (n=2) departments (Figure 12).

Spain

Spain is characterized by an average of 9 cases with a mean notification rate of 0.04 per 100,000 inhabitants over the period 2012-2022.

The total number of cases reported between 2012 and 2022 is 93, with most of the infections happening in 2020 (77) (Figure 13).

Geographically, WNV infections are located in the South-Western part of the Country, mainly in the Andalusia region (n=83) (Figure 14).

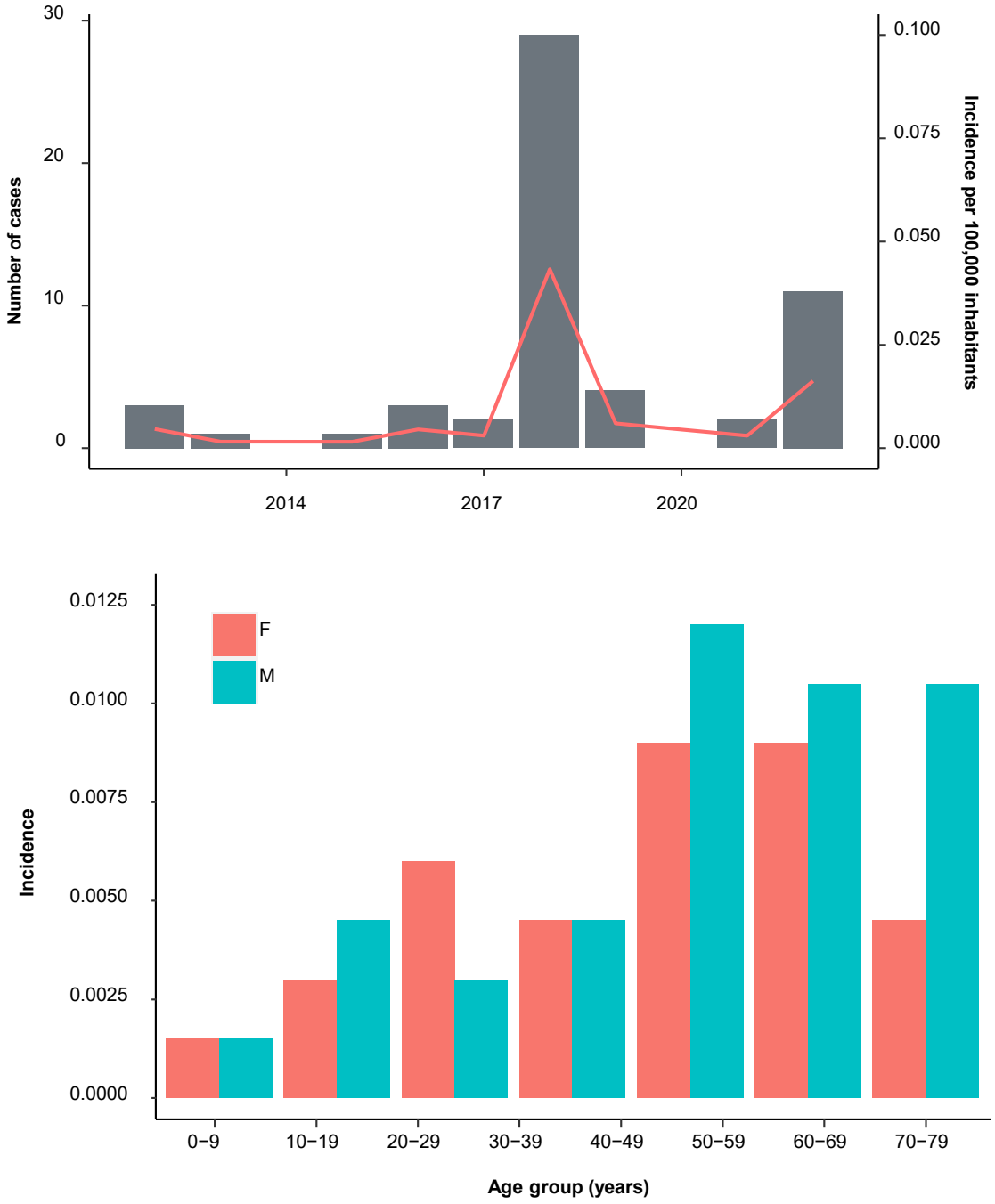

Figure 11. FRANCE - WNV: cases and incidence per 100,000 inhabitants by year and incidence by gender and age group (n = 56) (data from TESSy-ECDC).

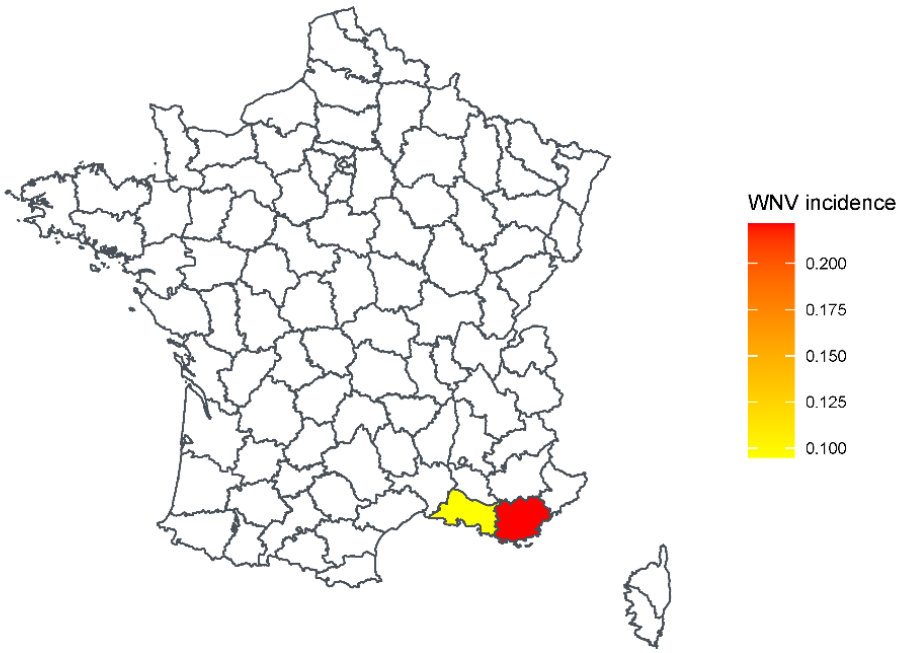

Figure 12. FRANCE - WNV average annual incidence rate per 100,000 inhabitants by NUTS3 (n=7, period 2012–2022) (data from TESSy-ECDC)

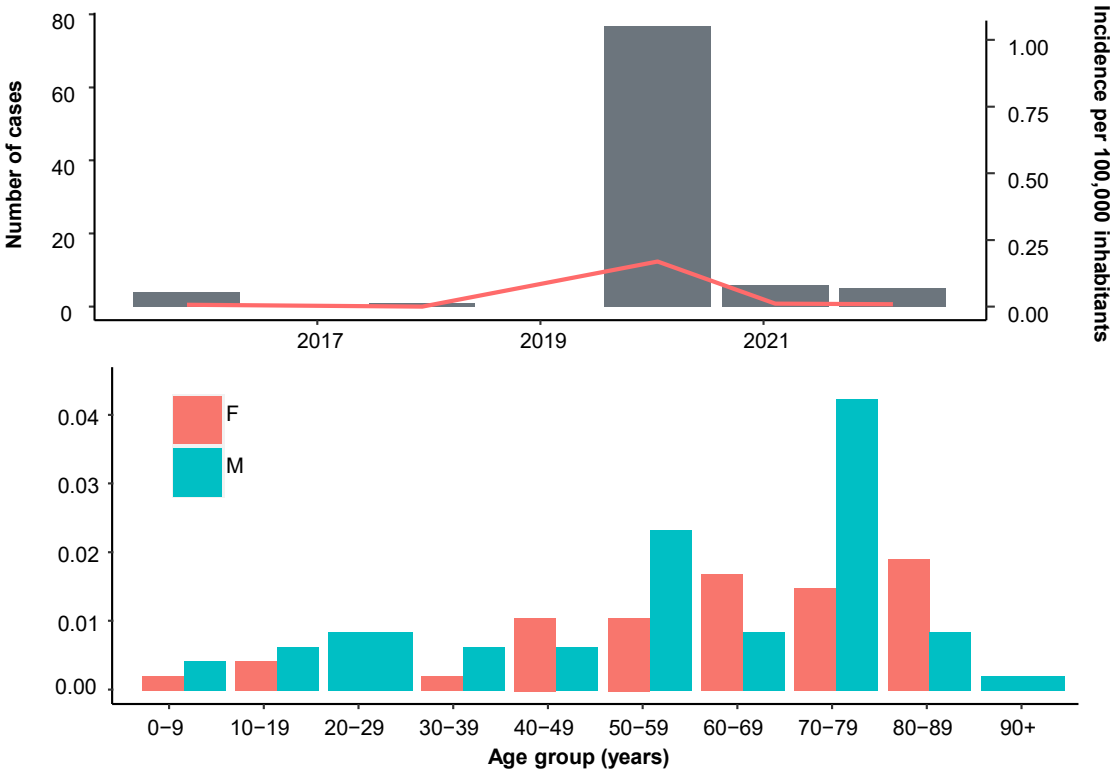

Figure 13. SPAIN - WNV: cases and incidence per 100,000 inhabitants by year and incidence by gender and age group (n = 93) (data from TESSy-ECDC)

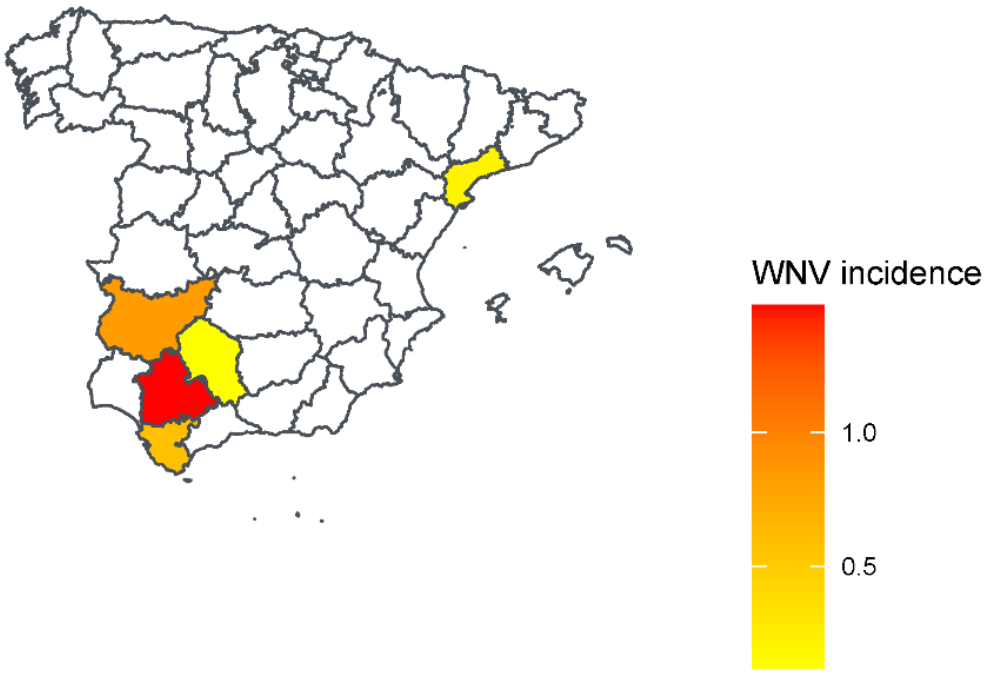

Figure 14. SPAIN - WNV: average annual incidence rate per 100 000 inhabitants by NUTS3 (n=87, period 2012–2022) (data from TESSy-ECDC).

Sociological and demographic dimension affecting susceptibility and exposure, including gender

Age and gender are among the most critical factors in determining the WNV infection risk. For example, the number of WNV reported cases is estimated to be greater in areas with a higher number of elderly (age > 65 years) people, consistently with the fact that age is one of the main risk factors for developing severe symptoms upon infection (120) (*see* also the paragraph "Availability of preventive, therapeutic and control measures, including licensed or pipelined vaccines").

However, several other factors such as economic conditions and sociodemographic characteristics are considered to be important in the WNV epidemiology. As reported in a number of studies, a range of local-level socio-economic factors such as income, sanitation, wastewater management, and population density tend to influence the distribution and intensity of mosquito-borne diseases, including WNV, both pre-infection and post-infection (39). Poorer communities are less likely to have air-conditioned homes, tap water and adequate drainage, and therefore may be more exposed to biting mosquitoes. However, factors associated with higher economic status can also bring humans into closer contact with mosquitoes, for example, homeowners with gardens and potted plants, swimming pools and ponds or having good access to recreational space where mosquitoes can breed (39). As for the land-use variables, regions with a larger proportion of arable land and wetlands are associated with higher WNV incidence. Humans are particularly at risk in areas close to rice paddies, irrigated agriculture and wetlands, since these areas tend to attract susceptible mosquitoes and birds (39). The percentage of discontinuous urban fabric

populated areas of low to medium density often characterized by peri-urban forest, gardens, parks, and ponds, such as residential suburbs and villages, are also often cited as a driver of WNV infections in humans (39, 44). For example, WNV infection outbreaks have been associated with urban settings in some countries such as Romania, where people living in the basements of tall buildings were more at risk than the rest of the population. This has been linked to a peri-domestic ecological behaviour of some mosquito vector species such as *Cx. pipiens,* which are urban mosquitoes commonly found indoors (2). Other risk factors include outdoor activities and travelling. In fact, it has been demonstrated that spending more time outdoors or travelling to endemic regions constitute a risk of contracting WNV since individuals might get more exposed to mosquito bites. The main outdoor occupations at risk appear to be farm workers, loggers, landscapers and groundskeepers, construction workers, painters, summer camp workers, pavers, soldiers and security guards, among others. Healthcare, laboratory workers, veterinarians, animal handlers, animal slaughterers, and butchers appear also to be at risk of contracting WNV due to the possibility of getting exposed to the virus via needlesticks, accidental cuts, or contamination of open wound coming in contact with WNV infectious materials (2).

Diagnostic procedures and notification systems used at local, national and European scale

Diagnosis

Cases of WNV infection should be notified following the EU case definition outlined to the ECDC. List of case definitions for reporting communicable diseases to the Community network follow the Commission Implementing Decision (EU) 2018/945 of 22 June 2018 on the communicable diseases and related special health issues to be covered by epidemiological surveillance as well as relevant case definitions:

– *Clinical criteria*
  At least one of the following:
  - any person with fever
  - encephalitis; and/or
  - meningitis

– *Laboratory criteria*
  - Laboratory test for case confirmation at least one of the following:
    - isolation of WNV from blood or cerebrospinal fluid (CSF).
    - detection of WNV nucleic acid in blood or CSF.
    - WNV-specific antibody response (immunoglobulin M; IgM) in CSF; and/or
    - WNV IgM high titre, detection of WNV IgG and confirmation by neutralisation
  - Laboratory test for probable case:
    - WNV-specific antibody response in serum.

– *Epidemiological criteria*
  At least one of the following epidemiological links:
  - Animal to human transmission (residing, having visited or having been exposed to mosquito bites in an area where WNV is endemic in horses or birds).
  - Human to human transmission (vertical transmission, blood transfusion, transplants).

– *Case classification:*

A. *Possible case*
Not applicable

B. *Probable case*
Any person meeting the clinical criteria and with at least:

- an epidemiological link; and
- a laboratory test for a probable case.

C. *Confirmed case*
Any person meeting laboratory criteria for case confirmation.

Note: Serological results should be interpreted according to previous exposure to other flavivirus infections and vaccination status. Confirmed cases in such situations should be validated by serum neutralisation or other equivalent assays.

Notification system

WNF is a notifiable disease in humans and in equids at the EU level. Notifications of human WNF cases in Europe are collected through the European Surveillance System (TESSy) of the ECDC. Between June and November, the period of high vector activity, the ECDC publishes weekly updated maps of human cases and complementary information on animal and vector WNV infections based on data provided by the World Organisation for Animal Health (WOAH) and European countries. The yearly analyses of TESSy data are published in the ECDC annual epidemiological report and jointly with the European Food Safety Authority (EFSA). EU countries report outbreaks of WNV encephalomyelitis in horses to the European Commission (EC) via the Animal Disease Notification System (ADNS) and regular summaries are posted online. The data from WNV monitoring in animals is reported annually by EU countries under Directive 2003/99/EC and presented in the annual EFSA/ECDC EU Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks. Animal WNF outbreak data reported to the WOAH are publicly available on the World Animal Health Information Database (WAHIS) interface (8).

In 2021, 25 Member States reported information on WNV infections in humans (146). Surveillance is mandatory in 23 countries; in France it is voluntary, while Germany did not specify its surveillance system. There is no surveillance system in place in Denmark and the disease is not notifiable or reported at EU level. The EU case definition was used by 23 Member States, France reported another case definition, while Germany did not specify the case definition used. All countries conducting surveillance had a comprehensive surveillance system with full national coverage, except Germany, which did not specify the surveillance system but had full national coverage. All countries reported case-based data.

WNV surveillance in animals involves mostly passive surveillance, including surveillance based on the diagnosis of neuro-invasive cases in equids, but some countries implement active surveillance of equids and/or captive birds and/or wild birds. Alongside EU Member States, Switzerland submits reports to EFSA on animal surveillance and monitoring activities in animals. Two sources of information are used to complete this report. Firstly, data are submitted to EFSA by EU Member States and Switzerland from annual surveillance and monitoring activities in accordance with Directive 2003/99/ EC. WNV is listed in Annex I, Part B (viruses transmitted by arthropods) as a virus to be monitored, if warranted by the epidemiological situation in an MS, in compliance with Article 4.1 of the same Directive. Secondly, it is mandatory for Member States

to notify outbreaks of equine and avian WNV to ADIS,41 in accordance with CIR (EU) 2020/2002.

Infrastructure capacity to identify pathogens for each Member State

Based on a survey conducted by the ECDC to collect information on the current WNV surveillance and control capacities across the EU/EEA countries, European Neighbourhood Policy (ENP) partner countries and EU candidate/potential candidate countries, eighty-three percent of the EU/EEA countries, ENP partner countries and EU candidate/potential candidate countries have implemented at least one method of WNV surveillance. The most common passive WNV surveillance method for all countries is the detection of human cases, followed by surveillance on dead animals. The most common method of active WNV surveillance is mosquito screening, followed by sentinel bird screening and sentinel equid screening. The majority of the countries conduct routine vector surveillance (abundance monitoring), but less than 15% perform pesticide resistance testing, and even where this is done, this is not implemented systematically.

Estimated influence of environmental change on the disease future trends

After the two major peaks observed in 2018 and 2022, which coincide with two of the hottest years recorded in Europe, changes in the epidemiological pattern of WNV circulation in Europe are expected to occur in the coming years (148).

As explained in the "drivers of disease emergence" section, global warming characterized by increased temperatures and extreme weather events is expected to create a suitable environment for diverse populations of mosquito species, as *Cx. pipiens,* and to increase the WNV transmission in Europe. This highlights the importance of European-wide integrated surveillance to cover human animals and vectors, detecting WNV host and vector distribution (118, 148).

Italy and other EU countries experienced an important peak of WNV circulation in 2022. Particularly, the Italian 2022 vector season was marked by an early onset of viral circulation in mosquitoes and birds while human infections started at the beginning of July, with a rapid increase in the number of cases (13). Furthermore, most human cases of WNND reported in Europe occurred in Italy in 2022.

Projections show up to 5-fold increase in WNV risk for 2040-60 in Europe, depending on geographical region and climate scenario, compared to 2000-20 (14). The proportion of disease-reported European land areas could increase from 15% to 23-30%, putting 161 to 244 million people at risk. Across scenarios, Western Europe appears to be facing the largest increase in the outbreak risk of WNV. The increase in the risk is not linear but undergoes periods of sharp changes governed by climatic thresholds associated with ideal conditions for WNV vectors. This will require a targeted public health response to manage the expansion of WNV with climate change in Europe (14).

References

1. Calisher CH, Gould EA. Taxonomy of the virus family Flaviviridae. *Adv Virus Res*. 2003;59:1-19. doi:10.1016/s0065-3527(03)59001-7.
2. Habarugira G, Suen WW, Hobson-Peters J, *et al.* West Nile Virus: an update on pathobiology, epidemiology, diagnostics, control and "One Health" implications. *Pathogens.* 2020;9:589.
3. Tuplin A, Evans DJ, Buckley A, *et al.* Replication enhancer elements within the open reading frame of tick-borne encephalitis virus and their evolution within the Flavivirus genus. *Nucleic Acids Res.* 2011;39:7034-7048.
4. Colpitts TM, Conway MJ, Montgomery RR, *et al.* West Nile Virus: biology, transmission, and human infection. *Clin Microbiol Rev.* 2012;25:635-648.
5. Zannoli, Sambri. West Nile Virus and Usutu virus co-circulation in Europe: epidemiology and implications. *Microorganisms.* 2019;7:184.
6. Green FM, Ronca SE. Safety procedures to work with West Nile Virus in biosafety level 3 facilities. In: Bai F (Ed.). *West Nile Virus*. New York, NY: Springer US, pp. 205-210.
7. Rizzoli A, Jimenez-Clavero MA, Barzon L, *et al.* The challenge of West Nile virus in Europe: knowledge gaps and research priorities. *Euro Surveill*. 2015;20(20):21135. doi:10.2807/1560-7917.es2015.20.20.21135
8. Gossner CM, Marrama L, Carson M, *et al.* West Nile virus surveillance in Europe: moving towards an integrated animal-human-vector approach. *Euro Surveill.* 2017;22(18):30526. doi:10.2807/1560-7917.ES.2017.22.18.30526.
9. Domanović D, Gossner CM, Lieshout-Krikke R, *et al.* West Nile and Usutu Virus infections and challenges to blood safety in the European Union. *Emerg Infect Dis.* 2019;25:1050-1057.
10. Young JJ, Coulombier D, Domanović D; European Union West Nile fever working group, Zeller H, Gossner CM. One Health approach for West Nile virus surveillance in the European Union: relevance of equine data for blood safety. *Euro Surveill.* 2019;24(16):1800349. doi:10.2807/1560-7917.ES.2019.24.16.1800349.
11. Dente MG, Riccardo F, Bolici F, *et al.* Implementation of the One Health approach to fight arbovirus infections in the Mediterranean and Black Sea Region: Assessing integrated surveillance in Serbia, Tunisia and Georgia. *Zoonoses Public Health.* 2019;66:276-287.
12. Young JJ, Haussig JM, Aberle SW, *et al.* Epidemiology of human West Nile virus infections in the European Union and European Union enlargement countries, 2010 to 2018. *Euro Surveill.* 2021;26(19):2001095. doi:10.2807/1560-7917.ES.2021.26.19.2001095.
13. Riccardo F, Bella A, Monaco F, *et al.* Rapid increase in neuroinvasive West Nile virus infections in humans, Italy, July 2022. *Euro Surveill*. 2022;27(36):2200653. doi:10.2807/1560-7917.ES.2022.27.36.2200653.
14. Farooq Z, Sjödin H, Semenza JC, *et al.* European projections of West Nile virus transmission under climate change scenarios. *One Health.* 2023;16:100509.
15. Reisen W. Ecology of West Nile Virus in North America. *Viruses.* 2013;5:2079-2105.
16. Chancey C, Grinev A, Volkova E, *et al.* The global ecology and epidemiology of West Nile Virus. *Biomed Res Int*. 2015;2015:1-20.
17. Jani C, Kakoullis L, Abdallah N, *et al.* West Nile virus: another emerging arboviral risk for travelers? *Curr Infect Dis Rep* 2022;24:117-128.
18. Bodjoski M, Bodjoski M, Glisic A, Boskovic R. (Arbovirus infections in Serbia). *Vojnosanit Pregl*. 1972;29(4):173-5 (In Serbian).

19. Medić S, van den Hoven R, Petrović T, *et al.* Serological evidence of West Nile virus infection in the horse population of northern Serbia. *J Infect Dev Ctries* 2014;8:914-918.
20. Hubálek Z, Halouzka J. West Nile Fever-a reemerging mosquito-borne viral disease in Europe. *Emerg Infect Dis.* 1999;5:643-650.
21. Vasić A, Răileanu C, Körsten C, *et al.* West Nile virus in the Republic of Serbia-Diagnostic performance of five serological tests in dog and horse sera. *Transbound Emerg Dis.* 2022;69(5):e2506-e2515. doi:10.1111/tbed.14593.
22. Nikolić N, Poluga J, Milošević I, *et al.* Neurological and neuromuscular manifestations in patients with West Nile neuroinvasive disease, Belgrade area, Serbia, season 2022. *Neurol Sci.* 2024;45(2):719-726. doi:10.1007/s10072-023-07025-y.
23. Mencattelli G, Iapaolo F, Polci A, *et al.* West Nile Virus Lineage 2 Overwintering in Italy. *Trop Med Infect Dis.* 2022;7:160.
24. Lu L, Zhang F, Oude Munnink BB, *et al.* West Nile virus spread in Europe: Phylogeographic pattern analysis and key drivers. *PLoS Pathog.* 2024;20(1):e1011880. doi:10.1371/journal.ppat.1011880
25. Barzon L, Pacenti M, Montarsi F, *et al.* Rapid spread of a new West Nile virus lineage 1 associated with increased risk of neuroinvasive disease during a large outbreak in Italy in 2022. *J Travel Med* 2022; taac125.
26. Fall G, Di Paola N, Faye M, *et al.* Biological and phylogenetic characteristics of West African lineages of West Nile virus. *PLoS Negl Trop Dis.* 2017;11:e0006078.
27. Fall G, Diallo D, Soumaila H, *et al.* First Detection of the West Nile Virus Koutango Lineage in Sandflies in Niger. *Pathog Basel Switz* 2021;10:257.
28. Mencattelli G, Ndione MHD, Silverj A, *et al.* Spatial and temporal dynamics of West Nile virus between Africa and Europe. *Nat Commun.* 2023;14:6440.
29. Bakonyi T, Ivanics É, Erdélyi K, *et al.* Lineage 1 and 2 Strains of Encephalitic West Nile Virus, Central Europe. *Emerg Infect Dis* 2006;12:618-623.
30. Jovanović Galović A, Weyer J, Jansen Van Vuren P, *et al.* West Nile Virus Lineage 2 Associated with Human Case in Republic of Serbia. *Vector-Borne Zoonotic Dis* 2017;17:780-783.
31. Haussig JM, Young JJ, Gossner CM, *et al.* Early start of the West Nile fever transmission season 2018 in Europe. *Euro Surveill.* 2018;23(32):1800428. doi:10.2807/1560-7917.ES.2018.23.32.1800428.
32. Riccardo F, Monaco F, Bella A, *et al.* An early start of West Nile virus seasonal transmission: the added value of One Heath surveillance in detecting early circulation and triggering timely response in Italy, June to July 2018. *Euro Surveill.* 2018;23(32):1800427. doi:10.2807/1560-7917.ES.2018.23.32.1800427.
33. Pacenti M, Sinigaglia A, Franchin E, *et al.* Human West Nile Virus lineage 2 infection: epidemiological, clinical, and virological findings. *Viruses.* 2020;12:458.
34. Beck C, Leparc Goffart I, Franke F, *et al.* Contrasted epidemiological patterns of West Nile Virus lineages 1 and 2 infections in France from 2015 to 2019. *Pathogens.* 2020;9:908.
35. García San Miguel Rodríguez-Alarcón L, Fernández-Martínez B, Sierra Moros MJ, *et al.* Unprecedented increase of West Nile virus neuroinvasive disease, Spain, summer 2020. *Euro Surveill*. 2021;26(19):2002010. doi:10.2807/1560-7917.ES.2021.26.19.2002010.
36. Busquets N, Laranjo-González M, Soler M, *et al.* Detection of West Nile virus lineage 2 in North-Eastern Spain (Catalonia). *Transbound Emerg Dis.* 2019;66:617-621.
37. Autorino GL, Battisti A, Deubel V, *et al.* West Nile virus epidemic in horses, Tuscany Region, Italy. *Emerg Infect Dis.* 2002;8:1372-1378.

38. Ziegler U, Angenvoort J, Fischer D, *et al.* Pathogenesis of West Nile virus lineage 1 and 2 in experimentally infected large falcons. *Vet Microbiol.* 2013;161:263-273.
39. Watts MJ, Sarto I Monteys V, Mortyn PG, *et al.* The rise of West Nile Virus in Southern and Southeastern Europe: A spatial-temporal analysis investigating the combined effects of climate, land use and economic changes. *One Health.* 2021;13:100315.
40. Pérez-Ramírez E, Llorente F, Jiménez-Clavero M. Experimental Infections of Wild Birds with West Nile Virus. *Viruses* 2014;6:752-781.
41. Dohm DJ, Sardelis MR, Turell MJ. Experimental vertical transmission of West Nile virus by Culex pipiens (Diptera: Culicidae). *J Med Entomol* 2002;39:640-644.
42. Kampen H, Holicki CM, Ziegler U, *et al.* West Nile Virus mosquito vectors (Diptera: Culicidae) in Germany. *Viruses.* 2020;12:493.
43. Reisen WK, Wheeler SS. Overwintering of West Nile Virus in the United States. *J Med Entomol.* 2019;56:1498-1507.
44. Marcantonio M, Rizzoli A, Metz M, *et al.* Identifying the environmental conditions favouring West Nile Virus outbreaks in Europe. *PLoS One*. 2015;10(3):e0121158. doi:10.1371/journal.pone.0121158
45. Chaskopoulou A, L'Ambert G, Petric D, *et al.* Ecology of West Nile virus across four European countries: review of weather profiles, vector population dynamics and vector control response. *Parasit Vectors.* 2016;9:482.
46. Coroian M, Petrić M, Pistol A, *et al.* Human West Nile meningo-encephalitis in a highly endemic country: a complex epidemiological analysis on biotic and abiotic risk factors. *Int J Environ Res Public Health.* 2020;17:8250.
47. Martínez-de La Puente J, Ferraguti M, Ruiz S, *et al.* Mosquito community influences West Nile virus seroprevalence in wild birds: implications for the risk of spillover into human populations. *Sci Rep* 2018;8:2599.
48. Vogels CB, Göertz GP, Pijlman GP, *et al.* Vector competence of European mosquitoes for West Nile virus. *Emerg Microbes Infect*. 2017;6:1-13.
49. Giesen C, Herrador Z, Fernandez-Martinez B, *et al.* A systematic review of environmental factors related to WNV circulation in European and Mediterranean countries. *One Health.* 2023;16:100478.
50. Fyie LR, Tronetti HR, Gardiner MM, *et al.* Potential for urban warming to postpone overwintering dormancy of temperate mosquitoes. *J Therm Biol.* 2023;115:103594.
51. Jansen S, Heitmann A, Lühken R, *et al.* Culex torrentium: a potent vector for the transmission of West Nile Virus in Central Europe. *Viruses.* 2019;11:492.
52. Wang L, Rosales Rosas AL, De Coninck L, *et al.* Establishment of *Culex modestus* in Belgium and a glance into the virome of Belgian mosquito species. *mSphere.* 2021;6:e01229-20.
53. Tolsá-García MJ, Wehmeyer ML, Lühken R, *et al.* Worldwide transmission and infection risk of mosquito vectors of West Nile, St. Louis encephalitis, Usutu and Japanese encephalitis viruses: a systematic review. *Sci Rep.* 2023;13:308.
54. Di Luca M, Toma L, Boccolini D, *et al.* Ecological Distribution and CQ11 Genetic Structure of Culex pipiens Complex (Diptera: Culicidae) in Italy. *PLoS One.* 2016;11:e0146476.
55. Kemenesi G, Krtinić B, Milankov V, *et al.* West Nile virus surveillance in mosquitoes, April to October 2013, Vojvodina province, Serbia: implications for the 2014 season. *Euro Surveill.* 2014;19(16):20779. doi:10.2807/1560-7917.es2014.19.16.20779.
56. Petrić D, Petrović T, Hrnjaković Cvjetković I, *et al.* West Nile virus 'circulation' in Vojvodina, Serbia: Mosquito, bird, horse and human surveillance. *Mol Cell Probes.* 2017;31:28-36.

57. Jansen S, Heitmann A, Uusitalo R, *et al.* Vector Competence of Northern European Culex pipiens Biotype pipiens and Culex torrentium to West Nile Virus and Sindbis Virus. *Viruses.* 2023;15:592.
58. Ferraguti M, Heesterbeek H, Martínez-de la Puente J, *et al.* The role of different Culex mosquito species in the transmission of West Nile virus and avian malaria parasites in Mediterranean areas. *Transbound Emerg Dis.* 2021;68:920-30.
59. Tsai TF, Popovici F, Cernescu C, *et al.* West Nile encephalitis epidemic in southeastern Romania. *Lancet.* 1998;352:767-71.
60. Engler O, Savini G, Papa A, *et al.* European surveillance for West Nile virus in mosquito populations. *Int J Environ Res Public Health.* 2013;10:4869-95.
61. Fortuna C, Remoli ME, Severini F, *et al.* Evaluation of vector competence for West Nile virus in Italian *Stegomyia albopicta* (= *Aedes albopictus*) mosquitoes. *Med Vet Entomol*. 2015;29:430-3.
62. Fortuna C, Remoli ME, Di Luca M, *et al.* Experimental studies on comparison of the vector competence of four Italian Culex pipiens populations for West Nile virus. *Parasites & Vectors* 2015;8:463.
63. Balenghien T, Vazeille M, Grandadam M, *et al.* Vector Competence of Some French *Culex* and *Aedes* Mosquitoes for West Nile Virus. *Vector-Borne Zoonotic Dis* 2008;8:589-596.
64. Kampen H, Walther D. Vector potential of mosquito species (Diptera: Culicidae) occurring in Central Europe. In: Benelli G, Mehlhorn H (Ed.). *Mosquito-borne diseases*. Cham: Springer International Publishing; 2018. p. 41-68.
65. Vogels CBF, Fros JJ, Göertz GP, *et al.* Vector competence of northern European Culex pipiens biotypes and hybrids for West Nile virus is differentially affected by temperature. *Parasit Vectors.* 2016;9:393.
66. Balenghien T, Vazeille M, Reiter P, *et al.* Evidence of laboratory vector competence of culex modestus for west nile virus. *J Am Mosq Control Assoc.* 2007;23:233-6.
67. Muñoz J, Ruiz S, Soriguer R, *et al.* Feeding Patterns of Potential West Nile Virus Vectors in South-West Spain. *PLoS One* 2012;7:e39549.
68. Mixão V, Bravo Barriga D, Parreira R, *et al.* Comparative morphological and molecular analysis confirms the presence of the West Nile virus mosquito vector, Culex univittatus, in the Iberian Peninsula. *Parasit Vectors.* 2016;9:601.
69. Kilpatrick AM, Kramer LD, Campbell SR, *et al.* West Nile Virus risk assessment and the bridge vector paradigm. *Emerg Infect Dis.* 2005;11:425-429.
70. Leggewie M, Badusche M, Rudolf M, *et al.* Culex pipiens and Culex torrentium populations from Central Europe are susceptible to West Nile virus infection. *One Health.* 2016;2:88-94.
71. Higgs S, Snow K, Gould EA. The potential for West Nile virus to establish outside of its natural range: a consideration of potential mosquito vectors in the United Kingdom. *Trans R Soc Trop Med Hyg.* 2004;98:82-87.
72. Hayes CG, Basit A, Bagar S, *et al.* Vector competence of Culex Tritaeniorhynchus (Diptera: Culicidae) for West Nile Virus1. *J Med Entomol.* 1980;17:172-177.
73. Brustolin M, Talavera S, Santamaría C, *et al. Culex pipiens* and *Stegomyia albopicta* (= *A edes albopictus*) populations as vectors for lineage 1 and 2 West Nile virus in Europe. *Med Vet Entomol.* 2016;30:166-173.
74. Blagrove MSC, Sherlock K, Chapman GE, *et al.* Evaluation of the vector competence of a native UK mosquito Ochlerotatus detritus (Aedes detritus) for dengue, chikungunya and West Nile viruses. *Parasit Vectors* 2016;9:452.
75. Goddard LB, Roth AE, Reisen WK, *et al.* Vector competence of California mosquitoes for West Nile virus. *Emerg Infect Dis.* 2002;8:1385-91.

76. Vermeil C, Lavillaureix J, Reeb E. (On the preservation and transmission of the West Nile virus by some arthropods). *Bull Soc Pathol Exot Filiales.* 1960;53:273-9.
77. Veronesi E, Paslaru A, Silaghi C, *et al.* Experimental evaluation of infection, dissemination, and transmission rates for two West Nile virus strains in European Aedes japonicus under a fluctuating temperature regime. *Parasitol Res.* 2018;117:1925-32.
78. Medlock JM, Snow KR, Leach S. Potential transmission of West Nile virus in the British Isles: an ecological review of candidate mosquito bridge vectors. *Med Vet Entomol.* 2005;19:2-21.
79. Camp JV, Bakonyi T, Soltész Z, *et al.* Uranotaenia unguiculata Edwards, 1913 are attracted to sound, feed on amphibians, and are infected with multiple viruses. *Parasit Vectors.* 2018;11:456.
80. Toma L, Cipriani M, Goffredo M, *et al.* First report on entomological field activities for the surveillance of West Nile disease in Italy. *Vet Ital.* 2008;44:483-497,499-512.
81. Hubálek Z, Rudolf I, Nowotny N. Arboviruses pathogenic for domestic and wild animals. *Adv Virus Res* 2014;89:201-75.
82. Vidaña B, Busquets N, Napp S, *et al.* The role of birds of prey in West Nile Virus epidemiology. *Vaccines.* 2020;8:550.
83. Llorente F, Gutiérrez-López R, Pérez-Ramirez E, *et al.* Experimental infections in red-legged partridges reveal differences in host competence between West Nile and Usutu virus strains from Southern Spain. *Front Cell Infect Microbiol* 2023;13:1163467.
84. Lim SM, Brault AC, Van Amerongen G, *et al.* Susceptibility of European jackdaws (Corvus monedula) to experimental infection with lineage 1 and 2 West Nile viruses. *J Gen Virol.* 2014;95:1320-9.
85. Del Amo J, Llorente F, Pérez-Ramirez E, *et al.* Experimental infection of house sparrows (Passer domesticus) with West Nile virus strains of lineages 1 and 2. *Vet Microbiol.* 2014;172:542-7.
86. Root JJ, Bosco-Lauth AM. West Nile Virus associations in wild mammals: an update. *Viruses* 2019;11:459.
87. Jeffrey Root J. West Nile virus associations in wild mammals: a synthesis. *Arch Virol.* 2013;158:735-52.
88. Escribano-Romero E, Lupulović D, Merino-Ramos T, *et al.* West Nile virus serosurveillance in pigs, wild boars, and roe deer in Serbia. *Vet Microbiol.* 2015;176:365-9.
89. Gómez A, Kramer LD, Dupuis AP, *et al.* Experimental infection of eastern gray squirrels (Sciurus carolinensis) with West Nile virus. *Am J Trop Med Hyg* 2008;79:447-451.
90. Pérez-Ramírez E, Llorente F, Del Amo J, *et al.* Susceptibility and role as competent host of the red-legged partridge after infection with lineage 1 and 2 West Nile virus isolates of Mediterranean and Central European origin. *Vet Microbiol.* 2018;222:39-45.
91. Komar N, Langevin S, Hinten S, *et al.* Experimental infection of North American birds with the New York 1999 strain of West Nile Virus. *Emerg Infect Dis.* 2003;9:311-322.
92. Pérez-Ramírez E, Llorente F, Jiménez-Clavero MÁ. Experimental infections of wild birds with West Nile virus. *Viruses.* 2014;6:752-781.
93. Spedicato M, Carmine I, Bellacicco AL, *et al.* Experimental infection of rock pigeons (*Columba livia*) with three West Nile virus lineage 1 strains isolated in Italy between 2009 and 2012. *Epidemiol Infect.* 2016;144:1301-1311.
94. Panella NA, Young G, Komar N. Experimental infection of Eurasian collared-dove (*Streptopelia decaocto*) with West Nile virus. *J Vector Ecol.* 2013;38:210-214.
95. Kostiukov MA, Gordeeva ZE, Bulychev VP, *et al.* (The lake frog (Rana ridibunda)--one of the food hosts of blood-sucking mosquitoes in Tadzhikistan--a reservoir of the West Nile fever virus). *Med Parazitol (Mosk).* 1985;49-50.

96. Molchanova EV, Prilepskaya DR, Negodenko AO, *et al.* Sensitivity of quails (Coturnix coturnix), siskins (Carduelis spinus), and frogs (Rana ridibunda) to West Nile Virus. *Bull Exp Biol Med.* 2021;171:461-463.
97. Xiao S-Y, Guzman H, Zhang H, *et al.* West Nile Virus infection in the golden hamster (*Mesocricetus auratus*): a model for West Nile encephalitis. *Emerg Infect Dis* 2001;7:714-21.
98. Van Der Meulen KM, Pensaert MB, Nauwynck HJ. West Nile virus in the vertebrate world. *Arch Virol.* 2005;150:637-57.
99. Root JJ, Bentler KT, Nemeth NM, *et al.* Experimental infection of raccoons (Procyon lotor) with West Nile virus. *Am J Trop Med Hyg.* 2010;83:803-7.
100. Austgen LE, Bowen RA, Bunning ML, *et al.* Experimental infection of cats and dogs with West Nile virus. *Emerg Infect Dis.* 2004;10:82-6.
101. Swayne DE, Beck JR, Smith CS, *et al.* Fatal encephalitis and myocarditis in young domestic geese (Anser anser domesticus) caused by West Nile virus. *Emerg Infect Dis* 2001;7:751-3.
102. Senne DA, Pedersen JC, Hutto DL, *et al.* Pathogenicity of West Nile virus in chickens. *Avian Dis.* 2000;44:642-9.
103. Allison AB, Mead DG, Gibbs SEJ, *et al.* West Nile Virus Viremia in Wild Rock Pigeons. *Emerg Infect Dis* 2004;10:2252-5.
104. Jiménez De Oya N, Camacho M-C, Blázquez A-B, *et al.* High susceptibility of magpie (Pica pica) to experimental infection with lineage 1 and 2 West Nile virus. *PLoS Negl Trop Dis.* 2018;12:e0006394.
105. Lim SM, Brault AC, Van Amerongen G, *et al.* Susceptibility of Carrion Crows to Experimental Infection with Lineage 1 and 2 West Nile Viruses. *Emerg Infect Dis.* 2015;21:1357-65.
106. Kramer LD, Bernard KA. West Nile Virus infection in birds and mammals. *Ann N Y Acad Sci.* 2001;951:84-93.
107. Lauriano A, Rossi A, Galletti G, *et al.* West Nile and Usutu viruses' surveillance in birds of the province of Ferrara, Italy, from 2015 to 2019. *Viruses.* 2021;13:1367.
108. Bravo-Barriga D, Aguilera-Sepúlveda P, Guerrero-Carvajal F, *et al.* West Nile and Usutu virus infections in wild birds admitted to rehabilitation centres in Extremadura, western Spain, 2017-2019. *Vet Microbio.l* 2021;255:109020.
109. García-Bocanegra I, Franco JJ, León CI, *et al.* High exposure of West Nile virus in equid and wild bird populations in Spain following the epidemic outbreak in 2020. *Transbound Emerg Dis.* 2022;69:3624-36.
110. Petrović T, Blazquez AB, Lupulović D, *et al.* Monitoring West Nile virus (WNV) infection in wild birds in Serbia during 2012: first isolation and characterisation of WNV strains from Serbia. *Euro Surveill.* 2013;18(44):20622. doi:10.2807/1560-7917.es2013.18.44.20622 P
111. Marzal A, Ferraguti M, Muriel J, *et al.* Circulation of zoonotic flaviviruses in wild passerine birds in Western Spain. *Vet Microbiol.* 2022;268:109399.
112. Feyer S, Bartenschlager F, Bertram CA, *et al.* Clinical, pathological and virological aspects of fatal West Nile virus infections in ten free-ranging goshawks (*Accipiter gentilis*) in Germany. *Transbound Emerg Dis.* 2021;68:907-19.
113. Bella A, Venturi G, Riccardo F, Iapaolo F, F. Monaco FF, Calistri P. *Sorveglianza integrata del West Nile e Usutu virus. Risultati nazionali. Bollettino n. 20 del 2 novembre 2022.* Istituto Superiore di Sanità, IZS Teramo; 2022.
114. Mencattelli G, Ndione MHD, Rosà R, *et al.* Epidemiology of West Nile virus in Africa: An underestimated threat. *PLoS Negl Trop Dis.* 2022;16:e0010075.

115. Sikkema RS, Schrama M, van den Berg T, *et al.* Detection of West Nile virus in a common whitethroat (Curruca communis) and Culex mosquitoes in the Netherlands, 2020. *Euro Surveill.* 2020;25(40):2001704. doi:10.2807/1560-7917.ES.2020.25.40.2001704

116. Brugueras S, Fernández-Martínez B, Martínez-de La Puente J, *et al.* Environmental drivers, climate change and emergent diseases transmitted by mosquitoes and their vectors in southern Europe: A systematic review. *Environ Res.* 2020;191:110038.

117. Farooq Z, Rocklöv J, Wallin J, *et al.* Artificial intelligence to predict West Nile virus outbreaks with eco-climatic drivers. *Lancet Reg Health Eur.* 2022;17:100370. doi:10.1016/j.lanepe.2022.100370.

118. Moser SK, Barnard M, Frantz RM, *et al.* Scoping review of Culex mosquito life history trait heterogeneity in response to temperature. *Parasit Vectors.* 2023;16:200.

119. Di Pol G, Crotta M, Taylor RA. Modelling the temperature suitability for the risk of West Nile Virus establishment in European Culex pipiens populations. *Transbound Emerg Dis.* 2022;69(5):e1787-e1799. doi:10.1111/tbed.14513.

120. Marini G, Poletti P, Giacobini M, *et al.* The role of climatic and density dependent factors in shaping mosquito population dynamics: the case of Culex pipiens in Northwestern Italy. *PloS One.* 2016;11:e0154018.

121. Marini G, Manica M, Delucchi L, *et al.* Spring temperature shapes West Nile virus transmission in Europe. *Acta Trop.* 2021;215:105796.

122. Marini G, Pugliese A, Wint W, *et al.* Modelling the West Nile virus force of infection in the European human population. *One Health.* 2022;15:100462.

123. Radojicic S, Zivulj A, Petrovic T, *et al.* Spatiotemporal Analysis of West Nile Virus Epidemic in South Banat District, Serbia, 2017-2019. *Animals (Basel).* 2021;11(10):2951. doi:10.3390/ani11102951.

124. Durand B, Tran A, Balança G, *et al.* Geographic variations of the bird-borne structural risk of West Nile virus circulation in Europe. *PLoS One.* 2017;12:e0185962.

125. Storch D, Koleček J, Keil P, *et al.* Decomposing trends in bird populations: Climate, life histories and habitat affect different aspects of population change. *Divers Distrib.* 2023;29:572-585.

126. Popović N, Milošević B, Urošević A, *et al.* Outbreak of West Nile virus infection among humans in Serbia, August to October 2012. *Euro Surveill.* 2013;18(43):20613. doi:10.2807/1560-7917.es2013.18.43.20613 P

127. Sević S, Stefan-Mikić S, Sipovac D, *et al.* Epidemics of the central nervous system infections caused by West Nile virus in the territory of the South Backa District, Vojvodina, Serbia. *Vojnosanit Pregl.* 2015;72:1098-1104.

128. Pierson TC, Diamond MS. The continued threat of emerging flaviviruses. *Nat Microbiol.* 2020;5:796-812.

129. Parkash V, Woods K, Kafetzopoulou L, *et al.* West Nile Virus Infection in travelers returning to United Kingdom from South Africa. *Emerg Infect Di.s* 2019;25:367-369.

130. Caldwell M, Boruah AP, Thakur KT. Acute neurologic emerging flaviviruses. *Ther Adv Infect Dis.* 2022;9:204993612211026.

131. García-Carrasco J-M, Muñoz A-R, Olivero J, *et al.* An African West Nile virus risk map for travellers and clinicians. *Travel Med Infect Dis.* 2023;52:102529.

132. Bampali M, Konstantinidis K, Kellis EE, *et al.* West Nile disease symptoms and comorbidities: a systematic review and analysis of cases. *Trop Med Infect Dis.* 2022;7:236.

133. Petersen LR, Brault AC, Nasci RS. West Nile Virus: review of the literature. *JAMA.* 2013;310:308.

134. Patel H, Sander B, Nelder MP. Long-term sequelae of West Nile virus-related illness: a systematic review. *Lancet Infect Dis.* 2015;15:951-9.

135. Montgomery RR. Age-related alterations in immune responses to West Nile virus infection. *Clin Exp Immunol*. 2017;187:26-34.

136. Murray KO, Garcia MN, Rahbar MH, *et al.* Survival analysis, long-term outcomes, and percentage of recovery up to 8 years post-infection among the Houston West Nile Virus Cohort. *PLoS One.* 2014;9:e102953.

137. Urošević A, Dulović O, Milošević B, *et al.* The importance of haematological and biochemical findings in patients with West Nile Virus neuroinvasive disease. *J Med Biochem.* 2016;35:451-457.

138. Popescu CP, Florescu SA, Hasbun R, *et al.* Prediction of unfavorable outcomes in West Nile virus neuroinvasive infection - Result of a multinational ID-IRI study. *J Clin Virol.* 2020;122:104213.

139. Saiz J-C. Animal and Human Vaccines against West Nile Virus. *Pathogens* 2020;9:1073.

140. Cavalleri JV, Korbacska-Kutasi O, Leblond A, *et al.* European College of Equine Internal Medicine consensus statement on equine flaviviridae infections in Europe. *J Vet Intern Med.* 2022;36:1858-71.

141. Sotelo E, Gutierrez-Guzmán AV, Del Amo J, *et al.* Pathogenicity of two recent Western Mediterranean West Nile virus isolates in a wild bird species indigenous to Southern Europe: the red-legged partridge. *Vet Res.* 2011;42:11.

142. Dridi M, Vangeluwe D, Lecollinet S, *et al.* Experimental infection of Carrion crows (Corvus corone) with two European West Nile virus (WNV) strains. *Vet Microbiol.* 2013;165:160-166.

143. Montecino-Latorre D, Barker CM. Overwintering of West Nile virus in a bird community with a communal crow roost. *Sci Rep.* 2018;8:6088.

144. Gould CV, Staples JE, Huang CY-H, *et al.* Combating West Nile Virus Disease — Time to Revisit Vaccination. *N Engl J Med*. 2023;388:1633-1636.

145. EFSA and ECDC. The European Union One Health 2018 Zoonoses Report. *EFSA Journal* 2019;17(12):5926, 276 pp. https://doi.org/10.2903/j.efsa.2019.5926

146. EFSA and ECDC. The European Union One Health 2021 Zoonoses Report. *EFSA Journal* 2022;20(12):7666, 273 pp. https://doi.org/10.2903/j.efsa.2022.7666)

147. EFSA and ECDC. The European Union One Health 2022 Zoonoses Report. *EFSA Journal*. 2023;21(12): e8442. https://doi.org/10.2903/j.efsa.2023.8442.)

148. Semenza JC, Rocklöv J, Ebi KL. Climate Change and Cascading Risks from Infectious Disease. *Infect Dis Ther* 2022; 11: 1371–1390.

ZIKA

Maria Bellenghi (a), Pachka Hammami (b), Elena Arsevska (b), Claudia Cataldo (a),
Francesca Dagostin (c), Marco Di Luca (d), Claudia Fortuna (d), William Wint (e), Busani Luca (a)
(a) Centro di Riferimento per la Medicina di Genere, Istituto Superiore di Sanità, Rome
(b) UMR Animals, Health, Territories, Risks, and Ecosystems (Astre), Department of Biological Systems (Bios), French Agricultural Research and International Cooperation Organization for Development (CIRAD), Campus International de Baillarguet, Montpellier
(c) Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige, Trento
(d) Dipartimento Malattie Infettive, Istituto Superiore di Sanità, Rome
(e) Department of Biology, Environmental Research Group Oxford Ltd, Oxford

Biological, ecological and molecular features of the causative agent

Disease name

Zika, Zika fever, Zika virus infection.

Disease agent

Zika virus (ZIKV).

Common, scientific and Latin name

The ZIKV is the causal agent responsible for Zika virus infection or Zika fever. Zika is an arthropod-borne viral disease transmitted by mosquito species of the *Aedes* genus, predominantly by *Ae. aegypti* and to a lesser extent by *Ae. albopictus*. ZIKV was first identified in Uganda in 1947 in a Rhesus macaque monkey that was caged in the canopy of Zika Forest, near Lake Victoria (1). The second isolation was made from *Ae. africanus* mosquitoes caught in the same forest in January 1948 (2). Thus, ZIKV received its name from the geographical area where the initial isolations were made.

Taxonomy

Zika virus is a small enveloped single-positive-stranded RNA virus belonging to the *Flavivirus* genus, of the *Flaviviridae* family. Its genome contains approximately 10.7 kb coding for three structural proteins forming the virion particle: the capsid (C), the pre-membrane/membrane (PrM/M), and the envelope (E), and seven non-structural proteins responsible for viral genome replication, modification of host cellular functions and immune responses: NS1, NS2A, NS2B, NS3, NS4A, 2K, NS4B, and NS5 (3,4). Beyond ZIKV, the genus *Flavivirus* comprises 52 other viral species, including the West Nile virus, yellow fever virus, dengue virus (DENV), chikungunya virus (CHIKV), tick-borne encephalitis, St. Louis encephalitis, Langat virus, the Modoc virus, Rio Bravo virus, the Powassan virus, and the Japanese encephalitis virus. ZIKV is genetically and antigenically related to all of them and more specifically to the Spondweni virus (5). Both viruses form a unique clade (clade X or Spondweni serocomplex) within the mosquito-

borne flavivirus cluster. Based on phylogenetic analyses ZIKV strains were grouped into three major lineages: East African, West African, and Asian/American (6, 7).

Physiochemical properties

Similar to other Flaviviruses, ZIKV is stable at slightly alkaline pH (8.0) and low temperatures (especially at -60°C or below) and for at least 6 h in liquid aerosol suspension at room temperature and 23–80% humidity. On the other hand, ultra-low temperatures preserve infectivity almost indefinitely and once freeze-dried they also survive almost indefinitely at room temperature (8). More recent experiments showed that routinely used disinfectants and alcohols are sufficient to inactivate ZIKV in the laboratory. Complete loss of infectivity was observed after virus exposure to 1% hypochlorite, 2% paraformaldehyde, and 2% glutaraldehyde (9). Exposure for 10 minutes entirely inactivated ZIKV in the presence of 2.5% FCS serum; increasing concentrations of serum reduced the antiviral effects of UV light. Gloves routinely used in BSL-2 laboratories protect against ZIKV (9).

Regarding the environmental stability of ZIKV, experiments showed that commonly used disinfectants and UV radiation can inactivate dried ZIKV. Additional experiments demonstrated that dried ZIKV remained infectious for >3 days suggesting that dried droplets can be infectious, and confirming that proper surface disinfection is essential. The virus was stable at temperatures up to 50°C but lost all infectivity at temperatures of >60°C. Thus, virus-contaminated materials such as surgical instruments can be decontaminated by heat. Experiments also found that ZIKV infectivity was highest after adjusting the stock to a pH of ≈9. In contrast, adjusting ZIKV to pH 12 or to <pH 4 abrogated infectivity (9).

Priority level for EU

ZIK is not endemic in Europe, but like other *Aedes*-borne viruses, it has the potential to emerge following the importation of the virus in the European regions and areas colonized by competent vectors: *Ae. aegypti* (currently established in Madeira) and to a lesser extend *Ae. albopictus* (currently established in 18 countries including Switzerland and UK, and 337 regions of mainland Europe) (10,11). Up to date, European Zika cases reported to the European Centre for Disease Control (ECDC) are sporadic and mostly travel-associated (travellers returning from endemic areas). To our knowledge, sporadic autochthonous sexually transmitted ZIKV infections were reported in France, Germany, Italy and Norway from 2016 to 2019 (12–15), three autochthonous vector-borne transmissions were identified in France in 2019 (16) and few unidentified transmission events occurred in Germany (17).

Considering the frequency of travellers between high-incidence areas in the world and the European Union (EU) and the past experiences in France (16), the risk of more ZIKV outbreaks in continental Europe is not unlikely. The large outbreak in South America in 2016 led to an increased concern about the virus getting introduced in the EU/European Economic Area (EEA) and potential local transmission. In March 2016, a surveillance of Zika started with the main objectives of detecting the locally acquired/autochthonous cases in the European Union/European Economic Area (EU/EEA) early, and timely reporting of travel-associated cases, particularly those residing in areas where *Ae. albopictus* is established, to trigger appropriate control measures (18).

The risk of larger-scale outbreaks in the European region, as the ones seen in South America in 2016, is currently considered moderate to low (as of 2022) mainly because *Ae. aegypti*, the main vector of ZIKV is not widely present in Europe, although it is established in limited areas,

such as Madeira Island and the north-eastern Black Sea coast. *Ae. albopictus*, a potential vector for ZIKV transmission is present in 18 European countries (as of February 2023), primarily in the Mediterranean Basin. However, the likelihood of ZIKV spread in countries where *Aedes* mosquitoes are present should be seen as high or moderate, since in several European countries there is a suitable climate for the establishment of competent mosquitoes, including some countries with a history of transmission of DENV or CHIKV, higher ship and flight connections as well as high population density and urbanization patterns favourable to anthropophilic populations of *Aedes*.

Distribution of the pathogen

ZIKV was first detected in 1947 in rhesus monkeys in the Zika jungle of Uganda. In humans, the first report was in 1952 from Uganda and the United Republic of Tanzania. Infections have since been reported in Africa, the Americas, Asia and equatorial Pacific Island countries. In 2015 and 2016, high temperatures and severe drought conditions provoked a major outbreak in South America. Although, the number of reported outbreaks decreased from 2017, in 2023, 92 countries and regions had already experienced current or past transmission events (19). As with every emerging arthropod-borne virus (arbovirus), ZIKV distribution and spread depend on the distribution and spread of its competent vectors. *Ae. aegypti* and *Ae. albopictus* are known to be the main vectors of ZIKV (20).

Ae. aegypti originated from Africa and has a worldwide distribution, mainly in tropical regions. This mosquito species was probably imported to America and the Mediterranean by ship from Africa (21). The ship carrying tanks of fresh water on board could retain colonies of *Ae. aegypti* favouring its dispersion from one continent to another. It was observed sporadically in Europe from the Atlantic coast (Britain, France, and Portugal) to the Black Sea, in the first half of the 20th century, which shows a wide dispersion compared to today (22). This species was common before the Second World War in the Mediterranean region (present in Spain up to 1953, Portugal up to 1956 and Madeira up until 1977-1979), and it disappeared from Southern Europe and North Africa after this period. This may be attributed to the use of DDT (dichloro-diphenyl-trichloroethane), a synthetic insecticide (now prohibited in many places due to its high impact on the environment and public health) used in malaria eradication programs (23). Sporadic occurrences were observed in Britain, France, Malta, Italy, Croatia, Ukraine, Russia and Turkey (24). It was recently re-established in the island of Madeira (Portugal) in 2004 and 2005 (24), observed for the first time in the Netherlands in 2010, in relation to imported used tires (25).

Ae. albopictus originally came from the tropical and subtropical areas of Southeast Asia; however, it spread to other countries through cold- and dry-resistant eggs in used tires and the plant Lucky Bamboo during the past three decades (26). It was first found in Europe in Albania in 1979 by a shipment of goods from China (27). Then, it was introduced to Italy through the port town of Genoa in used tires from Georgia (USA) in 1990. This species dispersed across mainland Italy, as well as parts of Sicily and Sardinia in 1990-1991. *Ae. albopictus* was then established in France in 1999, especially in the south. In 2002, it was also found in the tourist city on the island of Corsica. After that, the species spread in Belgium in 2000 and 2013, in Montenegro in 2001, in Canton Ticino in Southern Switzerland, Greece in 2003, 2004 Spain and Croatia in 2004, the Netherlands and Slovenia in 2005, and in Bosnia and Herzegovina in 2006 (28). The map of the distribution of *Ae. albopictus* in Europe in 2024 is presented in Figure 1.

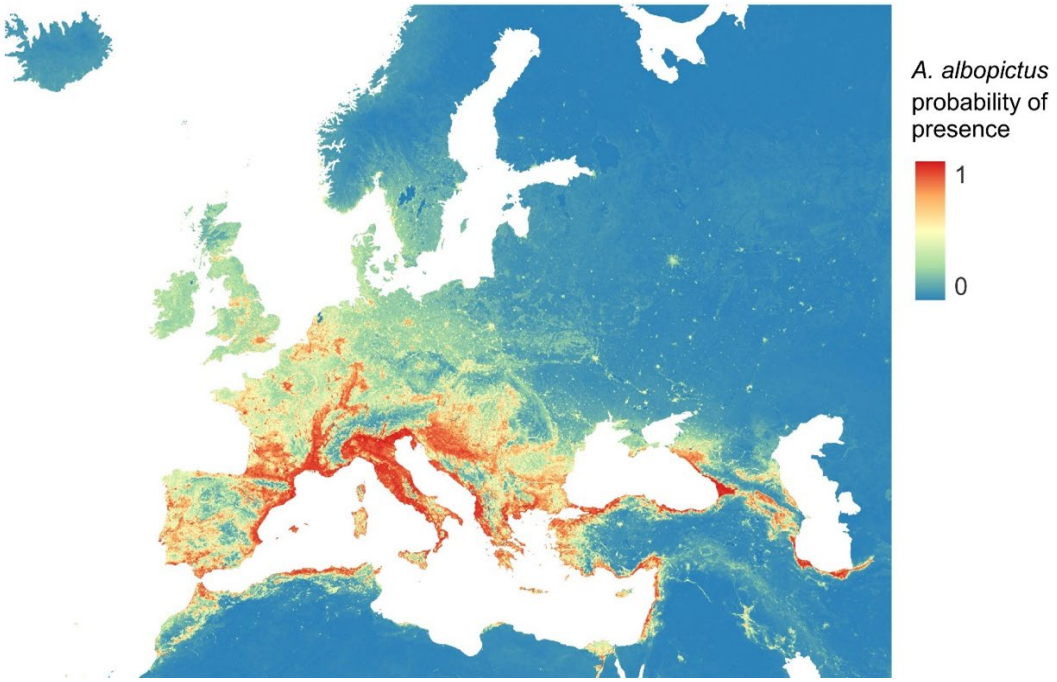

Figure 1. Current 1-km probability of presence of *Ae. albopictus* across Europe, produced using random forest and boosted regression trees analyses (source updated by ERGO for E4Warning Project)

Ecology and transmission routes

Zika virus (ZIKV) is a neurotropic flavivirus. In humans, the incubation period of ZIKV may vary from 3 to 14 days and the infectious period from 2 to 7 days. Most of the infections are asymptomatic. Symptomatic infections may cause from mild symptoms such as fever, rash, conjunctivitis, muscle and joint pain, malaise and headache to severe neurological manifestations in adults and the developing foetus. Neurological manifestations include Guillain-Barré like syndrome, neuropathy and myelitis in adults and children (29,30). In February 2016, the causal relationship between ZIKV infections and microcephaly in developing foetus was confirmed and considered until November 2016 as a Public Health Emergency of International Concern (PHEIC) by the World Health Organization (WHO). Mild symptoms are common to other arboviral and non-arboviral diseases; thus, the diagnosis of ZIKV infection requires laboratory confirmation. Transmission of ZIKV can be mosquito-borne, sexual, transfusion-based, or vertical (maternofoetal).

Mosquito-borne: The primary mode of transmission of ZIKV is mosquito-borne transmission with *Ae. aegypti* which is the major vector for ZIKV (31). *Ae. aegypti* is present in all tropical regions of the world, placing those at the highest risk of new outbreaks. The widely distributed *Ae. albopictus* can also transmit ZIKV but seems to play a minor role in the recent outbreaks (it was reported as the potential vector only in Gabon in 2007 and recently in sporadic cases in France) (32,33) (34). Other *Aedes* species with limited geographic distributions can also transmit Zika, for instance, *Ae. hensilli* on Yap Island or *Ae. polynesiensis* in French Polynesia (35, 36).

Conflicting studies suggest that mosquitoes belonging to other genera, mainly *Culex* mosquitoes, which transmit West Nile and Japanese encephalitis viruses, might be involved in ZIKV transmission (37-41).

Sexual: ZIKV sexual transmission has been suspected since 2008 when a traveller returning from an endemic area infected his partner who was in a region where vectors were absent (42). Sexual transmission is possible from both asymptomatic and symptomatic infections through genital, oral, and anal intercourse (43).

In men, ZIKV RNA shedding was demonstrated in two-thirds of semen samples tested within 30 days of illness onset, which decreased substantially within a month (44). ZIKV RNA can persist for several months in semen, but infective particles seem to be limited to the first weeks of illness, making the presence of ZIKV RNA an unreliable indicator of the presence of infectious ZIKV (44). The presence of ZIKV RNA in vaginal secretions is rare (around 2%) and its persistence is thus difficult to assess (45). The effect of Zika on male fertility is a matter of concern. The testis is an immune-privileged organ, protected by the blood-testis barrier to protect spermatogenesis. Despite this mechanism, pathogens such as ZIKV can persist in the male genital tract. The consequences of this on male fertility need to be determined. Acute Zika may alter the quality of sperm with evidence of a decreased sperm count between day 7 and day 60 after infection. Total motile sperm count decreased by 50% at day 60. Inhibin values increased until sperm count recovered (after 120 days) (46).

Transmission through Blood Transfusion: Given the presence of ZIKV in blood donors, and the report of four possible cases of transfusion-associated transmission of the virus, ZIKV should be classified as a potential transfusion-transmitted disease (47). This emerging disease is a challenge for blood banks, and strategies should be implemented to prevent transmission by blood transfusion.

Maternofoetal: Perinatal transmission of the ZIKV was first reported during the French Polynesian outbreak in 2013 (48). Viral RNA was detected in the amniotic fluid of pregnant women suffering from symptoms compatible with ZIKV infection, and later in foetal brains and products of miscarriages, supporting maternofoetal transmission of the virus (49,50). Vertical transmission will not occur in all pregnant women infected with the ZIKV and symptomatic congenital infection will not be observed in all exposed foetuses, similar to cytomegalovirus and toxoplasmosis (51). The exact rate of vertical transmission and congenital infection remains to be identified. Infectious viral particles have been detected in breast milk, but neonatal transmission by breastfeeding has so far not been described (52).

Drivers of the disease emergence and spread

Ecological drivers

ZIKV spreads primarily by vector-borne transmission. Therefore, its emergence and spread are conditioned, among other factors, by the distribution of the competent vectors. Mosquito population dynamics is itself affected by multiple components which partly depend on ecological drivers, such as temperature, precipitation or relative humidity (53, 54). Environmental conditions including land cover and urbanization also play a critical role in *Aedes* spp. establishment, favouring vector settlement and proliferation after its introduction (55). In urban environments, some facilities are favourable to the production of breeding sites, such as gardens, terraces and green spaces. Those facilities are conducive to the coexistence of high vector and human population densities (56). Although *Ae. albopictus* is known as less efficient to transmit the ZIKV than *Ae. aegypti*, its high level of eco-physiological plasticity allowed it to adapt to temperate environments and largely dispersed all over the world (57).

Moreover, the temperature seems to affect the vector/virus couple relationship including vector competence accelerating the ZIKV replication in the vector, the vector survival rate and

the hatching rate at high temperatures (58). Favoured by high temperatures, ZIKV vector transmission events appear to be stricter than those of other Aedes-transmitted viruses, such as dengue. One study showed that ZIKV transmission required a minimal temperature of 5°C warmer than DENV (59).

Natural history of disease in humans and animals, including symptoms, morbidity and mortality

Brief history of the pathogen and disease

ZIKV was first discovered in 1947 and is named after the Zika Forest in Uganda (1). In 1952, the first human cases were detected and since then, outbreaks of Zika have been reported in tropical Africa, Southeast Asia, and the Pacific Islands. Zika outbreaks have probably occurred in many locations. From the 1960s to the 1980s, sporadic human infections were detected across Africa and Asia. Since 2007, outbreaks of Zika have been recorded in Africa, the Americas, Asia, and the Pacific. Because the symptoms of Zika are similar to those of many other diseases, many cases may not have been recognized. The first European autochthonous Zika cases were reported in 2016 for sexual transmission and in 2019 for vector-borne transmission (12, 15, 16).

Disease in humans

Most ZIKV infections are asymptomatic or with mild clinical symptoms, however, infection during pregnancy can cause congenital microcephaly and other brain defects, Guillain-Barre syndrome, stillbirth and miscarriages (60). Serological evidence showed that ZIKV infection is associated with neurological disorders, such as increased intraocular pressure, resulting in damage to optic nerves which triggers a causal relationship with glaucoma (61). About 20-25% of infected patients usually develops, with an incubation period of about one week, the following symptoms: skin rashes, headache, fever, joint pains and conjunctivitis. Besides, some patients also vomit, have diarrhoea, redness of eyes, weakness, oedema, abdominal pain, loss of appetite, and experience of hematospermia (40). Detection of viral RNA in the amniotic fluid, placenta, and brain tissue of foetuses and infants with microcephaly, and the high rates of microcephaly among children born to mothers with proven acute ZIKV infection during pregnancy, provided strong evidence linking central nervous system anomalies to maternal infection (49,62).

Unlike some congenital pathogens, the ZIKV seemed to harbour a specific neurotropism (63). Major anomalies observed in foetuses and neonates include microcephaly, ventriculomegaly, diffuse calcifications, cerebral atrophy, signs of abnormal gyration, cortical development, and ocular anomalies that might lead to severe mental retardation and substantial motor disabilities, and (27) visual and auditory impairments (63). Microcephaly might not always be observed, as a normal head circumference was observed in 20% of ZIKV congenital syndromes (64); screening for microcephaly at birth is thus not sufficient to detect congenital syndromes (65). Head growth deceleration has been reported in infants born with a normal head circumference, leading to the development of microcephaly after birth (66). The clinical presentation of ZIKV infection is similar in pregnant and non-pregnant women, and congenital infection is possible even in asymptomatic women (64).

Availability of preventive, therapeutic and control measures, including licensed or pipelines vaccines

Therapy in humans

There is no antiviral-specific treatment.

Licensed or pipelined vaccines

As of 2022, no effective vaccine to prevent Zika infection is licensed and available (67). A wide variety of drug formulations are being studied; and among those being tested are live virus vaccines, inactivated vaccines, whole-virus vaccines, subunit vaccines, and messenger RNA (mRNA), DNA, protein, and vector-based formulations (67).

Other prevention measures

In the absence of therapeutic strategies and licensed vaccines, efficient vector control plays a crucial role in ZIKV prevention (68). Integrated anti-virus control is required and should include: a) epidemiological surveillance; b) environmental management focusing on educative actions to eliminate potential mosquito breeding sites and reduce standing water sites; c) chemical control using repellents (mainly for travellers and pregnant women) and insecticides, respecting the vectors resistance; and d) biological control against eggs, larvae and mosquitoes (69-72). In certain countries such as Germany, Italy, and Spain, tools or apps have been provided to citizens for reporting mosquito presence and biting incidents. Current guidelines also recommend 6 months with protected intercourse for travellers returning from endemic areas (73).

Disease-specific recommendations

WHO recommends that pregnant women avoid travelling to areas with ZIKV transmission, particularly during outbreaks, based on the increased risk of microcephaly and other severe congenital malformations. All residents of, and travellers to areas with ongoing or historical ZIKV transmission should be mindful of preventing mosquito bites and be able to make informed decisions on whether to practise protected intercourse, abstain from sex, or avoid/delay pregnancy. Pregnant women and their partners, and anyone planning pregnancy should be provided with comprehensive information about the risks associated with ZIKV infection, especially before travelling (17, 74).

Recommendations of the United States (US) Centres for Disease Control and Prevention (CDC) regarding preconception counselling are that men should use condoms for 3 months after returning from endemic areas (or after the last possible ZIKV exposure) and that women wait for two months before trying to conceive. These recommendations seem reasonable as the longest interval between infection and sexual male-to-female transmission ever reported is 44 days with a peak infectivity at 2 weeks. The risk of intrauterine transmission among ZIKV-infected women trying to conceive near the end of the recommended 8-week period is thought to be small as 95% have no detectable ZIKV RNA in serum after 6 weeks from disease onset. According to the US CDC, all pregnant women returning with possible ZIKV exposure should be tested, regardless of symptom status. Women living in areas of active virus circulation should avoid pregnancy, those

not living in endemic areas should be advised to avoid travelling in endemic countries, and all pregnant women should avoid sexual contact with partners with the same exposure risks.

Travellers who visit areas endemic for *Aedes*-borne diseases (e.g. CHIKV, DENV, ZIKV) and reside in areas of mainland EU/EEA where *Ae. albopictus* and/or *Ae. aegypti* mosquitos are established and should continue to apply personal protective measures after their return for a period of about two weeks (17).

Medical practitioners in travel clinics should be aware of the current ZIKV epidemiology during their pre-travel individual risk assessments. Health professionals providing antenatal care, obstetricians and paediatricians should also be aware of current ZIKV epidemiology to identify and investigate pregnant women exposed to ZIKV during their pregnancy and monitor the neurological development of their children. Clinicians should also remain alert to the risk of ZIKV infection in travellers returning from areas with ongoing or past transmission and the risk to their sexual contacts in the EU/EEA.

Epidemiological situation at different spatial scales: past and current trends

ZIKV circulated sporadically in Africa and Asia between 1964 and 2007 without major public health impact. The first outbreaks that significantly affected public health occurred in 2007, in Micronesia, and in 2013 in the Pacific islands (75, 76). In 2015, a major outbreak stroked South and Central America (77). To our knowledge, to date, ZIKV circulates in approximately 87 countries and territories of both tropical and subtropical regions (78). The increasing number of cases since 1964 might be associated with the expansion of urban populations, global travel and commerce, climate change, and a paucity of mosquito control programs. Following the same drivers, the low number of cases since 2020 might be associated with both underreporting and a reduction of global travel during the covid pandemic.

In link with the global epidemic of Zika and in particular the epidemic in South America, the highest number of cases in the EU/EEA countries were reported in 2016 (n=1,925; 20 countries). The majority of cases were travel-related (imported cases). In Europe, there was no autochthonous vector-borne transmission reported; however, there were 21 locally acquired cases via a sexual transmission (n=20) and vertical transmission (n=1). Over half of the cases were reported by France and most were linked with travel to the French overseas territories in the Caribbean (79). The number of cases reported by EU/EEA countries has been declining steadily since 2016 when the highest number of notifications was observed. In 2021, three EU/EEA countries reported seven human cases, the lowest number ever reported since the start of the EU/EEA surveillance in 2016 (17). Surveillance was mostly passive in reporting countries. The cases were reported by Spain (n=4), Germany (n=2) and Luxembourg (n=1). Among the cases reported in 2021, two were reported in January, two between May and June, and three from September to November. For 2021, all the cases were imported from outside the EU/EEA. The location of infection of the index case was known for six cases: two occurred in Cameroon, one in Benin, one in Sierra Leone, one in Cuba, and one in Thailand (17). The only autochthonous vector-borne transmission cases in the EU/EEA occurred in France, in 2019 (16).

Sociological and demographical dimension affecting susceptibility and exposure, including gender

As the ZIKV has circulated for several decades with sporadic or silent transmission to human beings and no reported epidemics, it was surprising when it suddenly emerged as a major public health problem. Although the epidemic potential of the virus has changed, allowing epidemic transmission, it is unclear whether the virulence has changed. Because of the close genetic and epidemiological relationship between DENV and ZIKV, the same demographic, societal, and technological factors that drove the emergence and spread of the pandemic dengue virus probably also were factors in the emergence and spread of ZIKV (80). Economic growth in tropical developing countries was a major driver of unprecedented and unplanned urban growth, which provided the ideal ecological conditions for increased *Aedes* mosquito populations living in intimate contact with crowded human populations (80).

This, combined with ineffective mosquito control and modern transportation, provided the ideal mechanism to transport both mosquitoes and viruses around the world. As with DENV, the resulting increased transmission expanded the probability of genetic change in ZIKV and thus led to the emergence of viruses with greater epidemic potential and virulence. Therefore, globalisation facilitated the geographical spread of these new viral strains.

Diagnostic procedures and notification systems used at local, national and European scale

Diagnosis

The ZIKV can be detected only in plasma or serum during acute illness. Compared with serum, urine was reported to increase the detection rate of viral RNA within the first week after symptom onset and expand the window of detection, as viral RNA was detectable up to 39 days after exposure (81). For Diagnosis of ZIKV infection, Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and serum IgM antibodies detection are the common practices. In pregnant women infected with the virus, both RT-PCR, on serum and whole blood, and serology should be considered at any time (82); however, laboratory confirmation of foetal infection during pregnancy is challenging. Detection of ZIKV RNA in blood, urine, and amniotic fluid can be negative or transient, despite the proven presence in the foetus (83).

Conversely, the virus can be detected in pregnant mothers or amniotic fluid without foetal abnormalities. Sensitivity, specificity, and negative and positive predictive values of detection of ZIKV RNA in amniotic fluids are unknown, challenging maternal counselling. For infants with possible congenital ZIKV infection, RT-PCR should be performed within the first 2 days of birth on both serum and urine, and IgM ELISA (Enzyme-Linked Immunosorbent Assay) should be performed on serum. Molecular and serology diagnosis tests are commercially available.

Infrastructure capacity to identify pathogens for each Member State

ZIKA is among the communicable diseases that according to the Commission Implementing Decision (EU) 2018/945 are covered by epidemiological surveillance. It means that EU Member States are required to establish a national capacity for the detection and reporting of human cases. The decision provides a case definition and laboratory criteria:

1. Probable case
   a. Detection of ZIKV-specific IgM antibodies in a serum sample.
2. Confirmed case at least one of the following:
   a. Detection of ZIKV nucleic acid in a clinical specimen;
   b. Detection of ZIKV antigen in a clinical specimen;
   c. Isolation of ZIKV from a clinical specimen;
   d. Detection of ZIKV specific IgM antibodies in serum sample(s) and confirmation by neutralization test;
   e. Seroconversion or a four-fold increase in the titre of Zika-specific antibodies in paired serum samples.

Diagnosis is routinely made by clinical microbiology laboratories, and there is no European-wide reference laboratory network or national laboratories in most EU countries.

Estimated influence of environmental change on the disease future trends

As for other *Aedes*-borne diseases, Zika distribution will be affected by climate change (52). The effect of climate change on El Niño was suggested has impacting ZIKV transmission (51). An assessment of the relationship between climate change, in particular the future temperature change and ZIKV transmission, showed that in the worst-case scenario, over 1.3 billion new people could face suitable transmission temperatures for ZIKV by 2050. Substantially increased ZIKV transmission temperature suitability was predicted in North America and Europe, where naïve populations might be particularly vulnerable. Mitigating climate change even to moderate emissions scenarios could significantly reduce the global expansion of climates suitable for ZIKV transmission (84).

References

1. Dick GWA, Kitchen SF, Haddow AJ. Zika Virus (I). Isolations and serological specificity. *Trans R Soc Trop Med Hyg*. 1952 Sep;46(5):509-20.
2. Dick GWA. Zika virus (II). Pathogenicity and physical properties. Trans R Soc Trop Med Hyg. 1952 Sep;46(5):521-34.
3. Bos S, Viranaicken W, Turpin J, El-Kalamouni C, Roche M, Krejbich-Trotot P, *et al.* Corrigendum to “The structural proteins of epidemic and historical strains of Zika virus differ in their ability to initiate viral infection in human host cells” (Virology 516 (2018) 265-273). *Virology*. 2019 Jan;526:233.

4. Liu ZY, Shi WF, Qin CF. The evolution of Zika virus from Asia to the Americas. *Nat Rev Microbiol.* 2019 Mar;17(3):131-9.
5. Leier HC, Messer WB, Tafesse FG. Lipids and pathogenic flaviviruses: An intimate union. *PLoS Pathog.* 2018;14(5):e1006952. doi:10.1371/journal.ppat.1006952.
6. Saiz JC, Vázquez-Calvo Á, Blázquez AB, Merino-Ramos T, Escribano-Romero E, Martín-Acebes MA. Zika Virus: the Latest Newcomer. *Front Microbiol.* 2016;7:496. doi:10.3389/fmicb.2016.00496.
7. Weaver SC, Costa F, Garcia-Blanco MA, Ko AI, Ribeiro GS, Saade G, *et al.* Zika virus: history, emergence, biology, and prospects for control. *Antiviral Res*. 2016 Jun 1;130:69-80.
8. Elveborg S, Monteil V, Mirazimi A. Methods of inactivation of highly pathogenic viruses for molecular, serology or vaccine development purposes. *Pathogens*. 2022 Feb 19;11(2):271.
9. Müller JA, Harms M, Schubert A, Jansen S, Michel D, Mertens T, *et al.* Inactivation and environmental stability of Zika Virus. *Emerg Infect Dis*. 2016 Sep;22(9):1685-7.
10. Emmanouil M, Evangelidou M, Papa A, Mentis A. Importation of dengue, Zika and chikungunya infections in Europe: the current situation in Greece. *New Microbes New Infect.* 2020 May;35:100663.
11. European Centre for Disease Prevention and Control. I*ncreasing risk of mosquito-borne diseases in EU/EEA following spread of Aedes species*. Stockholm: ECDC; 2023. Available from: https://www.ecdc.europa.eu/en/news-events/increasing-risk-mosquito-borne-diseases-eueea-following-spread-aedes-species
12. Grossi PA, Percivalle E, Campanini G, Sarasini A, Premoli M, Zavattoni M, *et al.* An autochthonous sexually transmitted Zika virus infection in Italy 2016. *New Microbiol*. 2018 Jan;41(1):80-2.
13. Venturi G, Zammarchi L, Fortuna C, Remoli ME, Benedetti E, Fiorentini C, *et al.* An autochthonous case of Zika due to possible sexual transmission, Florence, Italy, 2014. *Euro Surveill*. 2016 Feb 25;21(8):30148.
14. Fréour T, Mirallié S, Hubert B, Splingart C, Barrière P, Maquart M, *et al.* Sexual transmission of Zika virus in an entirely asymptomatic couple returning from a Zika epidemic area, France, April 2016. *Euro Surveill*. 2016 Jun 9;21(23):30254.
15. Frank C, Cadar D, Schlaphof A, Neddersen N, Günther S, Schmidt-Chanasit J, *et al.* Sexual transmission of Zika virus in Germany, April 2016. *Euro Surveill*. 2016 Jun 9;21(23):30252.
16. Giron S, Franke F, Decoppet A, *et al.* Vector-borne transmission of Zika virus in Europe, southern France, August 2019. *Euro Surveill.* 2019;24(45):1900655. doi:10.2807/1560-7917.ES.2019.24.45.1900655
17. ECDC. *Zika virus disease, annual Epidemiological Report for 2021*. Stockholm: European Centre for Disease Prevention and Control; 2023. (Annual Epidemiological Report for 2021).
18. Spiteri G, Sudre B, Septfons A, Beauté J; European Zika surveillance Network. Surveillance of Zika virus infection in the EU/EEA, June 2015 to January 2017. *Euro Surveill.* 2017;22(41):17-00254. doi:10.2807/1560-7917.ES.2017.22.41.17-00254.
19. Centers for Disease Control and Prevention. *Zika Travel Information. Travelers' Health*. CDC (Internet). (cited 2023 Jul 25). Available from: https://wwwnc.cdc.gov/travel/page/zika-information
20. Wong PSJ, Li M zhi I, Chong CS, Ng LC, Tan CH. Aedes (Stegomyia) albopictus (Skuse): A Potential Vector of Zika Virus in Singapore. Turell MJ, editor. *PLoS Negl Trop Dis*. 2013 Aug 1;7(8):e2348.
21. Weaver SC, Reisen WK. Present and future arboviral threats. *Antiviral Res*. 2010 Feb;85(2):328-45.
22. Ebi KL, Nealon J. Dengue in a changing climate. *Environ Res*. 2016 Nov;151:115-23.
23. Reiter P. Climate change and mosquito-borne disease. *Environ Health Perspect*. 2001 Mar;109(suppl 1):141-61.

24. Almeida AP, Gonçalves YM, Novo MT, Sousa CA, Melim M, Grácio AJ. Vector monitoring of Aedes aegypti in the Autonomous Region of Madeira, Portugal. *Euro Surveill.* 2007;12(11):E071115.6. doi:10.2807/esw.12.46.03311-en
25. Scholte E, Den Hartog W, Dik M, Schoelitsz B, Brooks M, Schaffner F, *et al.* Introduction and control of three invasive mosquito species in the Netherlands, July-October 2010. *Euro Surveil*. 2010 Nov 11;15(45):19710.
26. Paupy C, Delatte H, Bagny L, Corbel V, Fontenille D. Aedes albopictus, an arbovirus vector: From the darkness to the light. *Microbes Infect*. 2009 Dec;11(14-15):1177-85.
27. Adhami J, Reiter P. Introduction and establishment of Aedes (Stegomyia) albopictus skuse (Diptera: Culicidae) in Albania. *J Am Mosq Control Assoc.* 1998 Sep;14(3):340-3.
28. Boukraa S, Raharimalala FN, Zimmer JY, Schaffner F, Bawin T, Haubruge E, *et al.* Reintroduction of the invasive mosquito species *Aedes albopictus* in Belgium in July 2013. *Parasite*. 2013;20:54.
29. World Health Organization. *Zika virus*. Geneva: WHO; 2022. Available from: https://www.who.int/news-room/fact-sheets/detail/zika-virus; last visited May 2023.
30. Bhardwaj U, Pandey N, Rastogi M, Singh SK. Gist of Zika Virus pathogenesis. *Virology*. 2021 Aug 1;560:86-95.
31. Boyer S, Calvez E, Chouin-Carneiro T, Diallo D, Failloux AB. An overview of mosquito vectors of Zika virus. *Microbes Infect*. 2018 Dec;20(11-12):646-60.
32. Grard G, Caron M, Mombo IM, *et al.* Zika virus in Gabon (Central Africa)--2007: a new threat from Aedes albopictus?. *PLoS Negl Trop Dis.* 2014;8(2):e2681. doi:10.1371/journal.pntd.0002681
33. Parola P, Musso D. Zika, dengue, chikungunya and yellow fever infections in Europe? - Winter is over, warm days are coming - So hedge your bets. *Travel Med Infect Dis*. 2020 May;35:101614.
34. Di Luca M, Severini F, Toma L, Boccolini D, Romi R, Remoli ME, *et al.* Experimental studies of susceptibility of Italian Aedes albopictus to Zika virus. *Euro Surveill*. 2016 May 5;21(18).
35. Richard V, Paoaafaite T, Cao-Lormeau VM. Vector Competence of French Polynesian Aedes aegypti and Aedes polynesiensis for Zika Virus. Pimenta PF, editor. *PLoS Negl Trop Dis*. 2016 Sep 21;10(9):e0005024.
36. Ledermann JP, Guillaumot L, Yug L, Saweyog SC, Tided M, Machieng P, *et al.* Aedes hensilli as a potential vector of Chikungunya and Zika Viruses. *PLoS Negl Trop Dis*. 2014 Oct 9;8(10):e3188.
37. Guo X xia, Li C xiao, Deng Y qiang, Xing D, Liu Q mei, Wu Q, *et al. Culex pipiens quinquefasciatus* : a potential vector to transmit Zika virus. *Emerg Microbes Infect.* 2016 Jan;5(1):1-5.
38. Viveiros-Rosa SG, Regis EG, Santos WC. Vector competence of Culex mosquitoes (Diptera: Culicidae) in Zika virus transmission: an integrative review. *Pan Am J Public Health*. 2020;44:e7.
39. Wang J, Xu H, Song S, Cheng R, Fan N, Fu S, *et al.* Emergence of Zika Virus in Culex tritaeniorhynchus and Anopheles sinensis Mosquitoes in China. *Virol Sin.* 2020 Jul 2;36(1):33-42.
40. Foy BD, Kobylinski KC, Foy JLC, Blitvich BJ, Travassos da Rosa A, Haddow AD, *et al.* Probable Non-Vector-borne Transmission of Zika Virus, Colorado, USA. *Emerg Infect Dis*. 2011 May;17(5):880-2.
41. Boccolini D, Toma L, Di Luca M, Severini F, Romi R, Remoli ME, *et al.* Experimental investigation of the susceptibility of Italian Culex pipiens mosquitoes to Zika virus infection. *Euro Surveill*. 2016 Sep 1;21(35):30328.
42. Venturi G, Zammarchi L, Fortuna C, Remoli ME, Benedetti E, Fiorentini C, *et al.* An autochthonous case of Zika due to possible sexual transmission, Florence, Italy, 2014. *Euro Surveill*. 2016;21(8):30148.

43. Davidson A, Slavinski S, Komoto K, Rakeman J, Weiss D. Suspected Female-to-Male Sexual Transmission of Zika Virus — New York City, 2016. *MMWR Morb Mortal Wkly Rep*. 2016 Jul 22;65(28):716-7.
44. Mead PS, Duggal NK, Hook SA, Delorey M, Fischer M, Olzenak McGuire D, *et al.* Zika Virus shedding in semen of symptomatic infected men. *N Engl J Med*. 2018 Apr 12;378(15):1377-85.
45. Paz-Bailey G, Rosenberg ES, Doyle K, Munoz-Jordan J, Santiago GA, Klein L, *et al.* Persistence of Zika Virus in body fluids — Final report. *N Engl J Med*. 2018 Sep 27;379(13):1234-43.
46. Joguet G, Mansuy JM, Matusali G, Hamdi S, Walschaerts M, Pavili L, *et al.* Effect of acute Zika virus infection on sperm and virus clearance in body fluids: a prospective observational study. *Lancet Infect Dis*. 2017 Nov;17(11):1200-8.
47. Musso D, Stramer SL, Busch MP. Zika virus: a new challenge for blood transfusion. *The Lancet*. 2016 May;387(10032):1993-4.
48. Besnard M, Lastere S, Teissier A, Cao-Lormeau V, Musso D. Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. *Euro Surveill*. 2014 Apr 3;19(13):20751.
49. Calvet G, Aguiar RS, Melo ASO, Sampaio SA, de Filippis I, Fabri A, *et al.* Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. *Lancet Infect Dis*. 2016 Jun;16(6):653-60.
50. Van Der Eijk AA, Van Genderen PJ, Verdijk RM, Reusken CB, Mögling R, Van Kampen JJA, *et al.* Miscarriage Associated with Zika Virus Infection. *N Engl J Med*. 2016 Sep 8;375(10):1002-4.
51. Vouga M, Musso D, Van Mieghem T, Baud D. CDC guidelines for pregnant women during the Zika virus outbreak. *The Lancet*. 2016 Feb;387(10021):843-4.
52. Dupont-Rouzeyrol M, Biron A, O'Connor O, Huguon E, Descloux E. Infectious Zika viral particles in breastmilk. *The Lancet*. 2016 Mar;387(10023):1051.
53. Cai X, Zhao J, Deng H, *et al.* Effects of temperature, relative humidity, and illumination on the entomological parameters of Aedes albopictus: an experimental study. *Int J Biometeorol.* 2023;67(4):687-694. doi:10.1007/s00484-023-02446-y
54. Reinhold JM, Lazzari CR, Lahondère C. Effects of the environmental temperature on Aedes aegypti and Aedes albopictus mosquitoes: A Review. *Insects*. 2018 Nov 6;9(4):158.
55. Muhammad NAF, Abu Kassim NF, Ab Majid AH, Abd Rahman A, Dieng H, Avicor SW. Biting rhythm and demographic attributes of Aedes albopictus (Skuse) females from different urbanized settings in Penang Island, Malaysia under uncontrolled laboratory conditions. *PLoS One.* 2020 Nov 11;15(11):e0241688.
56. Benitez EM, Ludueña-Almeida F, Frías-Céspedes M, Almirón WR, Estallo EL. Could land cover influence Aedes aegypti mosquito populations? *Med Vet Entomol*. 2020;34(2):138-44.
57. Gómez M, Martinez D, Muñoz M, Ramírez JD. Aedes aegypti and Ae. albopictus microbiome/virome: new strategies for controlling arboviral transmission? *Parasit Vectors*. 2022 Aug 9;15(1):287.
58. Jian X yi, Jiang Y ting, Wang M, Jia N, Cai T, Xing D, *et al.* Effects of constant temperature and daily fluctuating temperature on the transovarial transmission and life cycle of Aedes albopictus infected with Zika virus. *Front Microbiol*. 2023 Jan 4;13:1075362.
59. Tesla B, Demakovsky LR, Mordecai EA, Ryan SJ, Bonds MH, Ngonghala CN, *et al.* Temperature drives Zika virus transmission: evidence from empirical and mathematical models. *Proc Biol Sci*. 2018 Aug 15;285(1884):20180795.
60. Kakooza-Mwesige A, Tshala-Katumbay D, Juliano SL. Viral infections of the central nervous system in Africa. *Brain Res Bull*. 2019 Feb;145:2-17.

61. Adebayo G, Neumark Y, Gesser-Edelsburg A, Abu Ahmad W, Levine H. Zika pandemic online trends, incidence and health risk communication: a time trend study. *BMJ Glob Health*. 2017 Aug;2(3):e000296.
62. Brasil P, Pereira JP, Moreira ME, Ribeiro Nogueira RM, Damasceno L, Wakimoto M, *et al.* Zika Virus Infection in Pregnant Women in Rio de Janeiro. *N Engl J Med*. 2016 Dec 15;375(24):2321-34.
63. Vouga M, Baud D. Imaging of congenital Zika virus infection: the route to identification of prognostic factors. *Prenat Diagn*. 2016 Sep;36(9):799-811.
64. França GVA, Schuler-Faccini L, Oliveira WK, Henriques CMP, Carmo EH, Pedi VD, *et al.* Congenital Zika virus syndrome in Brazil: a case series of the first 1501 livebirths with complete investigation. *The Lancet*. 2016 Aug;388(10047):891-7.
65. Eppes C, Rac M, Dunn J, Versalovic J, Murray KO, Suter MA, *et al.* Testing for Zika virus infection in pregnancy: key concepts to deal with an emerging epidemic. *Am J Obstet Gynecol*. 2017 Mar;216(3):209-25.
66. van der Linden V, Pessoa A, Dobyns W, Barkovich AJ, Júnior H van der L, Filho ELR, *et al.* Description of 13 infants born during October 2015-January 2016 with congenital Zika virus infection without microcephaly at birth — Brazil. *MMWR Morb Mortal Wkly Rep*. 2016 Dec 2;65(47):1343-8.
67. Poland GA, Ovsyannikova IG, Kennedy RB. Zika vaccine development: current status. *Mayo Clin Proc*. 2019 Dec;94(12):2572-86.
68. Huang YJ, Higgs S, Vanlandingham D. Biological control strategies for mosquito vectors of Arboviruses. *Insects*. 2017 Feb 10;8(1):21.
69. Hemingway J, Ranson H. Insecticide resistance in insect vectors of human disease. *Annu Rev Entomol*. 2000;45:371-91.
70. Benelli G. Managing mosquitoes and ticks in a rapidly changing world - Facts and trends. *Saudi J Biol Sci*. 2019 Jul;26(5):921-9.
71. Benelli G. Plant-borne ovicides in the fight against mosquito vectors of medical and veterinary importance: a systematic review. *Parasitol Res*. 2015 Sep;114(9):3201-12.
72. Islam J, Zaman K, Duarah S, Raju PS, Chattopadhyay P. Mosquito repellents: An insight into the chronological perspectives and novel discoveries. *Acta Trop*. 2017 Mar;167:216-30.
73. Counotte MJ, Kim CR, Wang J, Bernstein K, Deal CD, Broutet NJN, *et al.* Sexual transmission of Zika virus and other flaviviruses: A living systematic review. *PLoS Med*. 2018 Jul 24;15(7):e1002611.
74. ECDC. *Zika virus transmission worldwide*. Stockholm: European Centre for Disease Prevention and Control; 2019. (Rapid risk assessment report).
75. Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, *et al.* Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med*. 2009 Jun 1;360(24):2536-43.
76. Cao-Lormeau VM, Roche C, Teissier A, Robin E, Berry AL, Mallet HP, *et al.* Zika Virus, French Polynesia, South Pacific, 2013. *Emerg Infect Dis*. 2014 Jun;20(6):1085-6.
77. Ribeiro IG, Andrade MR de, Silva J de M, Silva ZM, Costa MA de O, Vieira MA da CES, *et al.* Microcephaly in Piauí, Brazil: descriptive study during the Zika virus epidemic, 2015-2016. *Epidemiol Serv Saude Rev*. 2018 Feb 1;27(1):e20163692.
78. Giraldo MI, Gonzalez-Orozco M, Rajsbaum R. Pathogenesis of Zika virus infection. *Annu Rev Pathol*. 2023 Jan 24;18:181-203.
79. ECDC. *Zika virus disease, annual epidemiological report for 2016*. Stockholm: European Centre for Disease Prevention and Control; 2018. (Annual Epidemiological Report for 2016).
80. Gubler DJ. Dengue, urbanization and globalization: the unholy trinity of the 21st century. *Trop Med Health*. 2011;39(4SUPPLEMENT):S3-11.

81. Bingham AM, Cone M, Mock V, Heberlein-Larson L, Stanek D, Blackmore C, *et al.* Comparison of test results for Zika Virus RNA in urine, serum, and saliva specimens from persons with travel-associated Zika Virus Disease — Florida, 2016. *MMWR Morb Mortal Wkly Rep.* 2016 May 13;65(18):475-8.

82. Baud D, Van Mieghem T, Musso D, Truttmann AC, Panchaud A, Vouga M. Clinical management of pregnant women exposed to Zika virus. *Lancet Infect Dis*. 2016 May;16(5):523.

83. Vedovello D, Witkin SS, Silva ACB, Fajardo TCG, Mello AS, Bertozzi APAP, *et al.* Detection of Zika virus in paired urine and amniotic fluid samples from symptomatic and asymptomatic women and their babies during a disease outbreak: association with neurological symptoms in newborns. *J Neurovirol*. 2020;26(1):70-6.

84. Ryan SJ, Carlson CJ, Tesla B, Bonds MH, Ngonghala CN, Mordecai EA, *et al.* Warming temperatures could expose more than 1.3 billion new people to Zika virus risk by 2050. *Glob Change Biol*. 2021 Jan;27(1):84-93.

Serie Rapporti ISTISAN
numero di settembre 2024, 2° Suppl.

Stampato in proprio
Servizio Comunicazione Scientifica – Istituto Superiore di Sanità

Roma, settembre 2024