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Atti del

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Sommario

Razionale del Congresso

Il Congresso Nazionale di Ecopatologia della Fauna è il principale appuntamento a livello nazionale nel campo dell'ecopatologia della fauna, che ha lo scopo di promuovere il confronto, tra ricercatori, operatori della sanità pubblica ed animale, tecnici faunistici, esperti di gestione, istituzioni ed appassionati, sugli aspetti ecopatologici della gestione e conservazione della fauna, sulle implicazioni di sanità pubblica e sanità animale legate alle popolazioni selvatiche, sui rapporti tra gli animali selvatici e le attività umane e, infine, sulla ricerca scientifica in ecopatologia.

Il Congresso è articolato in tre sessioni tematiche e in una a tema libero, che abbracceranno diversi temi, dalla conservazione e gestione alla ricerca, sempre in chiave ecopatologica.

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Università di Bologna, Dipartimento di Scienze Mediche Veterinarie

COMUNICAZIONI

Sessione 1

METODOLOGIE ED ESPERIENZE DI SORVEGLIANZA E MONITORAGGIO SANITARIO DELLE POPOLAZIONI SELVATICHE

Chairmen: Carlo Citterio – Cristina Esmeralda Di Francesco

La sorveglianza ed il monitoraggio delle popolazioni selvatiche sono oggi essenziali per acquisire informazioni sulla comparsa, diffusione e persistenza dei patogeni che possono rappresentare una minaccia per la salute degli animali, dell'uomo e dell'ambiente. Senza dimenticare i metodi di campionamento, diagnosi e analisi dei dati più classici, anche nuove tecnologie e metodologie possono migliorare e modificare le attività di sorveglianza e monitoraggio. È però indispensabile che questi nuovi approcci, spesso derivanti da molteplici discipline, dall'ecologia alla statistica, dalla genetica alla bioinformatica, solo per citarne alcune, contribuiscano insieme all' interpretazione dei risultati ottenuti, per una corretta definizione delle priorità, del rischio e della sua possibile mitigazione.

RELAZIONI AD INVITO

Integrated wildlife monitoring to prevent future pandemics.
Jorge Ramón López Olvera (Esperto di Ecopatologia della Fauna Selvatica, Barcellona, Spagna)

CONTRIBUTI LIBERI

African Swine Fever in Italy: an update of the epidemiological situation related to the presence of genotype II virus. ISCARO C., DI SABATINO D., CRESCI M., RUOCCO L., SORDILLI M., PLASMATI F., PACELLI F., FELIZIANI F.
Francesco Feliziani (Lab. di Referenza Nazionale PSA, Ist. Zooprofilattico Sperimentale Umbria e Marche)

Preliminary results of a field trial for wild boar cadavers to support passive surveillance for African Swine Fever detection. OBBER F., CELVA R., ASSIRELLI G., CARLIN S., ZACOMETTI C., DA ROLD G., FRANZOSO A., MAZZUCATO M., MULATTI P., CHIARELLO G., POLETTO E., MIAN G., CASARA A., VERIN R., GALLO M., ROCCA G., MICHELOTTO E., TATA A., ROSA G., POZZATO N., CITTERIO C.V.
Federica Obber (Istituto Zooprofilattico Sperimentale delle Venezie)

Spreading of antimicrobial resistance in Escherichia spp. strains isolated from hunted wild boar and farmed pigs in central Italy (Umbria and Marche regions). DI GIACINTO G., RUBIOLA S., TOPPI V., RANUCCI D., MEROLA C., CASAGRANDE PROIETTI P., GRASSI A., CHIESA F.
Giacomo Di Giacinto (Università degli Studi di Torino, Dipartimento di Medicina Veterinaria)

Evidence of the wide circulation of multi-drug resistant enteric strains in lesser kestrel (*Falco naumanni*). LOMBARDI R., SAMARELLI R., PUGLIESE N., PRIOLETTI M., ROMITO D., BOVE A., D'ONGHIA F., SALIERNO D., CASALINO G., CIRCELLA E., CAMARDA A.
Roberto Lombardi (Università degli Studi di Bari. Dipartimento di Medicina Veterinaria)

Tracking the health of wolves (*Canis lupus*) in Foreste Casentinesi national park: merging conservation and public health. FERRARO E., DA ROLD G., CASSINI R., CELVA R., DALLA LIBERA E., LEOPARDI S., DE BENEDICTIS P., CAPPAI N., DISSEGNA A., CITTERIO C., OBBER F.
Elisabetta Ferraro (Università degli Studi di Padova. Dipartimento di Medicina animale, Produzioni e Salute)

Monitoring potential pathogens harboured by the alien slider *Trachemys scripta* in the North of Italy collection Centers. MORETTI A., RAPI M.C., GRILLI G., D'INCAU M., ORIGGI F., FERRARI N.
Amanda Moretti (Università degli Studi di Milano. Dipartimento di Medicina Veterinaria e Scienze animali)

Dermatophytes and wildlife: a study of the European hedgehog (*Erinaceus europaeus*) in Piedmont. PASSARINO E., PRANDI I., BONAFFINI G., OTTINO C., SERPIERI M., MAUTHE VON DEGERFELD M., PEANO A., QUARANTA G.
Elena Passarino (Università degli Studi di Torino. Dipartimento di Scienze Veterinarie)

Dicrocoelium dendriticum in roe deer (*Capreolus capreolus*) in Central Italy. SCARCELLI S., BUONO F., OTTAVIANO M., DIAFERIA M., LEPRI E., PEPE P., MAURELLI M.P., D'ALESSIO N., FIORETTI A., VARUZZA P., SGROI G., VENEZIANO V.
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INTEGRATED WILDLIFE MONITORING TO PREVENT FUTURE PANDEMICS

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Integrated wildlife monitoring includes two main components: wildlife population monitoring and wildlife health surveillance.

Wildlife population monitoring provides information about the number of animals, and therefore the infection pressure they may pose for the wildlife population itself, domestic animals and humans, as well as their potential role in the epidemiology of shared diseases. Beyond population abundance or density, as much other information as possible is to be gathered (for example, species distribution, social structure, sex and age class structure, morphometry and body condition, etc.), since they may be useful as early indicators of potential prepathological changes in the population.

The potential for a wildlife species to act as disease reservoir or just being a spill-over or dead-end host is determined by the population-scale consequences of the individual-scale dynamic balance between pathogen and host, which in turn is influenced by changes in the environment and the introduction of new agents (either pathogens or hosts) in the system. The worldwide exponential increase of human population is coupled with biodiversity loss and an increase of the interface among humans, domestic animals and their pathogens, on the one hand, and the wildlife and pathogens from the less anthropized ecosystems, on the other hand. Such increased contact becomes an unavoidable origin for the apparition of emerging diseases on both sides, and the jump of those pathogens more adaptable among different susceptible host species, with epidemiological and demographic consequences for humans, domestic animals, and wildlife. Although in most of the cases the natural trend of a new disease in a new population or species is tending to become endemic, if the interspecific pathogen jump is too frequent to allow the systems to adapt and rebalance, the outcome may be deleterious and catastrophic.

From a One Health approach, wildlife population monitoring and wildlife health surveillance should allow the achievement of the knowledge required to implement management and control actions for wildlife populations and shared diseases. Such monitoring and surveillance are also the tools to assess the effect of the management and control measures carried out, allowing the modification of such measures to achieve and complete an adaptive management system that readjusts constantly to changes in the conditions of the system, including environment, pathogens, and hosts. Additionally, studying the eco-epidemiological relationships and the contact network among hosts for a given pathogen in a specific system allow to identify the key host to monitor the epidemiology of that pathogen. Therefore, focusing the monitoring and surveillance efforts on such key hosts allows the integrated monitoring of the whole system.

When managing wildlife diseases, the management actions and measures must aim at preventing the entry of a pathogen in a system where it is not present and controlling the disease where it is present, since not acting at all is not an option and eradicating an endemic disease in an abundant wildlife population is challenging and rarely succeeds. In any case, not worsening the situation through unplanned actions without a sound scientific basis and knowledge should be the first priority. The main management strategies focus on biosecurity aimed at preventing or decreasing pathogen transmission among the different compartments (humans, domestic animals and wildlife); on wildlife population management, either decreasing density or targeting individuals specifically relevant for disease transmission; and on the pathogen. Monitoring and surveillance are always essential and must accompany any management measure before, during, and after its application.

The attempts to control or eradicate diseases in wildlife diseases have yielded variable results, ranging from no effect or even worsening of the epidemiological situation to eradication in specific cases, altogether demonstrating the difficulty of managing diseases in wildlife free-ranging populations. Finally, once the knowledge allowing the establishment of management measures to control wildlife diseases is achieved, the possibility of implementing such measures successfully is strongly conditioned by sociological and political human factors, which may be also taken into account since they are most determinant for the success of fail of the whole management strategy.

AFRICAN SWINE FEVER IN ITALY: AN UPDATE OF THE EPIDEMIOLOGICAL SITUATION RELATED TO THE PRESENCE OF GENOTYPE II VIRUS

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Introduction

To date African Swine Fever (ASF) is the major challenge for pig production worldwide, due to the heavy damage both at sanitary and economic levels. Italy was firstly affected by ASF virus genotype II (ASFV) in 2022 and currently the spreading of infection involves 4 clusters in 9 regions and 17 provinces. In relation to the size of affected territories, submitted to restriction measures as per Regulation EU 2023/594, the largest cluster is the north-west one. It is also the most dynamic and serious, due to the contiguity with the most relevant site for pig production in the country and to the involvement of mountainous territory of the Apennines. The infection is spreading in wild boar populations in all regional cluster; the domestic pig sector is involved also, showing a clear seasonality in spring/summer: epidemic waves occurred in 2023 (Calabria and Lombardy) and in 2024 (Piedmont, Lombardy, Emilia Romagna).

Material and Methods

VETINFO is the portal for epidemic surveillance data management for the Italian Animal Health Official Services. ASF data collection is allowed by the implementation of such informative systems on VETINFO. Firstly, SINVSA collects surveillance data in the ASF free territories, including sampling data by the Veterinary Services and testing ones by the Official Laboratories. In SIMAN, eradication data from the affected regions, as well as data from suspected cases in free areas, are collected. Therefore, the Dashboard and the Storymap show real time data on surveillance and eradication activities in the national territory for wild boar and domestic pigs, as well as the prevention and control activities carried out in free zones and in restricted ones. Finally, the latest implemented informative system collects data about hunting activities in ASF free zone.

Results and Discussion

Current ASF infection clusters undergo different epidemic phases. In Latium region, Rome municipality, the epidemics began in May 2022 in the wild boar population and a silent phase is reported currently. Due to a heavy activity of search for wild boar carcasses, a relevant number of them have been removed and a reduction of viral pressure in the environment has been allowed. Therefore, a decreasing of wild boar density in the area has been registered, due to the mortality caused by the disease and to the effective trapping actions. Here the latest case was notified in June 2024. In the bi-regional cluster Campania/Basilicata, the disease was notified firstly in May 2023 in Campania region. To date the infection cluster is still active, even if the infected area has not been subjected to a significative enlargement after the last one occurred in June 2024. At the same time, local veterinary services are coordinating an intensive activity of search for carcasses and wild boar depopulation measures. In Basilicata region, lack of surveillance data are not indicative for the absence of infection. Also in Calabria region the disease was notified firstly in May 2023 and surveillance data in the restriction zone II are not enough to rule out the persistence of the infection in the wild boar population; on the other hand, the restriction zone III established following the outbreaks in 2023, has been definitively lifted in September 2024. In the North-West cluster, the wild boar infection area is very large compared to that in 2022 at the beginning of epidemics in Liguria and Piedmont. Therefore, further enlargement is ongoing: southward, where the rugged territories of central Apennines (Tuscany) became infected in July 2024; south-eastern ward, since April 2024, involving the territory overlooking the Po Valley (Emilia Romagna); and northward, where infection is spreading likely from Lombardy, due to the presence of natural parks and ecological corridors such as the Ticino Park. Otherwise, in Savona province, surveillance data showed a progressive reduction of infection parameters that allowed to lift the restriction zone II on September 2024. Regarding domestic pig population, two epidemic waves occurred in 2023 (9 outbreaks in Lombardy only) and in 2024 (29 outbreaks in Piedmont, Lombardy and Emilia Romagna), in commercial farms. For both waves, a spread of infection from wild boar to kept pigs has been largely suspected for the primary outbreaks, via direct or indirect contact; subsequently, breaches in the biosecurity systems have been strongly supposed for all secondary outbreaks, due a lack of awareness in the structural and functional biosecurity measures in the farms. The Italian epidemiological context for ASFV genotype II appears definitively challenging and complex to be managed by all the involved actors.

PRELIMINARY RESULTS OF A FIELD TRIAL FOR WILD BOAR CADAVERS TO SUPPORT PASSIVE SURVEILLANCE FOR AFRICAN SWINE FEVER DETECTION

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Introduction

African swine fever (ASF) is a viral disease of pigs and wild boar that causes high mortality in the affected animals. Although harmless to humans, this virus is causing significant socio-economic disruption in many countries. Definition of the post-mortem interval (PMI), entomofauna composition and the study of the decay process in the sylvatic environment in different microhabitats is crucial to improve surveillance and identify the range of possible spread of the virus after the detection of the index case.

Materials and methods

Two sampling sites, representative of environmental and climatic features of the study area, have been identified in the Colli Euganei Regional Park (Padua, Italy). Three wild boar cadavers were placed in each site across four consecutive seasons (summer and autumn 2023, winter and spring 2024), and monitored for 2 months. For each cadaver, consumption by scavengers was assessed through continuous photo-trapping. In both sites, environmental temperature and humidity were recorded using external data logger; in addition, internal data loggers were placed inside each cadaver, allowing to monitor changes in body temperature. The state of decomposition was periodically evaluated by filling a total body score (TBS) sheet, as well as by collecting and identifying necrophagous entomofauna. Periodically, a sample of skin/subcutis/muscle was collected from each cadaver for histology, while samples of muscle only were withdrawn for metabolomic analysis by Direct Analysis in Real Time High Resolution Mass Spectrometry.

Results and Discussion

Wildlife interactions with the corpses were recorded on several occasions, including wild boar. No consumption activity was recorded. Time to advanced decomposition ranged from 3 days (summer) to 24 days (winter). During the first and second sampling sessions, 491 and 323 entomological samples were collected, respectively. For the first sessions, taxonomic identification of entomofauna specimen has been carried out using dichotomous keys for adults, 3rd instar larvae and pupae: the predominant orders of insects were Diptera (7 families, 11 genera) and Coleoptera (7 families, 9 orders); amongst the latter, the Dermestidae family was first detected in chronological order (adults from day two). Samples collected during the other three sessions are currently being processed. Data collected so far are in line with previous literature, leading to validate the sampling method. Metabolomic analyses allowed to detect changes in muscle content in both volatile and non-volatile compounds during decomposition. An Accumulated Degree-Days calculator that integrates temperature, TBS, entomology, histology and metabolomics is under development.

The present study is expected to provide detailed information for the estimation of PMI of wild boars in Northern Italy, as a useful integration for ASF surveillance and management strategies. In a European perspective, data obtained in this study will increase the amount of available information useful to refine and validate epidemiological and surveillance/management models for ASF.

The present work is part of the research project IZSVe RC 06/2022 – Death boars funded by the Italian Ministry of Health.

SPREADING OF ANTIMICROBIAL RESISTANCE IN *ESCHERICHIA* SPP. STRAINS ISOLATED FROM HUNTED WILD BOAR AND FARMED PIGS IN CENTRAL ITALY (UMBRIA AND MARCHE REGIONS)

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Introduction

Antibiotic resistance (AR) is a growing global health concern that extends beyond human and domestic animal populations, posing a significant threat to wildlife and ecosystems. Monitoring AR in wildlife is essential for understanding the spread of resistant pathogens and their potential impact on both animals and humans. In this study, we focused on wild boar (*Sus scrofa*), a widespread species in central Italy, as a key indicator for AR surveillance in wildlife. Specifically, we targeted microorganisms belonging to the *Escherichia* genus, conducting a comprehensive analysis of antibiotic-resistant *Escherichia* spp. strains isolated from wild boar populations. To better understand the dynamics of AR, we compared these findings with *Escherichia* spp. strains isolated from farmed pigs raised in the same geographical areas. This comparative approach allowed us to evaluate the potential exchange of resistant bacteria between wildlife and livestock. A key aspect of our study is the investigation of the dissemination of antimicrobial resistance genes (ARGs) through whole genome sequencing (WGS), a method strongly recommended by both the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC). WGS enables a deeper understanding of the genetic mechanisms underlying AR, allowing for the precise identification of resistance genes and the tracking of their spread across different ecosystems and host populations.

Material and Methods

For this study, intestinal contents were sampled from 70 wild boar and 50 pigs. The isolation of microorganisms of interest was carried out using standard methods, and species identification was performed via mass spectrometry analysis using MALDI-TOF technology. All *Escherichia* spp. strains were subjected to a disk diffusion test to assess phenotypic antimicrobial susceptibility against 16 antibiotics belonging to 9 chemotherapeutic classes. The inhibition zones provided by the disk diffusion test were interpreted according to the reference CLSI manual. Following this screening, strains resistant to at least one class of antimicrobials were selected and subjected to whole genome sequencing (WGS) in order to identify AR genes.

Results and Discussion

From a total of 120 samples (70 wild boar and 50 farm pigs), 114 *Escherichia coli* strains and 4 *Escherichia marmotae* strains were isolated. *Escherichia* spp. strains isolated from wild boar and farm pigs showed resistance, respectively, to ampicillin (54% vs 52%), amoxicillin/clavulanate (10% vs 22%), tetracycline (3% vs 34%), gentamicin (0% vs 6%), and sulfamethoxazole/trimethoprim (1% vs 10%). No multidrug-resistant (MDR) *E. coli* strains were found in wild boar, whereas 19% of *E. coli* strains from farm pigs were MDR.

Our preliminary results confirmed the high prevalence of bacteria in farm pigs, especially those reared under intensive conditions, while the prevalence of AR bacteria in wild boar hunted in Umbria was lower compared to previous studies. Additionally, WGS provides critical insights that go beyond traditional phenotypic resistance tests, offering a more comprehensive view of the evolutionary and epidemiological pathways of AR. These findings highlight the potential for the dissemination of resistance genes through environmental contamination related to pig farming practices and their transmission via wildlife populations. Our research highlights the critical role of wildlife in the AR cycle and underscores the need for continuous monitoring to mitigate the risks associated with resistant pathogens. Further investigations are needed to understand the implications of this dynamic for public health.

EVIDENCE OF THE WIDE CIRCULATION OF MULTIDRUG-RESISTANT ENTERIC STRAINS IN LESSER KESTREL (*FALCO NAUMANNI*)

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Introduction

The lesser kestrel (*Falco naumanni*) is a small migratory raptor, whose distribution ranges from sub-Saharan Africa to southern and central Europe. During the breeding season, lesser kestrel forms colonies that colonize anthropogenic structures, such as attics and wall cavities, as nesting sites. The species' synanthropic nature enables it to prosper in human settlements, both urban and rural, where it finds a plentiful supply of prey, including orthopterans, coleopterans, lizards, and small rodents.

The increasing proximity between humans and this wild species has raised questions regarding the potential effects of this coexistence, particularly in relation to the spread of bacteria, and more specifically, antibiotic-resistant microorganisms. Among them, great attention is dedicated to fluoroquinolone-resistant strains, because of the critical importance of such antimicrobial class in human medicine, and to the increase in their circulation.

Therefore, the aim of this study was to evaluate the presence of fluoroquinolone-resistant strains of commensal and non-commensal Gram-negative bacteria colonizing lesser kestrels during the 2021 breeding season, in order to define the role and the potential impact of this raptor on spreading the antimicrobial resistance across the environment.

Material and Methods

Cloacal swabs were collected from 32 lesser kestrels (*Falco naumanni*) hosted at the Apulian Wildlife Rescue Centre in July 2021. All samples were collected upon admittance as part of the routine diagnostic procedures. Swabs were incubated in tryptic soy broth (TSB) with enrofloxacin (ENR), selected on McConkey agar supplemented with ENR, and identified biochemically or by MALDI-TOF. The susceptibility of isolates to the most common antibiotic classes was ascertained by the disk diffusion method.

Results and Discussion

Only one out of 32 samples did not produced growth of at least one ENR-resistant strain. From the remaining 31 birds, 41 ENR-resistant strains were isolated, identified as *Escherichia coli* (23), *Proteus mirabilis* (10), *Klebsiella pneumoniae* (6), *Citrobacter freundii* (1), and *Enterobacter cloacae* (1). Out of them, 39 (95.12%) were resistant to three or more antibiotics.

In detail, 35 strains were resistant to tetracycline, 32 to ampicillin, and 25 to sulfamethoxazole/trimethoprim or streptomycin. No colistin-resistant strain was isolated except for *P. mirabilis*, naturally resistant. No strain was resistant to imipenem, but 7 were resistant to the fourth-generation cephalosporin cefepime.

Those data suggest a wide circulation of multidrug-resistant (MDR) strains, some of them with clinical relevance, in birds that were never treated with antibiotics. Considering its position at the top of the food chain, *Falco naumanni* should be considered an important indicator of the flow of multidrug-resistant bacteria between anthropic and wild environments, providing useful information in a One Health view.

Additionally, considering *F. naumanni* habits, sampling resistant bacteria from the microbiome of these birds could provide valuable insights into the circulation of resistant strains and serve as an early warning system for levels of environmental anthropogenic contamination. Furthermore, as migratory birds, lesser kestrels can acquire microorganisms and transmit them to distant ecosystems, transporting them across multiple geographic areas and contributing to their spread.

TRACKING THE HEALTH OF WOLVES (*CANIS LUPUS*) IN FORESTE CASENTINESI NATIONAL PARK: MERGING CONSERVATION AND PUBLIC HEALTH

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Introduction

The grey wolf (*Canis lupus*, Linnaeus, 1758) population has expanded due to favorable conditions, such as protective legislation and ecological factors. However, wolves still face significant risk, mainly due to human-related threats, like poaching and hybridization with domestic dogs. In this context, the interaction between wolves and domestic dogs may facilitate the transmission of canine diseases, some of which can increase mortality rates among wild carnivores, raising concerns for the conservation of this species. On the other side, wolves can play an epidemiological role for different zoonotic diseases and therefore monitoring their health status is relevant also from a public health viewpoint. This study aimed to investigate the health status of a wolf population in the Foreste Casentinesi National Park (FCNP) (between Tuscany and Emilia-Romagna regions, Italy) by using a non-invasive method of environmental faecal sampling. The presence of viral genome of *Coronavirus* and *Parvovirus*, identified as pathogens at high risk for the conservation of the species, and the occurrence and intensity of parasitic infestations were explored, with a particular interest in the presence of *Echinococcus granulosus* and *E. multilocularis*, identified as pathogens of high interest for public health.

Material and Methods

Faecal sampling was performed between May 2019 and April 2020, following transects throughout all the territory of the FCNP. Opportunistic sampling was added, allowing more samples to be collected. For safety reasons, the samples were kept to a temperature of -80°C for 10 days before handling, and then stored at -20°C.

Genetic analysis was performed on fresh samples, following ISPRA protocols, allowing the identification of the species and individual wolves. One sample for each wolf was subjected to virological analysis for the identification of *Coronavirus* and *Parvovirus* genomes, by PCR. Copromicroscopic exam using a Zinc chloride solution (s.g. 1,300) was carried out on all samples available. Finally, a duplex real-time PCR for the detection of *Echinococcus granulosus* and *Echinococcus multilocularis* was conducted on the genotyped samples and the samples positive at the copromicroscopic exam for Taeniid eggs.

Prevalence (C.I. 95%) was computed. Samples were characterized based on sampling season (warm and cold), age (young and adult) and gender of wolves, if known. Pearson's Chi-square test and Fisher's Exact test were used to assess significant differences in prevalence among categories. To assess whether if wolves' age, gender, and sampling season affect the outcome of the coprological examination, generalized linear models with binomial distribution were used. $P<0.05$ was considered statistically significant. All statistical analyses were performed in R (R Core Team, 2023).

Results and Discussion

Overall, 261 samples were available for parasitological analysis. Among these, genetic analyses were successfully performed on 132 samples, resulting in the recognition of 81 individuals (1 dog, 1 hybrid wolf, 6 wolves with past introgression, and 73 wolves) clustered in 8 packs (a total of 11 packs are identified within the FCNP).

Out of 81 samples analysed for *Coronavirus* and *Parvovirus*, only one sample tested positive for *Coronavirus* (1.2%; 95% CI: 0.2-6.7). A total of 261 samples were analysed by copromicroscopic examination by flotation. The parasites with higher prevalence were *Capillaria* spp. (88.9%; 95% CI: 84.5-92.1), *Sarcocystis* spp. (41.8%; 95% CI: 35.9-47.8), Ancylostomatidae (28.0%; 95% CI: 22.9-33.7), Taeniidae (23.4%; 95% CI: 18.6-28.9). Coccidia, *Toxocara canis* and *Trichuris vulpis* showed prevalence values less than 3% (respectively, 1.1%; 95% CI: 0.4-3.3; 1.9%; 95% CI: 0.8-4.4; 2.7%; 95% CI: 1.3-5.4). Out of 104 samples analysed by RT-PCR for *E. granulosus* and *E. multilocularis* DNA, none resulted positive. Wolves' gender and age did not explain any parasite prevalence ($p>0.05$), while the cold season had a statistically significance with the excretion of *Capillaria* spp. ($p<0.05$).

Our study suggests that wolves in the FCNP do not significantly increase the risk of zoonotic parasitosis. Among viruses with a high impact on species conservation, only *Coronavirus* was detected, and its prevalence was low. However, wolves, domestic dogs, and other wild canids and carnivores share common pathogens, some of which were found at high prevalence in this study, such as *Capillaria* spp., Ancylostomatidae, and Taeniidae. Therefore, it is essential to deepen our understanding of the epidemiology of these parasites and their transmission between wildlife and domestic dogs. Continuous monitoring of wolf health is essential, especially given its potential to expand into peri-urban areas, increasing its interactions with domestic animals.

MONITORING POTENTIAL PATHOGENS HARBOURED BY THE ALIEN SLIDER *TRACHEMYS SCRIPTA* IN THE NORTH OF ITALY COLLECTION CENTERS

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Introduction

Trachemys scripta or "pond slider" is a freshwater turtle, comprised in the family Emydidae, native to United States and Mexico. This species is part of the "100 of the World's Worst Invasive Alien Species" IUCN list after its release worldwide during the last century.

In the attempt of preventing the release of domestic individuals into the wild, some Italian regions defined "collection centers" where citizen can bring their animals.

However, despite several investigations and the identification of a number of infectious agents harbored by these turtles, the epidemiological role of these chelonians as potential sentinels/reservoirs of these infectious agents in this context is still poorly defined. Accordingly, it is still relatively unclear if they could represent a menace as concerns public, domestic animals, wildlife health and the overall biodiversity.

This study aims to provide preliminary data concerning the occurrence of bacterial and viral infections, described in the literature for this species, in two collection centers located in Lombardy, a region in the north of Italy.

Material and Methods

A total of 136 *T. scripta* were sampled from two different centers in Lombardy in October 2023 and July 2024. The pathogens investigated were *Salmonella* spp., *Mycoplasma* spp., *Leptospira* spp., Herpesvirus, Adenovirus and Ranavirus.

Blood samples were collected from the occipital sinus to detect antibody titers against eight *Leptospira* serogroups through Microagglutination Test (MAT), according to the recommendations of World Organization for Animal Health standards.

Cloacal and oro-cloacal swabs were taken to assess the presence of the other pathogens mentioned. In particular, for the isolation of *Salmonella* spp., the samples were divided into pools (7 cloacal swabs per pool, totaling five pools for each site) and analyzed using the method outlined in International Standards ISO 6579-1:2017. The presence of the other pathogens was investigated through PCR assays.

Results and Discussion

The overall prevalence of antibodies against *Leptospira* spp. was 91.6% during both seasons. Specifically, the Tarassovi serogroup showed a prevalence of 90.8% and 92.4% in two centers. Lower prevalences rates were detected for the serogroup Australis, Icterohaemorrhagiae, and Ballum.

The prevalence of *Salmonella* spp. was 0% during the first sampling, however, 50% of the pools tested positive during the second one.

PCR testing yielded negative results concerning the presence of detectable Herpesvirus, Ranavirus, Adenovirus, and *Mycoplasma* spp. nucleic acids.

Further investigation is needed to clarify the actual clinical significance of *Leptospira* sero-prevalences. Furthermore, the dynamics of the infection of this spirochete in turtles are yet to be conclusively deciphered. In particular, the role of turtles as shedders and their role in the epidemiology of *Leptospira*'s environmental cycle requires clarification. Nonetheless, the observed prevalence aligns with those reported in other studies. Likewise, the prevalence of *Salmonella* spp. is similar with other described in the literature.

The absence of the remaining pathogens should be carefully evaluated, given that PCR positivity is dependent on shedding of the investigate agent, which is known to be heavily influenced by season, temperature and hormonal conditions. Accordingly, repeated sampling at different time during the season is needed to conclusively assess the presence or absence of these agents. As a matter of fact, reptiles in general are more prone to develop disease immediately prior and immediately after hibernation.

While further investigations are needed, these findings suggest that *T. scripta* can carry pathogens which can pose potential risks to other animal species and to humans.

For these reasons, the collection centers that gather these turtles may serve as potential source of infectious outbreaks. This raises considerations about the need for setting up appropriate health management plans for these centers and at the same time to assess the real opportunity to maintain these centers as a management option.

FUNDING. This project was funded by the Regione Lombardia DG Territorio e Sistemi Verdi, as part of agreement for the implementation of an experimental plan for the management of the invasive sliders *Trachemys scripta*.

DERMATOPHYTES AND WILDLIFE: A STUDY OF THE EUROPEAN HEDGEHOG (*ERINACEUS EUROPAEUS*) IN PIEDMONT

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Introduction

The European hedgehog (*Erinaceus europaeus*) is a cosmopolitan insectivore mammal broadly distributed in Europe. An increased presence of this animal in urban and suburban areas (private gardens, public parks) has been reported as the consequence of habitat loss due to human activities. The increased contact occasions with humans lead to a higher risk of transmission of various diseases, including dermatophytosis. Dermatophytosis is a fungal skin infection with substantial consequences on public health. Indeed, all animal-associated dermatophytes can infect humans. The most frequent species found on the hedgehog is *Trichophyton erinacei*. Other geophilic species – such as *Nannizia gypsea* – have been reported with low frequency. Highly inflammatory lesions characterize human infections that occur by direct contact with an infected hedgehog or contaminated materials such as their nest.

This study aimed to investigate the presence of dermatophytosis in the hedgehog population in the Piedmont area.

Material and Methods

From October 2020 to February 2024, 305 rescued hedgehogs submitted to CANC (Centro Animali Non Convenzionali, Department of Veterinary Sciences, University of Turin) were examined for the presence of dermatophytes. For each animal, general information was collected (place of origin, health status, arrival date). A clinical examination was performed to highlight skin lesions due to dermatophyte infection (erythematous/exfoliative dermatitis, crusty skin and spine loss).

The animals were sampled by brushing the skin surface using a disposable human toothbrush. Due to hedgehogs' back spines, and the rolling up defensive behaviour, sampling was focused on the back, the paws and the muzzle exposed areas. In animals with clinical signs requiring more in-depth observation, the clinical examination was performed under anaesthesia using isoflurane. Cultures were performed on Mycobios Selective Agar (Biolife, Milan, Italy), pressing the toothbrush onto the agar surface. Plates were incubated at 25°C and examined daily for ten days. The fungal identification was obtained by morphological analysis and sequencing of the internal transcribed spacer region (ITS), one of the molecular markers most employed in dermatophyte taxonomy studies.

Results and Discussion

Eleven out of 305 animals, equal to 3.6% of the samples taken, were positive for dermatophytes (*N. gypsea* n=9, 2.9%; *T. erinacei* n=1, 0.3%; *N. incurvata* n=1, 0.3%). Seven out of nine hedgehogs positive for *N. gypsea* and the one with *N. incurvata* presented skin lesions. This finding suggests that this genus of soil fungi could cause clinical manifestations in hedgehogs. On the contrary, the one with *T. erinacei* had no skin lesions, playing the role of asymptomatic carrier.

The prevalence of *T. erinacei* was lower in this study compared to recent works performed in the Northern part of France and in Poland (25% and 10.9%, respectively). However, the results agree with investigations carried out in Spain (*T. erinacei* = 0%). Different climatic or environmental conditions of the sampled areas may explain this discrepancy. Indeed, long warm and dry periods, characteristic of Southern European countries, could disadvantage the presence of the fungus. Unexpectedly, the prevalence of *N. gypsea*, opportunistic pathogens in animals, is higher than previously reported. This could suggest a progressive change in its ecological niche.

Notably, the infection by the geophilic dermatophyte *N. incurvata*, a recently described species, represents the first report in the hedgehog. These considerations show the necessity of further studies on the role of hedgehogs as carrier of dermatophyte fungi.

DICROCOELIUM DENDRITICUM IN ROE DEER (*CAPREOLUS CAPREOLUS*) IN CENTRAL ITALY

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Introduction

The roe deer (*Capreolus capreolus*) is a medium-sized generalist ungulate, living in a wide variety of terrestrial ecosystems due to its ecological plasticity. In Europe, it has been historically known for hunting activity, going through reintroductions, translocations, and local extinctions. In Italy, this cervid is a common species with a large distribution range and a gradually increasing number of individuals. As for other wild and domestic animals, the roe deer is affected by a plethora of infectious and parasitic agents resulting in animal's fitness and health status alterations. *Dicrocoelium dendriticum* is a trematode parasite that infects domestic and wild animals as definitive hosts, including roe deer. Furthermore, the biological cycle also involves two intermediate hosts such as snails and ants. The transmission of this parasite can occur in the wildlife-domestic interface. This study aims to investigate *D. dendriticum* infection in roe deer from central Italy (Tuscany, Marche and Umbria regions) and its epidemiological role, where it lives in sympatry with domestic ruminants.

Material and Methods

A total of 265 roe deer livers were collected by "convenience sampling" between 2021 and 2022: 198 from Tuscany, 7 from Marche, and 60 from Umbria regions. Data recorded at the time of sampling included culling area, sex, age class, and sampling season. The livers were subjected to various analysis, including accurate macroscopic visual examination, careful palpation to assess the presence of cysts and/or abnormal lesions, examination of liver sections for trematodes and subsequent counting of helminths present. At the same time, the Liver Lesions Score (LLS) was determined. Following collection, the helminths fixed in 70% ethanol were subjected to molecular and morphological analyses to confirm species identification. Animal-level prevalence for *D. dendriticum* was computed with the associated 95% confidence intervals (CIs). Data collected at the moment of the sampling (i.e., sex, age class, sampling season) were tested as independent variables in a univariable general linear models (GLMs) with a binomial distribution and logit link function to explore the relation between positivity to *D. dendriticum* and associated risk factors. The variables significant (P-value < 0.05) at the univariate analysis were tested in a multivariable regression model. Results were presented as adjusted odds ratios (OR) with 95% CI. Statistical analysis was performed using commercial software (SPSS, Version 22.0, Chicago, IL, USA).

Results and Discussion

The present study reports for the first time the infection of *D. dentriticum* (confirmed by morphological and molecular analysis) in roe deer from Italy, highlighting the circulation of this poorly investigated fluke species in wild ruminant populations of Europe.

At LLS examination, cysts and/or abnormal lesions were found, maybe on account of the low burden of *D. dendriticum* detected (i.e., mean intensity of infection 17.1 flukes/animal with a minimum and maximum of 1 and 354, respectively). This low parasite burden could suggest a limited role of roe deer in environmental contamination than domestic animals.

Overall, 52/256 (19.6%) animals were infected by *D. dendriticum*. Prevalence and mean intensity of *D. dendriticum* found in this study, compared to those in domestic animals (e.g., in sheep), could be related to the different feeding habits between domestic and wild hosts. However, the not negligible values of prevalence and mean intensity detected in roe deer indicate that, despite a fructivorous/granivorous diet, these wild ungulates are also inclined to a low vegetation feeding (e.g., grass and herbage), being exposed to the infection during grazing, through ingestion of infected ants. The higher mean intensity herein found in winter than in summer (i.e., 26.7 *vs* 8.3) is probably linked to the ingestion of metacercariae-infected ants during summer that, considering the *D. dendriticum* prepatent period (i.e., no less than 3 months), allows the develop of adult trematodes mainly in winter.

Although, it must be determined with further studies the roe deer's role as animal in refugia and how the interaction between domestic and wild animals, given the increase of the cervid populations in Italy and the phylogenetic relationship between wild and domestic ungulates, could influence anthelmintic resistance and the spread of this parasite.

Sessione 2

IMPATTO DEI CAMBIAMENTI AMBIENTALI SULLA SALUTE DELLE POPOLAZIONI ANIMALI E DELL'UOMO

Chairmen: Rudi Cassini – Roberto Viganò

Le modificazioni ambientali registrate negli ultimi decenni, associate all'abbandono delle attività tradizionali e all'aumento delle interazioni tra popolazioni selvatiche e uomo, connesse anche a fenomeni di sinantropia, hanno contribuito all'evolversi di quadri epidemiologici differenti e inaspettati. Nell'ambito delle interazioni tra animali selvatici, animali domestici e uomo, ad esempio, si affronta sempre più di frequente il tema delle patologie trasmesse da vettori (zecche, zanzare, flebotomi, *etc*…); troppo spesso però in ottica emergenziale, senza sviluppare metodi strutturati di raccolta dei dati e di monitoraggio epidemiologico. Quali esperienze abbiamo e quali obiettivi ci possiamo dare per raggiungere una maggior consapevolezza ed affrontare o prevenire adeguatamente i cambiamenti in corso?

RELAZIONI AD INVITO

Impact of climate change on the risk of transmission of endemic vector-borne diseases.
Annapaola Rizzoli (Fondazione Edmund Mach)

CONTRIBUTI LIBERI

Habitat restoration and biodiversity: an evaluation of mosquito community in urban parks of Milano. ARNOLDI D., BIGONI F., FESCE E., HAUFFE HC., TAGLIAPIETRA V., ROSSO F., MARINI G., DAGOSTIN F., FERRARI N., RIZZOLI A.
Fabio Bigoni (Università di Milano. Dipartimento di Medicina Veterinaria e Scienze Animali)

Ecological determinants of orthohantavirus infection in European small mammals: a systematic review. FABBRI D., MIROLO M., TAGLIAPIETRA V., BERALDO P.
Daniele Fabbri (Università di Udine. Dip. di Scienze agroalimentari, ambientali e animali. NBFC Palermo)

First evidence of a new *Flavivirus* circulation in Piedmont region: two cases in Alpine chamois and their ticks. NOGAROL C., MONNE I., MORONI B., GUARDONE L., GARCIA-VOZMEDIANO A., CASSINA G., ZOPPI S., VIGANÒ R., DE BENEDICTIS P., MANDOLA ML.
Chiara Nogarol (Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta)

Exposure of red deer *Cervus elaphus* to *Ixodes ricinus* ticks by immune response to tick symbiotic bacteria: age-related trend and geographical distribution in the Central Alps. NAVA M., CAFISO A., CIALINI C., CORLATTI L., PEDROTTI L., GUGIATTI A., BAZZOCCHI C., LUZZAGO C.
Matteo Nava (Università di Milano. Dipartimento di Medicina Veterinaria e Scienze Animali)

Catastrophic weather episodes affect patterns of vector and host community and disease risk in an alpine area: preliminary results. TAGLIAPIETRA V., FERRARI G., ROSSO F., ARNOLDI D., INAMA E., RIZZOLI A.
Valentina Tagliapietra (Fondazione Edmund Mach, NBFC, National Biodiversity Future Center, Palermo)

Unveiling blood parasites: first insights from wild canids in North-Eastern Italy. GRILLINI M., FERRARO E., BERALDO P., CASSINI R., ORIOLES M., BREGOLI M., SIMONATO G.
Marika Grillini (Università di Padova. Dipartimento di Medicina Animale, Produzioni e Salute)

Co-occurrence of *Angiostrongylus vasorum* and *Dirofilaria immitis* infections in golden jackals (*Canis aureus moreoticus*) from Friuli Venezia Giulia (Italy). BERALDO P., FABBRI D., PESARO S., BREGOLI M., DORIGO L., SACCÀ E., TOMÈ P., ORIOLES M.
Paola Beraldo (Università di Udine. Dipartimento di Scienze Agro-Alimentari, Ambientali e Animali)

Wolf presence as potential driver of *Echinococcus granulosus* s.s. transmission in sheep farms of Alto Maceratese area (Central Italy). HABLUETZEL A., PACIFICI L., PROPOGGIA G., RONCARATI A., CROTTI S., MANCIOLA G., PENNESI C., MORANDI F., GAVAUDAN S., GOBBI M., MORANDI B.
Annette Habluetzel (Università di Camerino, Scuola di Scienze del Farmaco e dei Prodotti della Salute)

IMPACT OF CLIMATE CHANGE ON THE RISK OF TRANSMISSION OF ENDEMIC VECTOR-BORNE DISEASES

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Introduction

Global warming is now unequivocal, as summarised in the latest IPCC reports, with unprecedented rate on a multi-decadal or even ultra-millennial scale. The Mediterranean region is considered one of the "hot spot" of climate change, with warming which exceeds by 20% the global average increase and a variation in the rainfall regime, with increase of extreme events. The impact of climate change on human and animal health is complex and depends on numerous factors, including the nature and route of transmission of the causal agent as well as the vulnerability of the affected population. However, the impacts deriving from other drivers must also be considered, such as changes in land use and loss of biodiversity. More than 50% of pathogens of relevance to human health are sensitive to climate variability. Among them, zoonotic pathogens, especially those transmitted by arthropods vectors, are particularly sensitive. In this presentation, some case-studies will be presented and discussed, with particular reference to viral infections transmitted by ticks and mosquitoes belonging to endemic species on the national territory.

Material and Methods

This presentation is the result of a scoping review integrated with unpublished data and the authors' personal opinion on the different topics covered.

Results and Discussion

Changes in climate, land use and biodiversity are considered among the most important drivers affecting parasites-host interaction and wildlife zoonotic disease emergence. In case of West Nile virus, we showed that the drastic increase in the human population at risk of exposure is partly due to historical changes in human population density, but that climate change has also been a critical driver behind the heightened risk of WNV circulation in Europe. In case of TBE, climate change is affecting several components of its transmission cycle, from the temporal and spatial distribution of the main vector, *Ixodes ricinus*, to the reservoir rodent host dynamics. Among climatic drivers, autumnal cooling and total precipitation appear to exert the major effect in affecting TBEV transmission and disease risk for humans. In conclusion, because of a number of factors implicated and their complex interactions, the forecasting of vector-borne disease risk under a global change scenario is particularly challenging and the development of predictive models require the intensification of the research efforts aimed at better understanding how changes in parasite-host interaction affect vectorial capacity with the establishment of long term eco-epidemiological studies based on a multidisciplinary approach under a One-Health framework.

HABITAT RESTORATION AND BIODIVERSITY: AN EVALUATION OF MOSQUITO COMMUNITY IN URBAN PARKS OF MILANO

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Introduction

Climate change and biodiversity loss are two of the major challenges that Europe is facing today. Building of green infrastructures, habitat, and biodiversity restoration represent some of the actions to be implemented to mitigate their impact. Vector borne diseases are particularly sensitive to changes in climate and variation in biodiversity and thus represent a model to test the effect of habitat restoration in terms of mitigation of zoonoses risk especially in urban areas. West Nile and Usutu viruses are two exotic pathogens introduced in Italy since the end of the last century and now endemic. Autochthonous dengue cases were also reported in Lombardy region. It is essential to compare data between different levels of habitat restoration and biodiversity to gain a clearer understanding of vector community composition and changes in space and time, especially in urban contexts. Cities are dynamic environments where various ecological renewal projects are underway, which can enhance animal biodiversity. However, these projects can also lead to an increase in the presence of vectors, consequently raising the risk of disease transmission.

Our initial goals, through mosquito sampling, are to investigate the demographic variations of different mosquito species, as one of the essential parameters necessary to estimate vector-borne disease risk. We also take into consideration environmental variables such as reforestation, temperature, rainfall, and relative humidity within an urban context.

Material and Methods

Mosquito sampling was conducted in urban parks of the Milan municipality from May to November 2023 and 2024. The first year, six sites were selected, four within Parco Nord Milano (PNM) and two in the Boscoincittà (BIC) area. In 2024, four additional sites were added: Giardini Indro Montanelli, Parco Sempione, Parco Don Luigi Giussani, and "La Goccia" observatory. In 2023 BG-Sentinel and CDC traps were used, while the following year we used only BG-sentinel traps. A motorized aspirator was used to increase the number of engorged mosquitoes. Traps were deployed at each site for 24 hours about once a month. Within few days after traps activation, aspiration was performed in mosquitoes resting sites. Collected mosquitoes were then identified and pooled by trap, species, and sex. Furthermore, environmental variables data (Temperature, Relative Humidity, Heat Index, Dew Point) were collected by using Kestrel Drop 2 data loggers placed in each sampling area at the beginning of the sampling season.

Results and Discussion

No significant differences were found in the number of mosquitoes collected in PNM and BIC between the two years. However, greater biodiversity was observed in 2023. Significant differences in relative abundances were noted among species sampled in both years. The most abundant species in the first year was *Ochlerotatus caspius*, followed by *Aedes albopictus* and *Culex pipiens*, while in the second year, the most abundant species was *Ae. albopictus*, followed by *Cx. pipiens* and *Oc. caspius*. Regarding sex, no significant differences were found in the number of females collected in the two years, whereas a higher number of males was observed in the second year. The majority of males collected in both years belonged to the species *Ae. albopictus*. A decrease both in number and species diversity was observed over the two consecutive years. In the two areas significant differences were noted regarding the abundance of *Ae. albopictus*, *Oc. caspius*, and *Cx. pipiens* both in 2023 and 2024. Moreover, in 2024, there was a marked decrease in *Oc. caspius* and an increase in *Ae. albopictus* and *Cx. pipiens*. Comparing the sites added in the second year of sampling, "Giardini Indro Montanelli" emerged as the site with the highest abundance of *Cx. pipiens* and *Oc. caspius*, while "Parco Don Giussani" had the highest abundance of *Ae. albopictus*. The sites within the Boscoincittà area were the only ones to report specimens of *Oc. geniculatus* and *Ae. vexans*. The monthly capture trend of all species reveals that in both years the peak occurred in July. However, at species level, this holds true for Cx. *pipiens* and *Oc. caspius* while *Ae. albopictus* peaked in August. Whilst *Cx. pipiens* gradually reached its peak starting from May, *Oc. caspius* showed a significant increase in June. Conversely, *Ae. albopictus* exhibited a gradual increase starting from June.

This study serves as a starting point for better understanding changes in mosquito species prevalence and abundance in urban areas. Our results demonstrate the high abundance of *Cx. pipiens*, *Ae. albopictus*, and *Oc. caspius*, which are important vectors for pathogens such as West Nile, Usutu, Dengue, Zika and Chikungunya viruses, and Dirofilaria nematodes. Further analyses (e.g., mosquitoes feeding preference and viral circulation) are necessary to monitor these vectors and potential viruses they can transmit and inform public health services against potential outbreaks of these pathogens.

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ECOLOGICAL DETERMINANTS OF ORTHOHANTAVIRUS INFECTION IN EUROPEAN SMALL MAMMALS: A SYSTEMATIC REVIEW

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Introduction

Orthohantaviruses are emerging zoonotic pathogens that cause severe human disease and pose a growing global public health threat. In Europe, the risk is heightened by the lack of effective vaccines, increasing human cases, and a rising prevalence of infected reservoir species. These viruses are naturally maintained in rodent populations and occasionally in other mammals, including bats. While research has linked human hantavirus infections to environmental factors and reservoir host prevalence using large-scale datasets, human case numbers do not always reflect the virus's distribution in its primary reservoirs. This discrepancy underlines the difficulty of accurately monitoring and predicting hantavirus prevalence. This review emphasizes the need for enhanced surveillance of reservoir hosts and better integration of environmental data. Understanding the dynamics between host population fluctuations, climate factors, and virus prevalence is essential for monitoring hantavirus spread and safeguarding public health.

Material and Methods

The "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA) method was adopted for this review. Searches covered entries from January 2003 to January 2024. The search was applied to four digital databases (Cabi Digital Library, PubMed, Jstor & SpringerLink), through the Boolean query: Hantavirus AND Europe AND "Small Mammals". After duplicate removal (=50), the research resulted in 364 citations. Studies using live trapping data of small mammal reservoir hosts assessed hantavirus prevalence (all species) and analyzed environmental, or population dynamics variables were included. After selection criteria were applied and additional articles recovered, 40 entries papers were considered for data extraction.

Results and Discussion

Environmental factors, host biology, and interactions with other species are the main ecological determinants of hantavirus prevalence in natural hosts. Aerosols from infected hosts are the primary mode of horizontal transmission, and the virus can persist outside the host for extended periods, particularly in moist and cold environments. This environmental stability is crucial for preventing pathogen fadeout and maintaining transmission potential during low host density intervals.

Infected individuals, although often asymptomatic, experience reduced survival and reproductive success due to the increased metabolic demands of immune stress. The impact of infection varies with host age: young females may favour reproduction over immune function, while older females tend to reduce their reproductive effort. Hantavirus prevalence typically increases with host age, as older individuals have more exposure opportunities due to their longer lifespan and increased contact with infected conspecifics. Although viremia peaks shortly after infection, it continues at lower levels, resulting in ongoing viral shedding. Newborns may receive temporary immunity through maternal antibodies, affecting seasonal virus fluctuations with lower infection rates during high birth periods.

Host density plays a critical role in hantavirus prevalence. Seasonal fluctuations in host populations affect the density and, also prevalence. Males generally have higher infection rates, attributed to reproductive behaviours, such as territorial marking and mate-seeking. The presence of predators can alter hantavirus dynamics by inducing behavioural changes in hosts or selectively preying on infected individuals.

Forest patches, especially those with dense vegetation and high humidity, are significant for hantavirus transmission. These environments enhance viral particle survival and support high densities of hosts, leading to increased prevalence. When habitat quality exceeds carrying capacity during rapid population increase phases, dispersing individuals spread the virus across the landscape.

Abiotic factors also influence prevalence, with mixed effects. Warmer winter temperatures may improve small mammal fitness and increase transmission, while colder temperatures can prolong viral survival in substrates. Similarly, snow cover can either increase transmission within communal burrows or reduce prevalence due to higher mortality from harsh conditions. Precipitation's impact is inconsistent, with some studies linking it to higher prevalence and others showing no effect. Generally, higher habitat humidity and the presence of water bodies support increased hantavirus prevalence by extending viral survival in the environment.

Overall, the dynamics of hantavirus transmission are complex, involving interactions between environmental conditions, host biology, predator activity, and habitat quality. Understanding the underlying determinants is essential for predicting and managing hantavirus outbreaks in different ecosystems.

FIRST EVIDENCE OF A NEW FLAVIVIRUS CIRCULATION IN PIEDMONT REGION: TWO CASES IN ALPINE CHAMOIS AND THEIR TICKS

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Introduction

Recent studies have demonstrated that climate change is the main cause of the globally observed decrease in biodiversity, also influencing the distribution and dynamics of ixodid tick populations, by accelerating their development and increasing their density, even at high altitudes. *Ixodes ricinus* is the most widespread tick species in Europe and is considered the primary vector of zoonotic pathogens (TBPs), including tick-borne encephalitis (TBEv), *B. burgdorferi* s.l., *A. phagocytophilum*, *C. burnetii*, *Rickettsia* spp. and *Babesia* spp.. Wild animals, in particular ungulate species, exert a considerable influence on ticks epidemiology, acting as reservoir or amplifiers of TBPs or alternatively as feeding or propagation host of ticks. These roles render wild ungulates a valuable sentinel species for the surveillance and monitoring of ticks and TBPs surveillance. The TBE virus (TBEv) is an RNA virus classified within the genus Flavivirus and member of Flaviviridae family, similarly to the etiological agents of dengue, yellow fever, and Japanese encephalitis. In this study, we present the first viral characterization of two cases involving a newly identified Flavivirus in Alpine chamois carcasses and their feeding ticks, from the Verbano-Cusio-Ossola province (VCO, Piedmont region), until now considered free of TBEv.

Material and Methods

Case 1. Ticks were collected from an Alpine chamois (*Rupicapra rupicapra rupicapra*) found dead in Malesco municipality (761 m a.s.l.), VCO province in May 2023. The carcass was sent to IZSPLV labs in the framework of the Ricerca Finalizzata GR-2021-12374932 and of the Piedmont regional plan for the monitoring and surveillance of wildlife. Brain and lung tissues were sampled from the carcass for TBEv PCR screening. Case 2. Ticks were collected from an Alpine chamois found dead in Formazza municipality (1280 m a.s.l.), VCO province in June 2024 and sent to IZSPLV labs as part of the Piedmont regional plan for the monitoring and surveillance of wildlife. In both cases, ticks were carefully removed with forceps and examined via stereomicroscopy for morphological identification as described by Estrada-Peña et al. 2017. Tick homogenates were prepared from either engorged female ticks, non-engorged adults or pools of up to 4 nymphs and/or larvae (grouped according to species, development stage, gender and host), followed by the Maxwell® RSC viral TNA Kit procedure. PCR amplifications for *C. burnetii*, *Anaplasma* spp., *B. burgdorferi* s.l. and *Rickettsia* spp. were performed using end-point PCR protocols, as described in Guardone et al. (2024); RT-PCR amplification for TBEv was carried out using the published method described by Schwaiger et al. (2003). Abundant rRNA and globin RNA was removed starting from 200ng of total RNA by the Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus (Illumina, San Diego, CA, USA). Ribodepleted samples then underwent library preparation and were processed on an Illumina NextSeq550 instrument at IZSVe.

Results and Discussion

Case 1. The seven adult ticks attached to the chamois were identified as *I. ricinus* (4 engorged females and 2 males) and *Haemaphysalis sulcata* (1 female). The chamois was found to be severely cachectic with mild bilateral conjunctivitis and showed incomplete molt. Due to the advanced decomposition status, it was not possible to perform histopathological examination of the brain and other organs. Ticks resulted negative for all bacterial pathogens but positive for TBE virus; only the brain resulted positive for TBEv with RT-PCR. Complete genome sequence (10,432 nucleotides) was obtained from the brain tissue of the chamois as well as from a female *I. ricinus* tick. Case 2. *I. ricinus* ticks removed from the chamois tested negative for all bacterial pathogens and positive for TBEv. The full genome analysis of the samples clearly identified the circulation of a novel Flavivirus strain, phylogenetically related to the TBE-like group, with a high genetic correlation with Louping ill virus and Spanish Goat encephalitis virus. To our knowledge, this is the first evidence of the occurrence of this new virus in our region. The two carcasses were found in the same area of Piedmont Region, the VCO Province, in a range of no more than 55 km, in an area very close to the Switzerland borders. The persistence of this new virus from May 2023 to June 2024, suggests the need of a monitoring plan for *Flaviviridae* viruses along the Alpine territories of the Northern part of Italy. Da Rold et al. (2022) described a case of clinical TBE in a roe deer (*Capreolus capreolus*) in Veneto region and Gaffuri et al. (2024) find out a high viraemic chamois in Lombardy: as in our cases, not only the animal, but also the attached ticks resulted positive. These data emphasize the need for further studies on the potential role of ungulates in the circulation of this new agent as well as its genetic and antigenic characterization. Additionally, identifying clinical signs and susceptible animal species is essential to better assess the risks posed to both animals and human.

EXPOSURE OF RED DEER *CERVUS ELAPHUS* TO *IXODES RICINUS* TICKS BY IMMUNE RESPONSE TO TICK SYMBIOTIC BACTERIA: AGE-RELATED TREND AND GEOGRAPHICAL DISTRIBUTION IN THE CENTRAL ALPS

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Introduction

Ticks are widely acknowledged as crucial vectors of arthropod-borne pathogens. In Europe, the most widespread and noteworthy species is *Ixodes ricinus*, known for transmitting various microorganisms and additionally harboring symbiotic bacteria, such as the endosymbiont *Midichloria mitochondrii*. This bacterium is present in all life stages of the tick and has been observed to be transmitted during blood meals, eliciting an immune response in parasitized vertebrates. The geographical distribution of *I. ricinus* is influenced by several factors, including host features and availability and environmental conditions. The alterations in these factors have resulted in upward shifts in the altitude limits of tick habitats and have also affected Alpine areas. The aim of this work was to indirectly assess the exposure of culled red deer to *I. ricinus* by evaluating antibodies against *M. mitochondrii* in the Stelvio National Park (SNP), where knowledge about *I. ricinus* presence and distribution of this tick is limited.

Material and Methods

Samples came from red deer culled inside the SNP and were collected from the end of November to the end of January 2017–18 and 2018–19. The culling plan included 30% calves (0.5 years, class 0), 16% yearling (1.5 years, class 1), 42% prime-age adult (2.5 ≤ 7.5 years, class 2), 12% senescent stage (≥ 8.5years, class 3). For each individual, data collection included the x- and y-coordinates, age estimated by the tooth erosion, dressed body mass (in kg), Kidney Fat Index (KFI). Blood was obtained from major blood vessels or from the cardiac clot. For the evaluation of circulating antibodies against *M. mitochondrii*, an in-house enzyme-linked immunosorbent assay (ELISA) test was performed. To analyze the variation in optical density (O.D.), we adopted an additive modeling approach. Specifically, O.D., the response variable, was a positive continuous variable and modelled assuming a Gamma conditional distribution with log-link. The explanatory variables were year and sex, the latter in interaction with age class, KFI and body mass. In addition, to model the geographical distribution of O.D., we fitted a smoothed trend surface using the x and y coordinates of each subject. In the current analysis it was not possible to establish a cut-off value to discriminate between positive and negative samples for anti-*M. mitochondrii* IgG. For these reasons, the O.D. value was used as a continuous variable, without predefining which age group could not have been exposed to ticks.

Results and Discussion

A total of 201 culled individuals, 84 males and 117 females, were included in the study: 59 animals of age class 0, 34 of class 1, 83 of class 2 and 25 of class 3. The results suggested that calves have significantly lower O.D. values compared to the other age-classes, while animals of age-class 1 show the highest value of O.D.. Finally, a decreasing trend in the O.D. values was observed from age-class 1 to age-class 3, although not statistically significant. It can be assumed that younger animals may not have been exposed to tick bites or have had limited exposure, resulting in an absent or limited antibody response against *M. mitochondrii* and a lower O.D.. It cannot be excluded that part of the low *M. mitochondrii*-antibody response of calves could be attributed to maternally inherited immunity. There is currently no information available on the *M. mitochondrii*-passive antibody kinetics in ruminants. On the other hand, referring to class 3 decreasing O.D. values, the results obtained could be explained by a process of immune senescence which has been reported previously in the wild in roe deer. However, antibody kinetics for *M. mitochondrii* in red deer need further investigations. The smoothed trend surface suggested that, after accounting for different age-class susceptibility to ticks, the highest values of O.D. were found in the Easternmost part of the study site. On the contrary, the Westernmost part of the culling area, showed a lower probability for red deer to present high O.D. values compared to the Easternmost area. The results span two well separated areas where the estimated abundance of red deer is comparable, although the extent of their pasturelands differs. The presence of grazing sheep and goat herds may facilitate the tick's presence in the Easternmost area. In conclusion, this study indirectly reported the exposure of red deer to tick bites in SNP, suggesting that further investigations are needed in this area.

CATASTROPHIC WEATHER EPISODES AFFECT PATTERNS OF VECTOR AND HOST COMMUNITY AND DISEASE RISK IN AN ALPINE AREA: PRELIMINARY RESULTS

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Introduction

Vaia windstorm occurred in the North-Eastern Italian Alps in 2018 causing 42 million trees falling over 41.000 hectares in few hours, hence producing new open fragmented patches. Changes in habitat are known to alter the composition and diversity of local animal communities with repercussions for pathogens transmission and risk of infection. The study area is part of the BEPREP project (https://www.beprep-project.eu/) which aims to understand how biodiversity, both undisturbed and restored, mitigates health threats from zoonotic and vector-borne diseases. Our aim is to evaluate the effect of the catastrophic meteorological event on rodents' community composition and tick density and the cascading consequences on tick-borne diseases prevalence transmitted by Ixodidae ticks.

Material and Methods

The study area is located in the Natural Park of Paneveggio-Pale di San Martino (Autonomous Province of Trento, Italy). Twelve sites were identified in three different habitat types (post-Vaia windthrow, forest, meadow) at low (about 1.100 m a.s.l.) and high altitude (about 1650 m a.s.l.). In each site we performed (i) Capture-Mark-Recapture live-trapping of small mammals and (ii) tick-dragging of host-seeking ticks along 100 m transects, in three different sessions during 2023. Bacteria and protozoa were detected in ticks using PCR-based methods combined with sequencing.

Results and Discussion

We collected 97 questing ticks belonging to *Ixodes* sp., 62% from Vaia and 38% from forest sites. Meadows were not monitored. We captured 248 small mammal individuals belonging to *Apodemus flavicollis*, *A. sylvaticus*, *Clethrionomys glareolus*, *Microtus* spp. and *Sorex* spp. All small mammal species were observed in Vaia sites, while only three in the forest ones. Similarly to ticks, 64% of the individuals were captured in Vaia windthrow, 20% in forests and 16% in meadows. We also observed a higher diversity of tick-borne pathogens in Vaia sites where *Anaplasma phagocytophilum* (2/60; 3.33%), *Babesia venatorum* (3/60; 5.00%), *Rickettsia monacensis* (2/60; 3.33%) and *Borrelia burgdorferi* s.l. were found. Only *Borrelia burgdorferi* s.l. was recorded in the forest sites with a slightly higher prevalence (10/37; 27.02%) compared to Vaia sites (12/60; 20.00%). At species level *Borrelia afzelii* was found both in Vaia (12/60; 20.00%) and in forest sites (6/37; 16.22%), while *Borrelia burgdorferi* s.s. was found only in the forest (2/37; 5.41%).

Vaia windthrows are undergoing natural reforestation with a complex vegetation structure and composition (e.g., ferns and brambles) that is providing an heterogenous habitat compared to mature forest sites. These successional habitats are favourable for ticks and small mammal species, while promoting their encounter. In this context, the recovered biodiversity following habitat disturbance due to climate change, favoured species with combination of physiological traits associated with increased disease risk. Consequently, circulation of zoonoses may be enhanced in Vaia windthrows until a state of climax is reached.

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UNVEILING BLOOD PARASITES:
FIRST INSIGHTS FROM WILD CANIDS IN NORTH-EASTERN ITALY

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Introduction

Tick-borne (i.e., *Hepatozoon canis* and *Babesia* spp.) and sand fly-borne (i.e., *Leishmania infantum*) protozoa are parasites that may pose significant health risks to canids worldwide causing severe disease in the infected hosts. Wild canids (i.e., golden jackals, grey wolves and red foxes) may also be vulnerable to these infections. Furthermore, the epidemiological role (e.g., accidental host, carrier, reservoir) of wild animals in most of these parasitic infections is still unclear.

In North-eastern Italy, the presence of these vector-borne pathogens in wildlife remains undetected despite the presence of several wildlife species. Among wild canids, golden jackals (*Canis aureus*) and wolves (*Canis lupus*) may be infected and they may serve as potential reservoirs for these parasites. This study presents preliminary data on *Hepatozoon canis, Babesia canis* and *Leishmania infantum* in golden jackals and wolves from different areas of Friuli-Venezia Giulia region (North-eastern Italy) using molecular screening techniques. These findings provide first data into the occurrence and the role of wild canids in the epidemiology of these vector-borne diseases in North-eastern Italy.

Material and Methods

Fifty-two wild canids (49 golden jackals and 3 wolves) found dead due to road accidents in different areas of Friuli-Venezia Giulia region in North-eastern Italy were collected and underwent necropsy. Bone marrow (or lymph node) and spleen samples were collected from all animals, and DNA was extracted. A conventional PCR targeting the 18S-rRNA gene, spanning the V4 region to detect protozoal pathogens (i.e., *Hepatozoon canis*, *Babesia canis*), along with a Real-Time PCR targeting the kinetoplast DNA minicircle for the detection of *L. infantum*. Subsequently, amplicons were sequenced, and sequences were compared to those in GenBank® dataset. Prevalence values and their 95% confidence intervals (95% CI) were calculated through EpiTools using the Wilson method.

Results and Discussion

The findings showed that a significant proportion of wild canids (34/52; 65.4%; 95% CI: 51.8-76.9) tested positive for *Hepatozoon canis*. Among the positive samples, 27 out of 34 jackal bone marrow samples (79.4%; 95% CI: 63.2-89.7) tested positive. Three golden jackals (8.8%; 95% CI: 3.1-23.0) were positive for *Hepatozoon canis* in both bone marrow and spleen, while one (2.9%; 95% CI: 0.5-15.0) tested positive only in the spleen. Two wolves out of 34 animals (5.9%; 95% CI: 1.6-19.1) had positive bone marrow samples and 1 (2.9%; 95% CI: 0.5-15.0) both spleen and bone marrow.

None of the individuals tested positive for *Leishmania infantum* or *Babesia canis* infection. The lack of *Leishmania*-positive samples is not surprising since Friuli-Venezia Giulia region is not typically considered an endemic region for both canine leishmaniosis and babesiosis.

Concerning the methodological approach, the results showed that bone marrow seems to be the best matrix to isolate *Hepatozoon*. The PCR approach adopted in this study allows for species identification, but it has limitation in detecting the co-infections as it only amplifies the predominant pathogen DNA present in the sample.

In conclusion, this preliminary study provides the first data on the prevalence and distribution of *Hepatozoon canis* in golden jackals and wolves in North-eastern Italy. Further research is needed to expand the sample size and explore additional molecular protocols.

CO-OCCURRENCE OF *ANGIOSTRONGYLUS VASORUM* AND *DIROFILARIA IMMITIS* INFECTIONS IN GOLDEN JACKALS (*CANIS AUREUS MOREOTICUS*) FROM FRIULI-VENEZIA GIULIA (ITALY)

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Introduction

The golden jackal has rapidly expanded its range across Europe, including Italy with numerous stable meta-populations established in Friuli-Venezia Giulia, necessitating the development of a regional health monitoring and surveillance network necessary. In fact, its biology and behavior create premises for their infection with a broad range of pathogens, including parasites. While co-infection by cardiopulmonary nematodes *Dirofilaria immitis* and *Angyostrongylus vasorum* in golden jackals was previously reported in Europe, their role as sylvatic hosts in Italy remains unreported and unclear. We report the first case of co-infection with *A. vasorum* and *D. immitis* in golden jackals from Friuli-Venezia Giulia (Italy), describing parasitological and anatomo-pathological findings and discussing some epidemiological features.

Material and Methods

Data regarding golden jackal corpses retrieved in FVG were recorded in the InfoFaunaFVG regional wildlife surveillance network. From 2020 to date, 109 carcasses underwent necropsy at University of Udine, jointly with the IZSVe and MFSN (Udine), to ascertain the causes of death and for anatomopathological and parasitological exams. Of 109 animals, 57 were deemed suitable for cardiopulmonary parasitological examinations using a total worm count approach. Parasites were morphologically identified and then DNA extraction, amplification, and sequencing were performed for identification of species.

Results and Discussion

Road-killed jackals consistently showed fractures of appendicular skeleton, haemorrhagic abdominal and thoracic effusions. Prevalence of *A. vasorum* and *D. immitis* was 29.8% (95% CI: 18.8-43.6%) and 7% (95% CI: 2.3-17.8%) respectively, and mean intensity was 6 (range 1-56) and 4 (range 3-6). *D. immitis* adults were mainly located in the heart and, in one case, both in the heart and pulmonary arteries. In the last two years, 3 out of 57 animals (5.3%, 95% CI: 1.8-14.4) were found co-infected with both *A. vasorum* and *D. immitis*. Macroscopic and microscopic lung lesions were similar in all cases. The lungs were hemorrhagic with multifocal, poorly defined nodules. Thrombotic infarcts were also present. *Dirofilaria immitis* adults were primarily found in the right heart chamber while *A. vasorum* adults in the lung blood vessels. Histologically, the lung parenchyma was multifocally effaced by clusters of granulomas, containing small central deposits of necrotic tissue and occasionally small, calcified areas. Primitive and embryonated nematode eggs and larvae were embedded within the inflammatory foci. The alveolar septa were thickened by lymphohistiocytic infiltration. In addition, adult nematodes were found in blood vessels concurrently with thrombotic lesions. To the best of the authors' knowledge, this is the first report of *A. vasorum* and *D. immitis* co-infection in golden jackals in Italy (second in Europe). This species, as other wild carnivores, are recognized hosts of *D. immitis* and *A. vasorum*, indicating the existence of their sylvatic cycle. Similarly to dogs, golden jackals are susceptible to potentially fatal cardiovascular and pulmonary complications due to these nematodes. Their adaptability and potential presence in urban contexts raise concern for increased parasite transmission, especially to non-endemic regions.

WOLF PRESENCE AS POTENTIAL DRIVER OF *ECHINOCOCCUS GRANULOSUS* S.S. TRANSMISSION IN SHEEP FARMS OF ALTO MACERATESE AREA (CENTRAL ITALY)

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Introduction

The diffusion of *Echinococcus granulosus* (*E.g.*) s.s. being strictly dependent from man – sheep – dog interactions is highly prevalent in areas with diffused pastoralism. However, the presence of wolves - sylvatic definitive hosts of *E.g.* - may interfere directly and/or indirectly with the transmission dynamics of cystic echinococcosis, increasing infection risks for humans. This study examines transmission risks associated to flock management changes introduced by breeders in response to wolf threats in sheep farms of the Alto Maceratese (Central Italy).

Study area and methods

In 2023, an in-person interview-based study was conducted in the Alto Maceratese (Marche region) area (mostly overlapping with the "Monti Sibillini National Park", 360-2476 m a.s.l.), where pastures and cultivated fields alternate with wooded and shrubby areas. Wolves are present at a density of around 10 individuals/100 km^2. Small-scale, mixed farming is prevalent. A questionnaire, with open and closed questions, was administered to 40 sheep breeders.

Results and discussion

The 40 sheep farmers (median of 150 sheep on 40 ha pastures/farm) keep their animals during summer on natural pastures and post-harvest forage cultivated fields. Fearing wolf attacks, about half of the breeders (22/40) recover flocks in stables at night, while the others use electric or mobile fences to protect the animals in the mountainous areas. As a primary defense measure, breeders keep considerable numbers of livestock guardian dogs (2.9 dogs/100 sheep), beside shepherd dogs (1.2 dogs/100 sheep). When not with grazing flocks, dogs are frequently left free to roam (31/39), thus, passing feces on pastures around farms and inside hay barns and stables. Only about half of the farmers (17/39) treat their dogs with anti-cestode drugs, and more often only once or twice a year. Several breeders (11/39) keep guardian dogs deliberately in stables to protect sheep from wolf attacks and let graze flocks in proximity of the farms to keep an eye on the animals while performing other farm work. Only about half of breeders (18/40) can afford to pay a temporary or permanent shepherd. Most of them (34/40) have been hit by wolf attacks. During the last 3 years, 26 breeders reported at least one attack, losing a median of 4 sheep/attack; according to them, other predation episodes remained undetected. About half of them (22/39) have occasionally observed dogs carrying pieces of sheep carcasses.

In 2022 and 2023 respectively, a mean of 6 and 8% (BDN-Banca Dati Nazionale) sheep of the flocks were notified as 'lost' in the study area, which implies that a substantial number of animals may end up providing potentially *E.g.* infected viscera to wolves and dogs.

As potential definitive sylvatic hosts of *E.g.*, wolves may directly enhance transmission by predating cyst-positive sheep and subsequently contaminate pastures with egg positive feces. As an indirect driver of transmission, through their predation activity, wolves may increase the probability of dogs to infect themselves on sheep carcasses with cysts. Furthermore, the constant threat of predation compels farmers to keep more guardian dogs, thus increasing the potential *E.g.* host population, and drives them to limit grazing to pastures around the farm, an area frequented by the free roaming dogs and potentially contaminated with infective feces.

In conclusion, the study highlighted transmission risks associated with the wolf threat in an endemic area for *E.g.*, as recently confirmed by the positive cases found in sentinel hosts such as cattle (5 and 9 positive cases in 2022 and 2023, respectively) and wild boars (0.12% and 0.41% positivity rates, same two-year-period).

Sessione 3

NUOVE INTERAZIONI TRA ANIMALI SELVATICI, DOMESTICI E UOMO

Chairmen: Nadia Cappai – Enrica Bellinello

Nel corso della nostra storia lo scenario delle interazioni uomo/animale è cambiato radicalmente. La domesticazione delle prime specie ha ridotto le distanze tra mondi un tempo separati. Ma cosa è cambiato negli ultimi anni del nostro rapporto con il mondo selvatico? La relazione tra animali selvatici, allevamento e uomo ha favorito nel tempo il fenomeno del salto di specie (spillover), e nell'arco di 3000 anni molte epidemie e pandemie hanno scandito la storia dell'umanità. Oggi, la grande mobilità delle persone e delle merci e il continuo sfruttamento delle risorse naturali rendono praticamente inevitabili l'alterazione della normale epidemiologia di malattie conosciute e l'insorgenza di nuove, sconosciute infezioni, che non avranno solo un impatto diretto sulla salute umana e/o animale, ma anche sul contesto socio-economico dei Paesi colpiti. Sarà pertanto necessario sviluppare metodi di valutazione del costo/beneficio della loro eradicazione/controllo che includano concetti come, ad esempio, il benessere animale, la salvaguardia del settore terziario e l'equità sociale.

RELAZIONI AD INVITO

If the bat changes habitat: possible impacts on public and animal health.
Stefania Leopardi (Istituto Zooprofilattico Sperimentale delle Venezie)

CONTRIBUTI LIBERI

African Swine Fever prevention, control and communication strategies in Veneto and Friuli-Venezia Giulia: results of a multi-stakeholder social research study. CROVATO S., VIO D., MASCARELLO G., LENZO F., ZAGO M., PINTO A., MANTOVANI C., CATTOLI G., RICCI A., ISCARO C., FELIZIANI F., CITTERIO C.
Stefania Crovato (Istituto Zooprofilattico Sperimentale delle Venezie)

Occurrence of HEV in red deer hunted in Lombardia and Piemonte regions. LORUSSO P., PANDISCIA A., MANFREDI A., VIGANÒ R., BESOZZI M., BONERBA E., TERIO V.
Patrizio Lorusso (Università di Bari. Dipartimento di Medicina Veterinaria)

Wolf expansion and its effect on parasitic infections. FESCE E., FERRARI N.
Elisa Fesce (Università di Milano. Dipartimento di Medicina Veterinaria e Scienze Animali)

Wolf mortality in Northeastern Italy in the period 2018-2024: forensic and one health reflections. VIEL L., ASSIRELLI G., LEONE S., PALLANTE I., CALDEROLA S., OBBER F., TRAMONTAN B., GALLOCCHIO F., BINATO G., GRADONI F., TONIOLO F., GAGLIAZZO L., TENUZZO MIRIAM, DELLAMARIA D., CEGLIE L., BREGOLI M., BERALDO P., DORIGO L., CITTERIO C.V., POZZATO N.
Laura Viel (Istituto Zooprofilattico Sperimentale delle Venezie)

Avian *Metapneumovirus* subtype b in a northern shoveler wintering in Italy: implications for the domestic-wild bird interface? GRAZIOSI G., LUPINI C., GENTILE N., QUAGLIA G., LIZZI G., PEDRAZZOLI S., TUCCIARONE C.M., FRANZO G., LEGNARDI M., BASTON R., CECCHINATO M., MENOTTI L., BRANDIMARTI R., AVITABILE E., CATELLI E.
Giulia Graziosi (Università di Bologna. Dipartimento di Scienze Mediche Veterinarie)

Anthropogenic factors affecting the prevalence of ESBL/ampC-producing *Escherichia coli* in wild boars. MASSERDOTTI M., FORMENTI N., SCALI F., GUARNERI F., BELLINI S., CHIARI M., ALBORALI G.L., FERRARI N.
Marta Massedrotti (Università degli Studi di Milano. Dipartimento di Medicina Veterinaria e Scienze Animali)

Changes in antibiotic resistance levels of *Escherichia coli* strains from European hedgehogs in a wildlife rescue centre. PRANDI I., PASSARINO E., BELLATO A., ROCH-DUPLAND O., BONAFFINI G., OTTINO C., SERPIERI M., MAUTHE VON DEGERFELD M., QUARANTA G., NEBBIA P., ROBINO P.
Ilaria Prandi (Università di Torino. Centro Animali Non Convenzionali, Dipartimento di Scienze Veterinarie)

Environmental detection of *Aspergillus fumigatus* spores in a wildlife rescue centre, University of Turin. BONAFFINI G., RIGAMONTI T., PASSARINO E., SERPIERI M., PRANDI I., OTTINO C., QUARANTA G., PEANO A., MAUTHE VON DEGERFELD M.
Giuseppe Bonaffini (Università di Torino. Centro Animali Non Convenzionali, Dipartimento di Scienze Veterinarie)

The conundrum of modelling parasite distribution when host range is unknown. MASSOLO A., SIMONCINI A., GIUNCHI D.
Alessandro Massolo (Università di Pisa, Dipartimento di Biologia Unità di Etologia)

IF THE BAT CHANGES HABITAT: POSSIBLE IMPACTS ON PUBLIC AND ANIMAL HEALTH

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Introduction

Bats are often cited as probable reservoirs of pathogens x and y, defined as microorganisms that already circulate undetected in nature and that will be responsible of an emerging infectious disease in the future, either in humans (pathogen x) or domestic animals (pathogen y) following spillover. The spillover is the first transmission of a microorganism from its natural host (possible reservoir) to an accidental host, regardless of the subsequent epidemic amplification and adaptation, which instead represents the completion of the host shift.

Spillover is a stochastic process in which the microorganism must cross various barriers, which can be summarized in 1. Presence of the natural host/reservoir; 2: infection of the natural host/reservoir; 3 eliminations of the virus and environmental resistance; 4 contacts between natural host/reservoir and accidental host; 5: receptivity of the accidental host. This scheme highlights how the distribution of wildlife and its relationship with humans and domestic animals are fundamental elements for defining the risk for their spillover of their associated microorganisms.

Many species of bats are subject to strong changes both in distribution and in the type of habitats occupied, following the climatic, environmental and urban changes typical of the Anthropocene. For example, the ongoing deforestation and fragmentation of the rainforest in Latin America is modifying the connectivity between populations of the vampire bat (*Desmodus rotundus*) with peaks of amplification of the Rabies Virus, of which they are the main sylvatic reservoir in the area. This factor is accompanied by the predation of cattle to the detriment of wild ungulates, which constitute easier and more readily available preys, with significant implications in terms of animal health. Another important example is the Nipah virus epidemic of 1998, which was favoured by the presence of orchards close to pig farms to increase the profit of agricultural areas. This factor, coupled with climatic events linked to El Nino, contributed to the massive movement of infected flying foxes into rural areas where, once again, they founded easy and readily available food whose residues contaminated by saliva led to the infection of pig populations raised outdoors and, subsequently, of farmers in contact with infected animals. The Nipah epidemic, in this case, was highly relevant in terms of both public and animal health. It is well known that South America and Southeast Asia represent hotspots for the emergence of human and animal infectious diseases. This correlates with clear hazards at all critical points of spillover events, including the loss of balance between environment, wildlife and humans. However, it is appropriate to consider how many critical points are also present in our territory, where wildlife is getting closer and closer.

Material and Methods

One of the objectives of laboratory of Emerging Viral Zoonoses at IZSVe is to study the critical points for the spillover of microorganisms circulating in Italian bats. In particular, several research projects aim at studying the Schreiber's bent winged bat (*Miniopterus schreibersii*), which hosts at least 3 lyssaviruses, 3 coronaviruses and 1 filovirus. The lines of research exploit virological and veterinary skills but also techniques and knowledge typical of ecology and wildlife monitoring, always considering the peculiar biology of bats. In particular, the study of the distribution of species and their contact with domestic animals and humans is carried out by combining camera trapping, bioacoustics, positioning and surveying of microchips and mathematical modelling. On the other hand, the level of exposure of domestic and wild animals to bat viruses can be carried out using different serological methods, including viral neutralization, immunofluorescence and ELISA methods.

Results and Discussion

From 2020 to now IZSVe to described the dynamics of infection of *Miniopterus schreibersii* with viruses belonging to the families/genera Lyssavirus, Coronaviridae and Filoviridae, all considered relevant for their zoonotic and/or epidemic potential. The bat species have been long considered purely cave dwelling and, therefore, was thought to be associated with a low risk of spillover due to the limited contact with people and domestic animals.

In 2020, IZSVe diagnosed, in collaboration with IZSLT, a case of rabies in a cat in the city of Arezzo, which resulted from the transmission of a lyssavirus whose reservoir host is *Miniopterus schreibersii*, namely West Caucasian Bat Lyssavirus. This event has fuelled a line of research on the ecological niche of this animal, with particular reference to its unexpected urban habits. From 2020, we have detected at least five urban colonies, with different seasonal characteristics, of which two were monitored for the presence of coronaviruses, filoviruses and lyssaviruses. Despite this, the national distribution model based on the bioclimatic model that we built using all the points of presence of the species at European level, has found that the urban environment has a low level of suitability for this animal, as per previous knowledge. This supports the need for further modelling investigations which, however, must be accompanied by field work aimed at identifying urban points of presence and absence in the territory, to improve computational accuracy. Serological analyses have not yet identified exposure to lyssavirus, even if the sample size to date is not sufficient yet to effectively study this process. More analyses are ongoing this year exploiting a collaboration with veterinary clinics and passive surveillance for rabies in carnivores.

AFRICAN SWINE FEVER PREVENTION, CONTROL AND COMMUNICATION STRATEGIES IN VENETO AND FRIULI-VENEZIA GIULIA: RESULTS OF A MULTI-STAKEHOLDER SOCIAL RESEARCH STUDY

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Introduction

African Swine Fever (ASF) is a severe threat to the health of pigs and wild boar and the pig supply chain. The management of the disease is very complex due to multiple factors: virus environmental resistance, the presence of wild boar, and different types of pig farming in the territory. A further critical factor is the diversity of stakeholders involved, at various levels, in managing the disease and control measures: farmers, hunters, surveillance services, municipal administrations, etc. In the framework of the ASF preparedness plan and to encourage the adoption of coordinated and effective actions, the Istituto Zooprofilattico Sperimentale delle Venezie has realized a social and participatory research project 'PSA-PRINCE' in 2021, funded by the Ministry of Health. The project was developed in the Friuli-Venezia Giulia and Veneto regions, which represent areas not affected by the disease yet.

Material and Methods

The project aimed to create and disseminate a document validated and shared by experts from health institutions and public administrations, containing useful indications for the prevention and proposals for the management of PSA on the territory, in line with the provisions of the regulations.

The document was designed through the application of qualitative, quantitative, and participatory social research methods. The social studies allowed the investigation of the perceptions and knowledge of selected stakeholders regarding the prevention and management of the disease. The stakeholders involved in the research were: official veterinarians of the Health Authorities, specialists in swine pathology and husbandry, chain/professional breeders, breeders of small local productions, hunters and representatives of hunting associations, forestry groups, and local public administrators. In particular, interviews were carried out with breeders (n=14) and hunters (n=20); focus groups with veterinarians and health service physicians (n=20), and with wildlife and hunting supervisory authorities (n=7); in addition, an online questionnaire was administered to professional pig breeders (n=91). Finally, a working group with local administrators was organized to discuss and share with veterinarians' critical issues and operational proposals for the prevention and management of ASF to be implemented on the territory.

Results and Discussion

The analysis revealed the stakeholders' high level of attention and concern about the disease. Participants were highly aware of the animal health issues related to ASF and its heavy impact on livestock economy and wildlife management. All actors highlighted the importance of biosecurity measures to be implemented during hunting practices and in livestock farms, especially small-scale farms, likely to be at higher risk. In addition to public institutions, the stakeholders agreed that all citizens can potentially contribute to limiting the spread of ASF and emphasised the importance of receiving up-to-date information on the disease through authoritative and easily accessible information channels.

The study provided useful insights of stakeholders' views on the disease in the territories considered. Starting from this information a document containing validated indications to manage disease preparedness in areas currently free and to increase the level of stakeholders' awareness was developed. The document ('Strategie di prevenzione, controllo e comunicazione della Peste Suina Africana in Veneto e Friuli-Venezia Giulia: indicazioni operative del progetto RC IZSVe 10/21 PSA PRINCE') was shared with institutions, trade associations, and citizens during the project final conference and was made available and disseminated through the IZSVe web channels.

OCCURRENCE OF HEV IN RED DEER HUNTED IN LOMBARDIA AND PIEMONTE REGIONS

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Introduction

Hepatitis E virus (HEV) is the etiological agent of acute viral hepatitis, a human disease caused by contaminated food or water consumption and transmitted by the faecal-oral route. In Europe, zoonotic transmission of HEV is associated with the consumption of raw or slightly cooked meat of pork and wild boars that are considered the main reservoirs. However, other wild animals, such as lagomorphs and ungulates, can also be a source of contamination. In this regard, a high-risk zone for the spread of the virus could be Northern Italy where there is a high consumption of meat and processed game meat products. To date, there are few scientific studies that carried out only serological investigations without the correlation between these data and the presence of HEV RNA in wild meat products in Northern Italy. In order to determine the role of red deer in the HEV human spread, the aim of the present study was to evaluate the presence of HEV (RNA) in liver and muscle samples from deer hunting during the 2022-2023 hunting season.

Material and Methods

One hundred seventy liver samples of red deer (95 female and 29 male) and forty muscle samples belonging to the same animals were collected during the 2022-2023 hunting season. Virus extraction was performed following the method described by Di Pasquale et al., 2019 with appropriate modifications. The RNA extracted was amplified by Touchdown RT-PCR and Touchdown hemi-nested PCR and was subjected to phylogenetic analysis.

Results and Discussion

In our study, 22/170 (12.94%) red deer tested positive for the presence of the HEV RNA in liver, while it was detected only in 3/40 (7.5%) muscle samples. All the samples tested positive were characterized as HEV-3c genotype.

Our study firstly reported the presence of HEV RNA in red deer (*Cervus elaphus*) circulating in Lombardy and Piemont regions and confirmed that the HEV-3 is the main genotype spreading in this animal group.

The presence of HEV in both liver and muscle of deer is a relevant finding for food safety since, in the Italian culinary tradition, deer meat is used to prepare fresh or cured sausages and other food products such as deer "bresaola". These traditional products were subjected to the curing process, whose effectiveness in inactivating and decontaminating viruses in food is not yet known. Therefore, future studies are needed to better understand the role of this technological treatment in reducing the risk of HEV human infection from meat products.

Based on data obtained in our study, the recent increase in the consumption of deer game meat in North Italy, in the form of carpaccio or tartare, could allow the human HEV spread. Further studies are needed to evaluate an appropriate risk assessment.

Interestingly, the HEV-3c strain genotype found in deer in our study, was identical to the one commonly found in pigs and wild boars in Italy, France and German, suggesting a possible transmission route between these species. Furthermore, our study did not find significant differences in HEV prevalence by sex, age, or geographic location of deer, but highlighted the widespread nature of the virus in the populations studied.

This research underscores the need to monitor HEV spread in wildlife and game meet products and human populations to better understand the zoonotic transmission and circulation to carry out properly risk assessment.

WOLF EXPANSION AND ITS EFFECT ON PARASITIC INFECTIONS

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Echinococcus spp. are parasitic cestodes that have a dixenic life cycle, involving definitive hosts (typically canids) and intermediate hosts (including herbivores and rodents). Humans can become infected with *Echinococcus* spp., leading to significant health issues such as organ dysfunction, anaphylaxis, or even death. *Echinococcus* spp., particularly *Echinococcus granulosus* and *Echinococcus multilocularis*, pose substantial health risks, thereby representing a global public health challenge. Among carnivores, foxes (*Vulpes* spp.) and wolves (*Canis lupus*) play crucial but distinct roles in the life cycle of the parasite. Both species act as definitive hosts, harboring adult parasites in their intestines, excreting parasite eggs in their feces, and serving as sources of infection for intermediate hosts. Despite these similarities, they differ in predatory behavior and habitat, which can influence transmission dynamics. Wolves are larger pack animals that primarily prey on larger herbivores, whereas foxes are more solitary and opportunistic feeders, often consuming small mammals and scavenging. In recent decades, wolf populations have significantly expanded across Italy, particularly in the Apennine and Alpine regions. This expansion may alter local ecological dynamics and change the roles of both species in disease transmission.

To explore the effects of wolf expansion on *Echinococcus* spp. prevalence, we developed a set of mathematical models based on the predator-prey framework. These models explicitly include mechanisms of infection transmission, allowing us to predict future scenarios of parasite transmission in wildlife. We simulated host-parasite dynamics in a fox population without wolves, then introduced two wolves into the area to examine how, and under what conditions, this introduction could alter parasite-host dynamics and *Echinococcus* spp. prevalence.

Our results confirmed that, without host mortality due to parasite presence, the two host species reach a new equilibrium, modulating the populations of herbivores and small mammals in the territory. Furthermore, *Echinococcus* spp. prevalence is strongly affected by the composition of the carnivore-host population. However, model outputs are highly sensitive to initial conditions and parameters, highlighting the urgent need for further research to predict and reduce the risk of human infection.

WOLF MORTALITY IN NORTHEASTERN ITALY IN THE PERIOD 2018-2024: FORENSIC AND ONE HEALTH REFLECTIONS

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Introduction

The presence of the wolf in the Central-eastern Alpine regions of Italy, starting from the settlement of the first stable pack in Lessinia mountains (Verona and Trento provinces) in 2013, has progressively increased reaching in 2020/21 an estimated population of 266 individuals, with 33 reproductive units distributed in a territory doubled compared to that occupied in 2017/2018. With the increase in the number of wolfpacks, whose distribution is still concentrated in the Trentino-Veneto piedmont belt, signs of the presence of individuals in hilly and flat areas have also increased over time, as well as the finding of dead or injured/sick individuals throughout the territory. The aim of this work is to describe the monitoring activity from a forensic and health point of view carried out on wolves delivered to Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe) diagnostic laboratories of Veneto, Friuli, Bolzano and Trento. This activity allowed us to obtain more information on the presence of the main circulating pathogens in the wolf population of the Central-eastern Alpine regions and on the most frequent causes of death, both intentional and accidental.

Material and Methods

In the period 2018-06/2024 years, 96 wolves underwent necropsy at the local laboratories of the IZSVe. In case of suspected poaching, the cadavers were subjected to radiographic examination to seek radiopaque material compatible with pellets/bullets. The autopsy was then performed according to the national guidelines for forensic autopsies in veterinary medicine in association with the diagnostic protocol defined by the IZSVe. This protocol includes the direct detection of rabies virus, distemper virus, influenza type A viruses, *Leptospira*, *Trichinella*, and cestodes. Antibodied against *Leptospira* were also investigated. Toxicological analyses were applied to determine carbamates, chlorinated and phosphorated pesticides, metaldehyde and strychnine in the gastric content and anticoagulant rodenticides in the liver. Finally, ticks were identified taxonomically and analyzed by PCR for *Anaplasma, Babesia, Theileria, Borrelia, Rickettsia* and Tick-Borne Encephalitis virus.

The analyses were performed in whole or in part depending on the state of decomposition of the animals, hence tissue availability, as well as the diagnostic suspicion formulated at the autopsy.

Results and Discussion

Out of 96 wolves examined, the majority died for anthropogenic causes, mainly from motor vehicle accidents (n= 71; 73.9%) but also from non-accidental causes as firearm injuries and pesticide poisoning (n=11; 11.4%). The prevalence of road accidents is evidently a direct consequence of the greater probability of finding carcasses on the roadside or in the immediate vicinity, compared to those in the natural environment where wolves usually live and, presumably, die from other causes. Non-anthropogenic causes were recorded in 11 cases (11.4%), 6 of which attributable to intraspecific competition, 2 to severe diffuse sarcoptic mange inanition, one to distemper, one to falling in a hydroelectric station channel and the latter, a postweaning puppy, to gastroenteric disease. In the remaining 3 cases it was not possible to confidently determine the cause of death.

As expected, based on the epidemiological situation, no wolf tested positive for the rabies virus. Also noteworthy is the fact that no positivity was observed for *Trichinella* spp.. Monitoring infectious diseases, particularly those of a zoonotic nature, becomes crucial in a context where an increasing interaction of the wolf with anthropogenic activities is expected.

Despite the results obtained do not represent the real distribution of the death occurrence in the wolf population, the application of standardized protocols among institutions allows a better representation of the information that is possible to collect, aiming at the improvement of environmental and health management of wild carnivores.

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AVIAN METAPNEUMOVIRUS SUBTYPE B IN A NORTHERN SHOVELER WINTERING IN ITALY: IMPLICATIONS FOR THE DOMESTIC-WILD BIRD INTERFACE?

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Introduction

Avian metapneumovirus (aMPV) is a pathogen with a growing impact on the poultry sector. aMPV mainly affects chickens and turkeys and it is associated with acute respiratory disease or reproductive disorders. To date, different aMPV subtypes have been recognized, based on genetic and antigenic profiles (aMPV-A, B, C, D) further showing diverse spatial distribution and host range. With respect to wild birds, molecular or serological evidence of aMPV-A, B or C infection has been reported for different species. Due to aMPV global spread over time and emergence in new niches, the role of migratory birds in the viral epidemiology has been also called into question. Given this background, a molecular survey was conducted on selected resident and migratory species hunted or found deceased in the river Po Valley of the Bologna province, Italy.

Material and Methods

Oropharyngeal swab samples were collected in the framework of the National Avian Influenza (AI) Surveillance plan from 2021 to 2023. The study area included private hunting grounds located in the river Po Valley, where densely populated poultry areas (DPPAs) intermingle with wetlands strategic for wintering and breeding of migratory bird communities. Setting the expected aMPV prevalence in wild birds at 6%, a minimum of 87 samples was required to find at least one positive sample in an infinite population with a confidence level of 95%. The samples were subjected to RNA nucleic acid extraction using a commercial kit and tested through a multiplex real-time RT-PCR to detect the presence of all known aMPV circulating subtypes. Subsequent RT-PCR protocols were used to amplify and sequence the positive sample. The obtained sequence was aligned to and compared with homologous sequences available on the GenBank® database and a maximum likelihood phylogenetic inference was performed. Percentages of nucleotide (nt) and amino acid (AA) homology between the obtained strain and its closest relatives, as identified by the phylogenetic investigation, were also computed.

Results and Discussion

A total of 250 oropharyngeal swabs were collected from wild aquatic birds sampled during three consecutive hunting seasons (October 2021 – January 2022, October 2022 – January 2023, and October – December 2023). Of the individuals included in the study, 91.6% (229/250) belonged to different species of dabbling ducks (*Anas crecca*, *Anas platyrhynchos*, *Spatula clypeata*, *Mareca penelope*), 4.8% (12/250) were Eurasian coots (*Fulica atra*), 2.4% (6/250) were Northern lapwings (*Vanellus vanellus*), and 1.2% (3/250) were common snipes (*Gallinago gallinago*). One oropharyngeal swab obtained from an adult female Northern shoveler (*S. clypeata*) sampled in November 2021 tested positive for aMPV subtype B through rtRT-PCR (aMPV/B/Italy/Northern_shoveler/80/21). A partial glycoprotein (G) gene sequence of 360 bases was obtained by RT-PCR and sequencing. The phylogenetic tree demonstrated that the aMPV/B/Italy/Northern_shoveler/80/21 strain clustered together with Italian aMPV-B strains obtained from chickens and turkeys during 2014-2019. A 98-100% nt and AA homology was observed with Italian strains detected in domestic birds from 1987 to 2019.

Previously published experimental studies proved domestic ducks resistant to aMPV-B infection. The herein detection of aMPV-B in a waterfowl of the genus *Spatula* spp. might therefore indicate a varying susceptibility among duck species.

In Italy, field studies in poultry have revealed a widespread circulation of aMPV-B in DPPAs in the Northeastern part of the country, where the above-mentioned sampling activities occurred. Considering that the phylogenetic analysis showed the close relationships among the hereby obtained aMPV-B strain and field strains circulating in Italian poultry from 2014 to 2019, a virus spillover at the domestic-wild bird interface might have occurred. aMPV presence in waterfowl opens to the existence of a wild niche, where viral strains might circulate, evolve, or be maintained, as attested by the present retrieval of an "old" Italian strain, possibly constituting a permanent viral source for the poultry sector. In the light of the confirmed presence of aMPV-B in waterfowl, dedicated surveys involving viral isolation and whole genome sequencing will set the premises for further studies investigating the biological features of aMPV strains detected in wild hosts through *in vitro* and *in vivo* studies.

ANTHROPOGENIC FACTORS AFFECTING THE PREVALENCE OF ESBL/AMPC-PRODUCING ESCHERICHIA COLI IN WILD BOARS

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Introduction

Antimicrobial resistance (AMR) represents a global public health threat, involving many host species, bacteria carrying resistance-genes and transmission routes. According to the World Health Organization (WHO), among the AMR pathogen of critical importance is *Escherichia coli*, specifically, *E. coli* producing β-lactamases, extended-spectrum-lactamases (ESBLs) and other β-lactamases such as AmpCs.

Although antimicrobial use (AMU) is a major driver of AMR and wild species are supposedly antibiotic-free, wildlife is shown to carry AMR bacteria. The occurrence of such bacteria is influenced by host and environmental factors, including the anthropization level of the host ranging area. In particular, the presence of landfills and the inadequate treatment of wastewater from urban centers and livestock farms seems to play an important role, but the extent of their effect is rarely defined. Hence, the interpretation of wildlife epidemiological role as indicator or reservoir has emerged as key question. In Lombardy, wild boars (*Sus scrofa*) are a species of particular interest, given their population density and their ranging in proximity to farms and anthropized environments. With almost 24,000 km^2, Brescia is the largest province in Lombardy and is characterised by several highly anthropized areas (1.3 million inhabitants) and extensive animal production (1.1 million pigs and half a million cattle), but also by large wilderness areas with a high presence of wild boar.

The present study aims to investigate factors affecting the prevalence of ESBL/AmpC-producing *E. coli* in free-ranging wild boar in the province of Brescia in relation to human population density, livestock presence (pigs and cattle) and host factors.

Material and Methods

We analyzed a total of 287 wild boar's faecal samples collected during the 2022/2023 hunting season in 21 municipalities within the Province of Brescia (Lombardy, Northern Italy). For each wild boar, sex and age were registered.

Given the consistent hunting behaviour, the total number of wild boars hunted per year was used as a proxy of the wild boar abundance in the investigated areas. The human population density was obtained by the data available on the Italian Statistical Institute (ISTAT) website by dividing the total number of inhabitants in the municipalities by the municipalities area in km^2. Data on farms and livestock (number of farms, animals and biomass) and AMU for pigs and bovines were obtained from the national surveillance system (ClassyFarm). AMU was expressed in Defined Daily Dose Animal for Italy (DDDAit).

The identification of ESBL/AmpC-producing *E. coli* was performed through a double synergy diagnostic method: positive growths on inoculated MacConkey agar supplemented with 1 mg/L cefotaxime were selected on the basis of staining and morphological characteristics and subjected to subsequent molecular characterization. The detection of resistance genes was performed with a panel of PCR reactions.

We used logistic regression models (GLM) to define the effects of host- and environment-related explanatory variables on the probability of wild boar to be positive for ESBL-/AmpC-producing *E. coli*. As host-related variables, we considered sex and age class, while as environment-related explanatory variables we considered for each municipality wild boar abundance, human population density, farms density, livestock density, biomass density and AMU. Statistical analyses were carried out using R software version 4.3.3. Results were considered statistically significant if the p-value (p) was less than 0.05.

Results and Discussion

The probability of wild boars to be positive for ESBL-/AmpC-producing *E. coli* was not influenced by hosts' age and sex, but emerged to be significatively associated with the environmental-related factors.

The positive association with human density, swine and bovine farms density, bovines' density and AMU in swine emphasize the impact of human and farm activities on the spread of AMR in wild boars. These results underscore the importance of responsible AMU and antimicrobial disposal, both in humans and farmed animals, to limit the spreading of AMR bacteria in the environment and wildlife. The negative association with wild boars' abundance might be explained by dilution effects due to competition.

Overall, these findings show the effects of anthropogenic activities on AMR bacteria emergence and spreading and highlight the importance of including the various human-influenced environmental factors in the analyses.

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CHANGES IN ANTIBIOTIC RESISTANCE LEVELS OF *ESCHERICHIA COLI* STRAINS FROM EUROPEAN HEDGEHOGS IN A WILDLIFE RESCUE CENTRE

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Introduction

Antimicrobial resistance is an emerging threat to human and animal health worldwide. It is enforced by the use of antimicrobials and leads to the acquisition of AMR genes in both pathogenic and commensal bacteria, that can exchange resistance genes through horizontal gene transfer. *Escherichia coli* is a Gram-negative bacillus that colonies the gastrointestinal tract of birds and mammals but is also widely distributed in the environment. Its ubiquitous behaviour makes it the ideal target bacterium to monitor AMR levels and acquisition.

Wildlife is not directly exposed to antimicrobials but human sources, agricultural facilities, and associated contaminated human environments can promote the acquisition of AMR commensal bacteria. European hedgehogs (*Erinaceus europaeus*) are nocturnal insectivores frequently found in urban areas, where they come into strict contact with humans and domestic animals. Hedgehogs are one of the most common mammals admitted to wildlife rescue centres (WRCs). In domestic animals, a hospitalisation period promotes the acquisition of AMR strains, and this could happen also in WRCs, where wild species are hosted for several days until recovery.

This study aimed to evaluate AMR levels and the consequence of a hospitalisation period on AMR changes in faecal *E. coli* isolated from wild hedgehogs admitted to Centro Animali Non Convenzionali (C.A.N.C.), section of the University of Turin.

Material and Methods

In 2023, rectal samples were collected from European hedgehogs at the time of arrival (T0) at C.A.N.C., as well as 10 days afterwards (T1). *E. coli* strains were cultured on MacConkey 3 Agar and confirmed by Matrix-Assisted LaserDesorption/Ionization – Time of Flight (MALDI-TOFF) analysis. A pool of *E. coli* strains collected from each sample was evaluated for resistance to a panel of 18 antibiotics using the Sensititre® system with MICs interpreted by CLSI and EUCAST breakpoints. Statistical analysis was performed with R Studio software (v. 4.3.2).

Results and Discussion

Thirty-nine hedgehogs were sampled at admittance in the study period. Of them, 24 were still hospitalised after 10 days and another rectal swab was collected. The remaining 15 animals died or were released before that time point. The present results refer to these 24 hedgehogs. A total of 48 rectal swabs were processed and the obtained *E. coli* strains underwent antimicrobial susceptibility testing. At T0, all animals (100.0%) were resistant towards at least one antimicrobial agent. The highest resistance levels were shown towards first-generation cephalosporins (n=24, 100.0%, 95% CI = [85.8-100.0]), ampicillin (n=12, 50.0%, 95% CI = [29.1-70.9]), fluoroquinolones (n=7, 29.2%, 95% CI = [12.6-51.1]) and amoxicillin-clavulanic acid (n=6, 25.0%, 95% CI = [9.8-46.7]). Resistance to the other antibacterials was not higher than 12.5% (n= 3, 95% CI = [2.7-32.4]). Twelve out of 24 animals were multi-drug resistant (MDR) (50.0%, 95% CI = [29.1-70.9]), defined as resistant to at least one agent in three or more antimicrobial classes. Three animals presented Extended-spectrum β-lactamases (ESBL)-producing *E. coli* (n= 3, 12.5%, 95% CI = [2.7-32.4]).

After 10 days of hospitalisation, six (25.0%, 95% CI = [9.8-46.7]) animals acquired resistance towards aminoglycosides, three towards fluoroquinolones (12.5%, 95% CI = [2.7-32.4]), two towards chloramphenicol (8.3%, 95% CI = [1.0-27.0]) and other two towards piperacillin/tazobactam (8.3%, 95% CI = [1.0-27.0]). Overall, 11 animals (45.8%, 95% CI = [25.6-67.2]) acquired resistant strains towards at least one new antimicrobial agent. ESBL-producing *E. coli* were detected in one more animal after 10 days of hospitalisation (n= 4, 16.7%, 95% CI = [4.7-37.4]).

Studies on AMR in *E. coli* collected from hedgehogs are scarce. A previous study performed on Long-Eared hedgehogs in Iran detected 35.0% MDR *E. coli* strains, showing the highest resistance towards ampicillin and tetracycline (53.3%; n = 31). This highlights the role of hedgehogs as carriers of AMR bacteria. Given their urban distribution (e.g., public parks, private gardens), they could represent a risk factor for the acquisition of resistant bacteria by humans and domestic animals.

Moreover, this study demonstrates an increase in the carriage of resistant *E. coli* to several antibacterials following hospitalisation, thus suggesting that a prolonged period at WRCs plays an important role in the probability of acquiring AMR. A study previously performed in the same facility on wild birds also detected the acquisition of new resistances during a ten-day hospitalisation period. This highlights the importance of applying, also in WRCs, all the recommended strategies to diminish the spread of bacteria and pathogens in hospital settings (handwashing, barrier clothing, disinfection of surfaces, proper antimicrobials administration).

ENVIRONMENTAL DETECTION OF ASPERGILLUS FUMIGATUS SPORES IN A WILDLIFE RESCUE CENTRE, UNIVERSITY OF TURIN

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Introduction

Aspergillus fumigatus is a filamentous fungus capable of causing severe diseases in both humans and animals. One of the main routes of infection is the inhalation of conidia dispersed in aerosols. The fungus is an opportunistic pathogen that causes severe forms in immunocompromised patients. However, exposure to high environmental loads of spores can also cause problems in individuals without specific predisposing factors. Although there are several active substances against this fungus, the increasing presence of resistant strains is a serious risk factor for public health. This study aimed to investigate and quantify the presence of *A. fumigatus* spores within the Centro Animali Non Convenzionali (CANC) of the University of Turin, and the possible presence of drug-resistant strains.

Material and Methods

Using a portable sampler (Surface air system – SAS), air samples were collected at various points in the CANC from June 2023 to May 2024. The plates were incubated under conditions that selected the growth of *A. fumigatus* (antibiotic-containing media and incubation at 50°C). The identification of the obtained colonies was carried out using morphological keys and molecular techniques.

In vitro antifungal sensitivity to widely used azole drugs such as voriconazole (VCZ), itraconazole (ITZ), posaconazole (PSZ) was assessed by agar immunodiffusion tests using commercially available reactive strips. Statistical analysis was performed using R Studio software (V. 4.3.2). Correlation between spores concentration and environmental parameters (temperature and humidity) was evaluated by means of Spearman test.

Results and Discussion

In total, 61 out of 286 plates were positive, equal to 21% of the samples taken, with a total of 146 colonies of the fungus. Out of 22 sampling days, on 16 occasions (73.3%) there was at least one positive point. The average concentration detected at all sampling points was 29.8 CFU/m³ (colony-forming units). Considering only the indoor sampling points, the average was 7 CFU/m³, whereas the average for the outdoor sampling points was 66 CFU/m³. An increasing trend in CFU per cubic meter could be observed with increasing humidity and decreasing temperature. However, this correlation was not statistically significant.

This study represents the first report that investigates the environmental presence of *Aspergillus fumigatus* spores in a wildlife rescue centre. The concentrations detected were mostly within values normally found in the environment and considered not dangerous for healthy individuals. Only occasionally were higher concentrations observed, up to 1500 CFU/m³.

Ten strains tested for in vitro sensitivity could be considered sensitive to VCZ, while one strain was resistant to ITZ. For PSZ, 7 strains had values close to the threshold considered for resistance.

The results are fairly reassuring regarding the environmental levels of *A. fumigatus* spores, indicating a "healthy" environment for both human operators and animal inhabitants. On the other hand, some occasionally found "peaks" suggest the need to maintain high attention to possible exposure to high spore loads. The situation appears more concerning regarding potential resistance phenomena in commonly used drugs.

THE CONUNDRUM OF MODELLING PARASITE DISTRIBUTION WHEN HOST RANGE IS UNKNOWN

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Introduction

Identifying the distribution of zoonotic parasites is challenging due to a paucity of information and sampling bias, not least, for most parasites, to unknown host range. Moreover, the complex link between hosts distributions, habitat characteristics (e.g., vegetation, land cover, elevation, orography), climate and the too often neglected non-host (animal) species, makes such host-parasite interactions very dynamic in fast-changing scenarios of climate and land use changes as the current ones.

Echinococcus multilocularis is responsible for alveolar echinococcosis, one of the most important food-borne zoonoses worldwide, and recent data indicated the expansion of its European range and of its strains across the Northern hemisphere. Despite its relevance, its surveillance is still not a priority in most areas where the parasite is not historically present. Our study then aimed to aid in the risk assessment and distribution modelling of this parasite by estimating the probability of being suitable to *E. multilocularis* infections of murid and cricetid species whose susceptibility and competence for this parasite are unknown.

Material and Methods

We used data on susceptibility and competence for *E. multilocularis* of Holarctic cricetid and murid rodents, its main intermediate hosts, to predict the probability of being a susceptible intermediate host, based on ecological and morphological traits as well as phylogeny using two alternative modelling approaches: Bayesian Generalized Linear Mixed Models (Bayesian GLMM) and Presence-Unlabeled Learning (PU-L), a machine learning approach that treats unlabeled data as a mixture of true and false absences. Once the probability of being susceptible to the parasite was estimated, we compared the two model outcomes and mapped potentially susceptible rodents in geographical, phylogenetic and trait space.

Results and Discussion

Many species without previous information were predicted as unreported hosts, suggesting that the current risk scenario for *E. multilocularis* may be optimistic. Predicted richness of intermediate hosts peaked in Central-Eastern Europe, Western North America and Central Asia, whereas the proportion of predicted intermediate hosts over the total richness of all reported rodents spiked at northern latitudes and in the Tibetan Plateau. Phylogeny and body mass were the most important predictors of susceptibility. Two species predicted as highly susceptible were confirmed susceptible with a targeted search, suggesting that our modelling approach, which aided in identifying undetected and under-reported *E. multilocularis* intermediate hosts, can be likely adapted to other host-parasite systems whose host range is unknown.

Tavola rotonda
QUADRO NORMATIVO E PROBLEMATICHE PROCEDURALI DELLA GESTIONE SANITARIA DELLA FAUNA SELVATICA

Chairmen: Simone Angelucci – Camilla Smoglica

I cambiamenti ecologici in atto, conseguenti al profondo decremento delle attività agrozootecniche nelle aree interne, e con essi l'incremento numerico delle popolazioni animali selvatiche e dei loro areali di distribuzione, inducono un cambio di approccio di visione e metodologico che non è sostenuto da un altrettanto decisiva innovazione normativa. Dalle leggi nazionali di tutela della fauna e della biodiversità, concepite in gran parte negli anni '90, ai quadri normativi regionali sulla gestione venatoria e sui centri di recupero, ed oggi, anche con l'evoluzione della Animal Health Law, la gestione sanitaria della fauna selvatica in Italia va faticosamente conformandosi in forza di iniziative che, seppur produttive e talvolta innovative su base locale, risentono di una mancata armonizzazione e di una frammentazione amministrativa che rallentano processi conoscitivi e ritardano adeguamenti procedurali.
Ne parliamo con professionisti e rappresentanti delle istituzioni, citando le più recenti esperienze sviluppate in tal senso, e non sottraendoci alla necessità di evidenziare vuoti normativi e difficoltà procedurali e di stimolare lo sviluppo di un dialogo interistituzionale ed un coordinamento tecnico-procedurale non più rimandabile.

Partecipano rappresentanti delle istituzioni competenti e gestori:

Ugo Santucci Direttore Tutela del benessere animale, igiene zootecnica e igiene urbana veterinaria. Ministero della Salute
Barbara Franzetti Istituto Superiore per la Protezione e la Ricerca Ambientale
Alessandro Bettosi Colonnello Carabinieri Unità Forestali Ambientali ed Agroalimentari, Medico Veterinario
Sonia Braghiroli Settore Attività Faunistico-Venatorie, Pesca e Acquacoltura, Regione Emilia-Romagna
Sandro Nicoloso D.R.E.A.M. Italia
Elisa Berti Centro Tutela e ricerca fauna esotica e selvatica Monte Adone
Coordina Simone Angelucci Servizio Veterinario Parco Nazionale della Maiella – Wildlife Research Center

Sessione 4
SESSIONE A TEMA LIBERO

Chairmen: Michele Vicari – Walter Mignone

CONTRIBUTI LIBERI

The HPAI outbreak 2021 – 2022 from the point of view of the Lipu Wildlife Rescue Centres network. PACINI M.I., CECCHERELLI R., TERREGINO C., BORTOLAMI A., SILVA L.
Maria Irene Pacini (Lipu Bird Life Italia, Parma)

Coronavirus infection in European hedgehog. Epidemiological survey in the regional wildlife rescue center of Apulia. PRIOLETTI M, LOMBARDI R., PUGLIESE N., SCIANCALEPORE D., SCHIAVONE A., ROMITO D., D'ONGHIA F., BOVE A., CASALINO G., CIRCELLA E., CAMARDA A.
Michela Prioletti (Università degli Studi di Bari. Dipartimento di Medicina Veterinaria)

Invaders and co-invaders: a survey on the parasitofauna of the red eared slider *Trachemys scripta* in a protected area in Northeastern Italy. MARCHIORI E., MARCER F., FERRARO E., GRILLINI M., BERALDO P., PASOTTO D., PESARO S.
Erica Marchiori (Università degli Studi di Padova. Dipartimento di Medicina animale, Produzioni e Salute)

Second outbreak of Sarcoptic mange in ibex population of the Friulan Dolomites regional nature park. FREGA B., FAVALLI M., DINI F.M., BORDONI T., DANELIN G., GRANZIERA E., GALUPPI R.
Beatrice Frega (Università Di Bologna. Dipartimento di Scienze Mediche Veterinarie)

Mycobacterium avium subsp. *avium* in a red deer (*Cervus elaphus*) population of Eastern Italian alps. BREGOLI M., DE ZAN G., DEOTTO S., CADAMURO A., PALEI M., ZANARDELLO C., LEVAN R., D'INCAU M.
Marco Bregoli (Istituto Zooprofilattico Sperimentale delle Venezie)

African Swine Fever and the role of wolves as spreader or reducer: a preliminary investigation. MORONI B., CARELLA E., CARISIO L., BEATO M.S., ROBETTO S., BICCHERI R., ORUSA R., ISCARO C., ROSSI L., ZOPPI S.
Barbara Moroni (Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta)

Is a wild boar meat supply chain possible in the context of the African Swine Fever emergency in the Piedmont region? VIGANÒ R., DEMARTINI E., BESOZZI M.
Roberto Viganò (Studio Associato AlpVet, Busto Arsizio VA)

Molecular characterization of five *Sarcocystis* species in red deer hunted for human consumption in Italy. RUBIOLA S., VIGANÒ R., GRIGLIO E., CIVERA T., BERTOCCI F., CHIESA F.
Selene Rubiola (Università degli Studi di Torino, Dipartimento di Medicina Veterinaria)

Parasitological evaluation in Mesola deer (*Cervus elaphus italicus*): focus on *Elaphostrongylus cervi*. BELLINELLO E., ANGELUCCI S., STANCAMPIANO L.
Enrica Bellinello (AUSL Modena - Dipartimento Sanità Pubblica)

Toxicity of lead ammunitions on both wildlife and game meat. Nostalgia for lead hunting despite evidence since 1786? FERRI M.
Mauro Ferri (Medico Veterinario, SIEF)

THE HPAI OUTBREAK 2021 – 2022 FROM THE POINT OF VIEW OF THE LIPU WILDLIFE RESCUE CENTRES NETWORK

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Introduction

The highly pathogenic avian influenza virus has recently spread widely in wild birds and has been detected in more than 400 avian species. Wildlife Rescue Centres (WRCs) admit large numbers of animals from numerous different species and may therefore be very supportive in the management and surveillance plans for infectious diseases. Lipu is an Italian environmental association and manages 9 WRCs throughout the country.

The purpose of our work is to describe how Lipu and its WRCs responded to the 2021-2022 HPAI epidemic.

Material and Methods

The main events of the last avian influenza epidemic in Europe and Italy, and the activities carried out by Lipu, both centrally and in the WRCs, were reviewed.

Results and Discussion

During 2021-2024 years, Lipu established a communication network with its WRCs, supervised their activities and developed partnerships with the main Italian health authorities. WRCs implemented the measures from the Ministry of Health, created useful isolation rooms and collected swabs and carcasses for the surveillance plan. Between 2022 and 2024, 4833 samples were collected from 110 species, 4725 carcasses and 108 swabs, and 22 samples from 6 species tested positive.

In conclusion, a central coordination between WRCs is feasible with satisfying results in managing a health emergency. Moreover, given that the species affected by bird flu are constantly increasing, data collected from WRCs can be helpful in the avian influenza surveillance.

CORONAVIRUS INFECTION IN EUROPEAN HEDGEHOG. EPIDEMIOLOGICAL SURVEY IN THE REGIONAL WILDILIFE RESCUE CENTER OF APULIA

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Introduction

Coronavirus is a heterogeneous group of positive-sense, single-stranded RNA viruses capable of infecting a wide range of vertebrates. A distinguishing feature of these viruses is the relatively large size of their non-segmented genome, whose plasticity makes it capable to adapt to new environments through mutation and recombination processes. Among adaptive processes, differentiation of tissue tropism and widening of their host range are the most challenging for the medical and scientific communities.

The emergence of SARS-CoV in 2002, responsible for a sudden and large epidemic break, led to significant increase in scientific interest in coronaviruses, further enhanced by the most recent pandemics of SARS-CoV2. Phylogenetic studies highlighted the animal origin of those viruses, also emphasizing the relationship with other mammalian infecting coronaviruses, such as bat and hedgehog coronaviruses. The latter has been found to widely circulate in the wildness. The synanthropic behavior of hedgehogs (*Erinaceous europaeus*), together with the constant expansion of human settlement toward the rural areas, has increased the frequency of interactions between humans and Erinaceinae, posing a significant risk factor for the circulation of infectious agents that may threaten human health.

This study aims to analyze the presence and circulation of coronavirus in the common hedgehog (*Erinaceus europaeus*) population of Apulia.

Materials and Methods

Biological samples were collected and analyzed from 111 common hedgehogs admitted to the wildlife rescue center in Bitetto (Bari), affiliated with Osservatorio Faunistico Regionale (OFR) of Puglia. Sampling was performed between October 2019 and April 2021 using rectal and oropharyngeal swabs, which were appropriately refrigerated immediately after collection. Of the sampled hedgehogs, 66 were released into the wild after the required treatments, while the remaining 45 died within a few days after admission. None of the examined hedgehogs showed symptoms compatible with respiratory or gastrointestinal viral infections. The collected samples were tested for the presence of Erinaceus coronavirus (EriCoV) by RT-PCR. In the case of positivity, amplicons were sequenced and phylogenetic analysis was performed to infer relationships with other EriCoV, bat coronavirus (BatCoV) and MERS-CoV strains.

Results and discussion

Coronavirus was detected in 27 out of the 111 sampled individuals (24.32%). The virus was present in the feces of all positive animals and, in 59.26% of cases, it was also detected in oropharyngeal swabs. Genomic analysis revealed that 24 of the examined subjects were positive for the same strain of EriCoV. Three samples did not produce reliable sequencing results and were excluded from further analysis. Phylogenetic analysis confirmed they belonged to EriCoV species, more distantly related to MERS-CoV and Bat-CoV. Sequences from Apulian strains formed a homogeneous cluster, indicating a monoclonal circulation of EriCoV, separated but related with those isolated in the North of Italy.

This may be explained with the solitary behavior of hedgehogs, which does not favor the circulation and intraspecific recombination of different viral lineages. This limits the circulation of virus and the introduction of new viruses into the area.

On the other side, positivity in both oropharyngeal and rectal swabs suggests that the virus can be transmitted both by respiratory and enteric routes. Also, the positivity often observed among the members of the same family groups (76% of subjects belonging to family groups were positive), suggests that contagion could occur in the nest through contact with the parents through feces or other contaminated matter. Such a hypothesis and would require further verification, which, however, is difficult to implement due to the wild nature of the hedgehog.

Finally, no evidence was found supporting the pathogenicity of EriCoV for infected animals, none of the positive subjects showed evident clinical symptoms.

All the above considering, the research results have shown that EriCoV population circulating in Apulian hedgehog population is stable, as in other Italian regions. Due to the wide spread of EriCov, and the high evolution rate of coronaviruses, monitoring should be extended to include more individuals and other species too, to understand the dynamics of coronavirus infections and to rapidly identify recombinant strains that could acquire the capability to infect humans.

INVADERS AND CO-INVADERS: A SURVEY ON THE PARASITOFAUNA OF THE RED EARED SLIDER TRACHEMYS SCRIPTA IN A PROTECTED AREA IN NORTHEASTERN ITALY

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Biological invasions represent a major threat to the conservation of biodiversity, and the establishment of new, self-sustaining populations of non-native organisms in a natural ecosystem is the most evident example of this phenomenon. Impact on the autochthonous fauna by non-native organisms may be associated to direct competition but also to alteration of disease dynamics. In particular, co-introduction of parasites with animal invaders may result into a spill-over of parasites to phylogenetically related native species, immunologically naïve to those species; alternatively, alien species may host local parasites, diluting or amplifying infection risk for native host species.

Following commercialization and illegal release of pet turtles in the natural environment, the red-eared slider turtle *Trachemys scripta*, native of Northern and Central America, has successfully invaded natural freshwater ecosystems across all Europe, becoming a stronger competitor with autochthonous emydid turtles. Nevertheless, indirect effects of its expansion mediated by parasites have been poorly studied. Few spillover events of parasites from *T. scripta* to the European pond turtle, *Emys orbicularis*, are reported in Spain, France and Switzerland but descriptions of the parasitofauna of the two turtle species are completely lacking in Italy.

In this survey, a first analysis of the parasitofauna of *T. scripta* is reported, from an area in which the species lives in sympatry with the autochthonous *E. orbicularis*, namely Isola della Cona, Riserva Naturale Regionale Foce dell'Isonzo (GO), in Friuli-Venezia Giulia region (Italy).

In the summer 2023 and 2024, an overall number of 62 animals were collected by basking traps and humanly euthanized following published protocols. A complete parasitological examination was carried out after the isolation of organs during dissection, here including the entire gastrointestinal tract (GIT), urinary bladder, lungs, heart and major vessels, spleen and liver. All organs were carefully opened or sectioned and observed under the stereomicroscope for detecting eggs of cardiovascular flukes or related lesions. The content of GIT and urinary bladder was washed and let to sediment in conic flasks for helminths isolation. A fecal pellet was also collected when possible for copromicroscopic examination. All helminths detected were counted, morphologically identified following keys in literature and stored in ethanol. Molecular identification was performed by amplification and sequencing of the 28S gene in selected specimens. Consensus sequences were assembled and compared with data available in GenBank.

Overall, 40 turtles were positive for at least one helminth taxon in the GIT. Taxa recovered included trematodes of the genus *Telorchis* sp. (Digenea: Telorchiidae) (28/62, P=45.16% [95%CI 32.77-57.55%]), nematodes of the species *Serpinema microcephalus* (Nematoda: Camallanidae) (13/62, P=20.97% [10.83-31.10%]), and monogeneans of the family Polystomatidae, the only taxon recovered exclusively at gastric level (4/62, P=6.45% [95%CI 0.34%, 12.57%]). Monogeneans were also recovered in the urinary bladder of nine turtles (9/62, P=14.52% [95%CI 5.75-23.28%]) and were morphologically identified as *Neopolystoma* sp. Neither cardiovascular flukes nor lesions related were found. Granulomatous pancreatitis was detected in 4 animals, three of them hosting *Serpinema microcephalus* in the gut.

BLAST search identified the 28S sequences from a specimen of *Telorchis corti* from Mexico as the closest relative to the sequences of *Telorchis* sp. from the current study; similarly, sequences from *Neopolystoma* sp. from the urinary bladder had the highest identity with those of *Neopolystoma orbiculare* isolated in the USA from a *Chrysemis picta*. Molecular characterization of nematodes of the genus *Serpinema* supported the inclusion in this genus, the sequences showing the highest identity with *Serpinema* sp. from the USA and excluding their identification as the Nearctic species *Serpinema trispinosum* (identity 94.4%). Morphological identification as *Serpinema microcephalus*, the Palearctic species, is therefore considered furtherly supported, being sequences of this species absent in the database. Copromicroscopic examination revealed oocysts of *Eimeria* spp. and eggs of *Telorchis* sp. in 10 and 6 out of 49 fecal samples retrieved, respectively. Additionally, eggs of *Capillaria* spp. and *Physaloptera* sp. were found in two and one turtle respectively.

The data here reported offer the first evidence of the presence of alien parasites in an Italian population of *T. scripta*. A wide survey on the diversity of Polystomatidae occurring in freshwater turtles in Southern Europe already reported *N. orbiculare* from *C. picta*, *T. scripta* and *E. orbicularis* in France and Spain. A wide spread of polystomes of Nearctic origin in exotic and autochthonous turtles in Europe is confirmed by the present findings. Other exotic species of the genus *Telorchis* have been reported in red eared slider in Spain, nevertheless, this represents the first detection in the continent of the species *T. corti*, the most widely distributed species of its genus in America. Finally, spill-back of *Serpinema microcephalus* to *T. scripta* is described in Spain and a correspondent phenomenon is reported in this study, presenting associated pathology, coherently with previous reports. The data here presented give a first interesting insight into the parasitofauna of allochthonous turtles in Italy and shed light on the presence of exotic parasites in Italian freshwater ecosystems. Parasitological data from the native species *E. orbicularis* would be essential to reveal spillover events in the area and quantify indirect effects mediated by parasitic diseases between the two emydid turtles.

SECOND OUTBREAK OF SARCOPTIC MANGE IN IBEX POPULATION OF THE FRIULAN DOLOMITES REGIONAL NATURE PARK

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Introduction

The ibex (*Capra ibex*) colony in the Friulian Dolomites Nature Park was established between 1985 and 1987, with the introduction of 26 individuals from the remaining population of the Gran Paradiso National Park. To increase both the number of animals and genetic diversity, from 2002 to 2007, a total of 30 ibex was released, comprising 16 females and 14 males from the Maritime Alps Nature Park, three different Parks in Switzerland and also from the Gran Paradiso National Park.

Initially, the colony grew steadily, but since 2011, a gradual population decline has been observed due to the emergence of sarcoptic mange. The parasite-host co-evolutionary relationship creates a cyclical pattern of epidemic outbreaks, though with a reduced incidence rate. This study seeks to evaluate the current population size and examine the trends of the sarcoptic mange epidemic within the ibex colony in the Friulian Dolomites Nature Park.

Materials and Methods

The study started with a retrospective review of data from annual censuses conducted between 2012 and 2023. Following this, a longitudinal survey took place from February to October 2023 in the Salta-Borgà area, situated in the southwestern section of the Park. This involved monthly monitoring of ibex along a predefined route, which consisted of direct observation with the support of optical instruments. Observations were carried out daily over multiple consecutive days, with intervals of one month between sessions. Each session included photographic documentation, which was later used to evaluate the animals' health, categorizing them into four levels of mange severity based on visible skin conditions. During the survey, environmental and carcass samples showing mange-associated lesions were collected. These samples underwent laboratory testing to identify *Sarcoptes scabiei*, using a sodium hydroxide maceration process followed by a flotation method.

Results and Discussions

The findings confirmed a second outbreak of sarcoptic mange, which began in 2019 in the Salta-Borgà region. Although this outbreak caused a less pronounced demographic decline compared to the initial one, it still negatively impacted the population trends. Laboratory tests detected *S. scabiei* in both carcasses and environmental samples, indicating possible transmission through indirect sources. Despite the presence of milder skin lesions, this second wave of sarcoptic mange spread across the entire ibex population in the Park. Given the population’s characteristics (relatively young, genetically limited, and geographically isolated), it is expected that future cyclical outbreaks with low incidence will occur due to the disease’s natural progression. As a result, the study recommends boosting genetic diversity by reintroducing individuals from colonies with long-term exposure to sarcoptic mange, as these ibexes may show a more adaptive coevolutionary response to the parasite.

MYCOBACTERIUM AVIUM SUBSP. *AVIUM* IN A RED DEER (*CERVUS ELAPHUS*) POPULATION OF EASTERN ITALIAN ALPS

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Introduction

Since 2018, red deer (*Cervus elaphus*) populations of Eastern Italian Alps (Friuli-Venezia Giulia Region) have been monitored according to the local health surveillance plan, which included, among other targeted disease, mycobacterial infections, primarily tuberculosis and paratuberculosis. This Region is officially free from bovine tuberculosis since 2006 and paratuberculosis has been only sporadically diagnosed in cattle, whereas no cases of avian tuberculosis were detected in domestic or wild birds during the last decade.

Material and Methods

Four red deer (2 male fawns, 1 adult female and 1 adult male) were found emaciated and legally euthanized, except for the adult male that was found dead. We carried out complete necropsies on these subjects and further bacteriological (including the search of *Yersinia* spp.) and histopathological investigations were added according to the macroscopic lesions. We investigated mycobacteria using cultural methods and real time PCR analysis. The latter included *Mycobacterium tuberculosis* complex (MTC) and *Mycobacterium avium* complex (MAC) PCR from consistent lesions and a specific real time PCR for *Mycobacterium avium* subsp. *paratuberculosis* (MAP) from ileocecocolic junction and lymph nodes.

Results and Discussion

All the subjects showed severe weight loss and different patterns of distribution of gross necrotic-purulent-granulomatous lesions consistent with a mycobacterial infection. Both young males and the female had generalized visceral granulomas referable to a systemic form of mycobacteriosis. Whereas, in the adult male granulomatous lesions were restricted to the intestinal tract and mesenteric lymph nodes, therefore resembling paratuberculosis. Histologically, all the granulomas consisted of epithelioid macrophages and multinucleate giant cells were present along with the evidence of plenty of intracellular acid-fast bacilli at Ziehl-Neelsen stain. Bacteriological cultural analyses were negative but *Staphylococcus aureus* that was isolated from a lymph node of the adult female. All the individuals presented concurrent parasite infections: ectoparasites *Lipoptena cervi* and *Ixodes ricinus* and bronchopulmonary and gastrointestinal strongyles.

The search for MTC and MAP was negative in all the subjects. However, MAC was detected in both the adult deer and identified as *Mycobacterium avium* subsp. *avium* (MAA).

In the bordering areas of Slovenia, pulmonary lesions by *Mycobacterium microti* in red deer and asymptomatic infection by MAA in alpine ibex (*Capra ibex*) were previously detected. Moreover, the municipalities of origin of these infected deer border with Austrian Carinthia's region where disease caused by MAA was also reported in red deer populations. These deer or a shared contaminated environment may therefore represent possible sources of infection for our cases. Crowding at feeding sites may provide conditions for the maintenance of infection. It should be taken into account that active surveys in European countries demonstrated the occurrence of asymptomatic infections; furthermore, clinical symptoms can be generally observed in generalized MAA infected animals that may shed mycobacteria in the environment.

Wildlife regional surveillance system highlighted the presence of mycobacterial systemic infections by MAA in an alpine red deer population of Eastern Italian Alps. Forestry workers and hunters were updated about these results during formative activities in order to enhance the detection of eventual further cases and to keep them aware of the zoonotic, although not frequent, issue of MAA infection.

AFRICAN SWINE FEVER AND THE ROLE OF WOLVES AS SPREADER OR REDUCER: A PRELIMINARY INVESTIGATION

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Introduction

African Swine Fever (ASF) is a highly contagious infectious disease affecting wild and domestic pigs worldwide. ASF virus (ASFV) may remain infectious in wild boar carcasses for several months. For these reasons, scavenging of carcasses and predation of ASFV-infected wild boars by carnivores have deemed potential drivers for either ASFV spread or reduction in the environment. ASFV spread would occur through the release of contaminated material, such as carcass residues and/or infected feces into the environment, while reduction might occur through carnivore removal of infected material and its digestion, with consequent virus inactivation. The Italian wolf (*Canis lupus italicus*), which was nearly extirpated from Italy, has successfully recolonized a large part of its former range, including the Northwest Italy, becoming the principal wild boar predator. Wolves might be the most important players, among carnivores, in reducing or increasing the secondary ASFV spread; however, there is still no conclusive evidence defining the role of wolves. The aim of the present study was to assess whether wolves may act as either ASFV spreaders or reducers in a currently ASFV-endemic area in Northwest Italy.

Material and Methods

From March 2023 to February 2024, wolves found dead in ASF-infected areas in Northwest Italy and their surroundings (Alessandria province, Piedmont) were collected as part of the sanitary passive surveillance for infectious diseases provided by the Piedmont Region in collaboration with the Istituto Zooprofilattico Sperimentale Piemonte, Liguria, Valle d'Aosta. For each wolf, we determined sex, age, cause of death, and collected three samples: i) gastric content, ii) feces, and iii) footpad swabs. The gastric content was visually inspected to determine the prey species consumed. All collected samples were tested for ASFV by real-time PCR, and in case of positive results samples were shipped to the ASFV National Reference Laboratory (NRL) for confirmation and virus isolation in primary cell cultures and Sanger sequencing attempts. Using official data from Italian ASF surveillance, we estimated the ASF prevalence in the wild boar population considering only wild boar found dead, within a buffer area of 6 km from the collection area of dead wolves as a proxy of its home range.

Results and Discussion

Sixteen wolves (11 males, 5 females) in good conservation status were examined. The stomach of examined wolves resulted empty in 9 wolves (68%), while in 7 wolves presented remains of livestock (18%) or wild ruminants (12%). All interdigital pads tested negative for ASFV. One out of 16 gastric contents from a young female in good body condition resulted ASFV positive. This gastric content was macroscopically identified as wild boar remains (muscle, skin, hair). Given the high ct values obtained (34) by Real-Time PCR, Sanger sequencing was inconclusive, and virus isolation was negative following three blind passages. All feces collected from wolves tested ASFV negative. This result may support the hypothesis that wolf digestion processes are capable of deactivating ASFV, suggesting that wolves might act primarily as ASFV reducers. For six out of the sixteen wolves, ASFV prevalence in their home ranges was higher than 40%, including the wolf with the ASFV-positive gastric content, indicating that infected wild boars were present in their home range. Overall, our preliminary results may suggest a potential role of wolves in wild boar ASFV infected areas, being the principal predators. However, whether wolves may act as ASFV mechanical reducers or spreaders need further field investigations. In the context of the debate on the impact of wolves, data presented herein highlight the ecological significance of wolves in the wild board transmission cycle. This study was supported by the projects "Ricerca corrente" (grant numbers: IZSPLV RC 05/22, 09/24 CUP J19I22001140001) funded by the Italian Ministry of Health.

IS A WILD BOAR MEAT SUPPLY CHAIN POSSIBLE IN THE CONTEXT OF THE AFRICAN SWINE FEVER EMERGENCY IN THE PIEDMONT REGION?

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The first case of African Swine Fever (ASF) in Italy was confirmed on January 7, 2022, in a wild boar carcass found in the municipality of Ovada, Piedmont. This event highlighted weaknesses in wild boar management policies at both local and national levels. For several years, there had been discussions about the need for new regulations to govern hunting activities for this species, supported by technical guidelines that were often left unimplemented, partly due to the corporate attitudes of hunting associations. Wildlife control efforts, which have increased significantly in recent times, need further incentivization, along with an increase in wild boar culling.

With the rising number of animals to be harvested in the coming years, the opportunity to develop a supply chain for game meat is becoming clear. Establishing a professional market for wild boar meat is expected to have at least three positive outcomes. First, it will help to reduce the number of animals and control the spread of ASF. Second, it will generate a new source of income for professional hunters and, eventually, funds for compensation measures related to the damages caused by wild boar overpopulation. Lastly, including hunters in the food supply chain and ASF control efforts are expected to increase their involvement and accountability within their local communities.

Despite the potential for developing local supply chains for wild boar meat, the literature shows that hunters often do not perceive themselves as food producers, and various regulations at the European and national levels hinder this development, especially in areas at high risk for ASF. Given the opportunities and risks of developing a wild boar meat supply chain, this research aimed to estimate the economic value of the product in Piedmont during the 2022/2023 hunting season and to identify local regulations that could support the creation of a professional supply chain, especially for animals culled in ASF-restricted areas.

Assuming that 30% of the hunted animals will enter the market, with an average net carcass weight of 23.0 kg, it was estimated that 8.0 kg of meat per head could be used for low-temperature cooking (reaching a core temperature of 60°C for at least 4 hours), 10.0 kg for hamburger preparation (cooked at 68°C for 35 minutes and grilled at 300°C), and 5.0 kg for ragout or stews. Applying average retail prices of € 25.0/kg for low-temperature cooking cuts, € 20.0/kg for hamburgers, and € 14.0/kg for ragout and stew cuts, the gross economic value was calculated to exceed € 4.4 million annually. Based on these estimates, the value of wild boar hunted in ASF-restricted areas from early 2022 to October 2023 would be approximately € 0.4 million per year at the slaughterhouse and € 2.9 million per year in restaurants.

However, several legal constraints must be considered for the commercialization of wild boar meat. According to European regulations, game meat products must pass through an approved Game Handling Establishment (GHE). In ASF-risk areas, the GHE must comply with Reg. (EU) 687/2020 for industrial thermal treatment, meaning that wild boar meat will be sold to large-scale retail distributors fetching low prices, ranging from € 0.5 to € 2.5/kg. On the other hand, European regulations allow hunters in ASF-restricted areas to keep their animals for personal consumption, with no further traceability requirements or ASF risk prevention measures.

Given this legal framework, which restricts the development of a professional market but permits hunters to consume potentially "risky meat," some opportunities have been identified in the Piedmont Region's DGR 4805/2022 and DD 1759/2022 regulations. These regional laws state that, in specific emergency situations (e.g., ASF), where culling large numbers of animals is necessary and there is no zoonotic risk, exceptions can be made regarding the definition of "small quantity commercialization" and the destination of harvested game meat. In such cases, wild boar meat could enter the market through registered structures without the need for further EU certification, as demonstrated in the Province of Verbania (Piedmont) under the "Filiera Eco-Alimentare" project. This could facilitate the development of local supply chains, providing income and encouraging hunters to sell part of their meat within a professional market. Small or medium-sized batches of animals would not require equipment for industrial-level thermal processing, but rather simple, dedicated professional ovens, making it economically feasible for local retail or dining businesses.

Based on this analysis, the creation of a wild boar meat supply chain is both feasible and desirable during the ASF crisis. Commercializing game meat in a professional market would ensure better product traceability, reduce the wild boar population in ASF-restricted areas, generate local income, and promote hunters' role as bioregulators in the eyes of consumers.

MOLECULAR CHARACTERIZATION OF FIVE SARCOCYSTIS SPECIES IN RED DEER HUNTED FOR HUMAN CONSUMPTION IN ITALY

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Introduction

Sarcocystis species are protozoan parasites with a two-hosts life cycle affecting mammals, birds and reptiles. Among the over 220 species recognized, at least three species can use humans as definitive hosts, that is *Sarcocystis hominis* and *Sarcocystis heydorni*, which can affect humans through the consumption of raw or undercooked beef, and *Sarcocystis suihominis*, which can affect humans through the consumption of raw or undercooked pork. Together with the zoonotic potential of some *Sarcocystis* spp., a growing attention has been paid to this genus of parasites due to their supposed association with macroscopic pathological alterations leading to carcass condemnation and economic losses for the meat sector; among these gross lesions, eosinophilic myositis, a myopathy characterized by the presence of green-grey focal or diffuse patches on muscle tissue, is the most frequently detected, and has been reported in association with some *Sarcocystis* spp. in cattle and red deer. Furthermore, a *Sarcocystis*-induced food poisoning event associated with the consumption of venison has been recently reported. In this context, to the best of the authors knowledge, the identification of *Sarcocystis* spp. in red deer in Italy has never been performed. Therefore, the aim of the present study is to investigate and molecularly characterize the presence of *Sarcocystis* spp. in red deer meat intended for human consumption.

Materials and Methods

Between October and December 2023, 75 red deer (*Cervus elaphus*) were culled in three Alpine Hunting Districts (Comprensori Alpini di Caccia – CAC): VCO2 Ossola Nord (n=23), VCO3 Ossola Sud (n=23) and Bergamo (n=29). A portion of the semimembranosus muscle, intended to be consumed raw (meat tartare or carpaccio), was individually collected from each red deer, frozen at -20°C and sent to the Department of Veterinary Sciences of the University of Turin. Each sample was checked for the presence of grossly visible alterations possibly associated with *Sarcocystis* spp. and processed by microscopic examination in order to detect and collect individual sarcocysts. Each sample was categorized based on the number of sarcocysts observed in each plate (low: 1–2 cysts; moderate: 3–5 cysts; abundant: ≥ 6 cysts). Cyst or cyst-portions were submitted to DNA extraction and molecularly characterized amplifying and sequencing the *cox1* mtDNA gene using the primer pair SF1-SR9. A chi-squared test was performed to compare the occurrence of *Sarcocystis* spp. according to hunting areas, sex and age class. Values of p <0.05 were considered significant.

Results and Discussion

Out of 75 red deer muscle samples, 45 revealed the presence of at least one *Sarcocystis* spp. (45/75, 60%, C.I.95%: 48.7%-70.3%). Among the positive red deer, 71.1% showed a low *Sarcocystis* spp. infection rate (32/45), 20% showed a moderate *Sarcocystis* spp. infection rate (9/45) and 8.9% revealed a high *Sarcocystis* spp. infection rate (4/45). All the cysts observed were microscopic, fusiform and showed smooth thin cyst wall or a thin wall with hair-like protrusions. None of the collected red deer muscle samples revealed the presence of grossly visible alterations such as eosinophilic myositis or macroscopic cysts. The sequencing of the *cox1* mtDNA gene gave good sequencing results for 26 cysts, generating 418-1003 bp sequences. Based on the *cox1* mtDNA gene, up to five different *Sarcocystis* species were detected in the collected muscle samples, which were identified as *Sarcocystis hjorti* (n=12), *Sarcocystis iberica* (n=7), *Sarcocystis linearis* (n= 4), *Sarcocystis venatoria* (n=2) and *Sarcocystis truncata* (n=1). No statistically significant differences in the occurrence of the infection according to hunting areas, sex and age class of the sampled red deer were found.

To the best of the authors knowledge, this is the first study identifying *Sarcocystis* spp. in red deer meat in Italy; although none of the detected *Sarcocystis* spp. is zoonotic, *S. truncata* has been previously associated with food poisoning events, and *S. hjorti*, *S. venatoria*, *S. iberica* and *S. linearis* have been associated with eosinophilic myositis alterations leading to carcass discard. As the popularity of locally hunted wild game meat is growing, thanks to its nutritional, ethical and environmental positive attributes, the risk for the consumer of raw or undercooked meat cannot be excluded.

PARASITOLOGICAL EVALUATION IN MESOLA DEER (*CERVUS ELAPHUS ITALICUS*): FOCUS ON *ELAPHOSTRONGYLUS CERVI*

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Introduction

The Italian deer (*Cervus elaphus italicus*), also known as the Mesola deer, represents the only population of deer truly native to the Italian peninsula, an entity of high biological significance, morphologically and genetically different from other populations of red deer. In order to conserve this taxon, according to the indications of the 2010 Mesola Deer National Conservation Program and the 2021 ISPRA feasibility study, a reintroduction was carried out in the Serre Regional Natural Park (VV) in Calabria. Among the endoparasites occurring in cervids, gastrointestinal nematodes are the most frequent and seldom have a significant impact on animal health, only if present in high numbers. Other important parasites in ruminants are the bronchopulmonary strongyles that can be divided into two large categories: the large strongyles (*Dictyocaulidae*) and the small lungworms (*Protostrongylidae*). Among the *Protostrongylidae* family, the genera *Elaphostrongylus* and *Parelaphostrongylus* include parasites adapted to the central nervous system and the muscles of cervids and occasionally of small ruminants. *Elaphostrongylus* spp. mainly parasitizes European ruminants of the Cervidae family. The infection with this parasite can cause three forms of disease: a neurological form -with spinal ataxia, asymmetric paraparesis, tetraparesis, blindness, circular movements, head tilt and mental confusion-, a form with pneumonia due to the migration of the larvae, and a chronic form. Cervid species that coevolved with the parasite rarely show clinical signs, but non-native cervid species and domestic small ruminants that graze in the same area as red deer can develop serious neurological diseases. The main aim of this study is to carry out a first overall evaluation of the endoparasites of the Mesola deer (*Cervus elaphus italicus*) as highlighted by the objectives of the Mesola Deer National Conservation Program, such as internal parasites responsible for gastro-intestinal and pulmonary forms, in particular *Elaphostrongylus cervi* and *Dictyocaulus sp*.

Materials and Methods

The Gran Bosco della Mesola, on the southern edge of the Po Delta, in the province of Ferrara, has a total surface area of approximately 1058 hectares, where, according to data of the 2021 ISPRA feasibility study, there are about 300 deer. Founding individuals were captured using dart guns in free-ranging. During the first captures for the reintroduction project, on 24-26th February 2023, it was possible to obtain faecal samples, blood, blood smears and ectoparasites. The faeces used for this study were collected from the rectal ampoule directly with a glove until reaching 50 grams of matrix, then placed in cellophane bags and kept at refrigeration temperature until analysis at the University of Bologna. During the captures it was possible to collect fecal samples from 18 subjects out of a total of 20 that were subsequently translocated, 5 males and 13 females respectively. For two subjects it was not possible to carry out the sampling due to a total lack of faecal material inside the rectal ampulla. For the analysis, different solutions for the flotation technique were used, in order to highlight not only nematode and tapeworm eggs that float in solutions with a specific gravity between 1,200 – 1,300, but also trematode eggs that require solutions with specific weight of 1,400 because they are heavier. The Baerman apparatus was used to search for bronchopulmonary nematode larvae.

Results and Discussion

During the inspection before the captures, on 28th April 2022, it was possible to observe a male individual in the central area with neurological symptoms: head tilt, apathy and uncertain gait with slight ataxia of the hindquarters. An interview with the forestry workers, who take care of the animals daily, highlighted that they already observed some other individuals with a rotated head and other neurological symptoms. During the capture in February 2023, it was possible to observe another male, frequenting the same area as the individual of April 2022, with his head tilt and an uncertain gait. The copromicroscopic examination of the feces with Baermann technique highlighted L1 larvae with dorsal spine attributable to the *Elaphostrongylus* genus with a prevalence of 61.1% out of the 18 animals examined. Furthermore, larvae of small and large bronchopulmonary strongyles were highlighted with a prevalence of 72.2%. No trematode eggs were observed, while 64.7% of the examined samples had gastrointestinal strongyles; the number of eggs per gram of faeces was within the norm for healthy free-living ruminants. Only one animal was positive for *Trichuris* sp. In two deer who died in Calabria after translocation, a histological examination at the Istituto Zooprofilattico Sperimentale del Mezzogiorno - U.O.C. Patologia Generale U.O.S. Istopatologia, Portici (NA) showed lesions and sections of adult nematode compatible with *E. cervi* and with the clinical signs observed in two animals in Mesola. Health factors can impact both the source population in Mesola and the reintroduced nucleus in Serre Nature Reserve (VV). For this reason, it would be essential that all the parasitological results obtained from the samples of live animals during captures, from any subsequent deaths and other non-invasive/opportunistic samples are carefully considered as an integral part of the process aimed at the conservation of the only deer subspecies native of the Peninsula Italian: *Cervus elaphus italicus*.

TOXICITY OF LEAD AMMUNITIONS ON BOTH WILDLIFE AND GAME MEAT. NOSTALGIA FOR LEAD HUNTING DESPITE EVIDENCE SINCE 1786?

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Introduction

The Museum of Natural History in Brescia and the Po Delta Regional Park of Emilia-Romagna recently proposed again the meritorious exhibition on "hunting lead" by the Civic Museum of Natural Sciences in Bergamo (2023), events with two interesting conferences (Bergamo, 2023; Comacchio, 2024). The meetings were an opportunity for new contributions and interventions, but also for second thoughts by representatives of hunters on choices to ban lead bullets, in a time frame influenced by politicians who support the impatience of a part of hunters and by infodemics about the toxicity of hunting lead. Owing to the above, ten years after my retirement I felt the need for a new assessment, at least to update my expert judgement.

Materials and methods

I reconsidered references I privileged since the 90s, relating to the impact of lead ammunitions from the aspects of conservation on scavengers and waterfowl, and then those focusing on game meat safety during my commitment as Official Veterinarian at the AUSL of Modena, and as a member of the SIEF (in support of the Emilia-Romagna Region and its specific public campaigns, in 2011-2012). To the review I added works recently co-published with colleagues (2017, 2022, 2024) on risk assessment for contaminants related to qualitative-quantitative habits of game meat consumption among Italian hunters, on the presence of lead pellets in gizzards of flamingos from a massive mortality and on gizzards from hunted ducks, both cases related to the same area and period. Then I searched for historical references on lead pellets ingested by ducks and on the dispersion of lead pellets in the depths of lagoons around Italian hunting lodges for waterfowl, and finally I searched also for news useful for fact checking initiatives, highly desirable for countering incorrect and/or improper information among hunters and political representatives and to give priority to information toward citizens and consumers.

Results and discussion

Lead poisoning has been known since ancient Greece, but it was Benjamin Franklin (1789) who accounted the human poisoning in the lead inadvertently taken in some professions and in the intake of beverages that have been in contact with this metal. Paolo Savi (1831) instead gave the first explicit account of some massive duck mortalities during 1786-1829 that he and other naturalists and local hunters attributed to the lead pellets consumed in their gizzards. Starting from the second half of the twentieth century, began a dense literature on waterfowl poisoned by lead pellets actively ingested as grit, covering North America, Europe, Australia and Italy, and equally early works (including experimental) arrived on the expected mortality rate for waterfowl ingesting lead pellets, and on higher chance of being easily shot for birds weakened by the early stages of poisoning. In addition, date back to the 90s important records on the density of lead shots in sediments around waterfowl Italian hunting lodges, that unfortunately are still without follow-up although European agencies warn on lead dispersal from sport and hunting projectiles and fishing lines, with an assumed accumulation of 876,000 tons of lead in the next years if no further restrictions are introduced on top of the current bans on lead hunting in wetlands. And above all, is now strongly documented the risk of assuming Pb by consuming game meat contaminated by bullet fragments and this is only partially countered by a growing number of hunters, also in Italy, voluntarily adhering to the use of "lead-free bullets" and by regulations anyway not enough to have large effects on safety in the management of food risk, of wildlife and environment, and unfortunately all this recently under a social context that sees the return of political pressure to relax the bans on lead hunting. But how can we forget that this rethinking overlooks the fact that the movement against hunting lead was started and actively promoted by hunters in the 70s, in North America, anticipating the first regulations? A lack of or weak information on the toxicity of lead loosens awareness among politicians, citizens and consumers while on the other hand the toxicity inherent in the overall cycle of "hunting lead", constitutes a serious problem from the point of view of the One Health vision, to be supported with maximum energy, without hesitation, now more than ever.

POSTER

10 YEARS OF CANINE DISTEMPER IN THE ABRUZZO AND MOLISE REGION

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Introduction

Canine distemper (CD) is a highly contagious disease characterized by a high morbidity and mortality. CD virus (CDV) exhibits a broad host range globally, with species from the Canidae and Mustelidae families being the most commonly impacted. However, CDV has also been identified in members of the Felidae, Viverridae, Procyonidae, and Ursidae families. It is a multi-host pathogen, a trait that becomes particularly significant within wildlife ecosystems in an area which hosts critically endangered species. Several outbreaks have been reported in the last decades in wild carnivores in Italy mainly in the Alpine regions of Northeastern Italy, particularly the Trentino-Alto-Adige, Veneto, and Friuli-Venezia Giulia regions. In 2013, along the Apennines, predominantly in the Abruzzi region, a widespread distemper outbreak driven by an Arctic-lineage strain occurred. The epidemic resulted in clinical disease in domestic and shepherd dogs, Apennine wolves (*Canis lupus*), and various other wild carnivores. In subsequent years, a CDV strain from the European-wildlife lineage was also detected in a fox found deceased in the same region, indicating the co-circulation of another CDV lineage within the area. Since 2010, as part of the wildlife monitoring program for the Abruzzo and Molise regions, all wild carnivores found dead and systematically submitted to the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" in Teramo (IZSTeramo) undergo anatomo-pathological, histological, and virological examinations to screen for the presence of CDV.

Given the potential impact of CDV on wildlife population dynamics, particularly among the critically endangered small population of brown bears (*Ursus arctos marsicanus*), we focused on analyzing the results of 10 years (2013-2023) of CVD monitoring in wild carnivores in Abruzzo and Molise regions.

Material and Methods

For each carcass of wild carnivores delivered to IZS-Teramo the following information are collected and stored in a specific section of the Laboratory Information Management System: species, sex, age, site of collection by coordinates, the suspected cause of death, and the sampling context. All submitted carcasses undergo a full pathological examination, and organs displaying lesions are subjected to histopathological analysis. A lung fragment is collected from all wild carnivores and tested by real time RT-PCR specific for CDV, even in the absence of macroscopically visible lesions. In cases of suspicious lesions consistent with CDV, all affected organs are sampled for further molecular analysis. A total number of 83 CDV positive samples were selected for metagenomic shotgun (mSG) approach. Total RNA purified from samples were used for the assessment of SISPA protocol. Library was prepared by Illumina DNA prep kit following the manufacturer's instructions and deep sequenced on the NextSeq1000 by the NextSeq 1000/2000 cartridge (300 cycles) and standard 150 bp paired-end reads. Illumina reads were analyzed by metagenomic tool of CZ ID platform (https://czid.org/), while CDV consensus sequences were obtained by a bioinformatic pipeline implemented in GENPAT platform of IZSTeramo.

Results and Discussion

Between 2013 and 2023, 137 out of 1360 wild carnivores tested CDV positive by real time RT-PCR. Two picks of cases were reported in 2013 and between 2021 and 2022, while only sporadic reports were recorded in the remaining years. Species involved were foxes (73/541), wolves (42/461), Marsican brown bears (2/46), badgers (18/234), beech marten (1/34) and polecat (1/9). Carcasses of wild carnivores were collected all over the study area, even if 65% of samples were collected in L'Aquila province. CDV positive wolves were mainly located across the Apennine chain while other CDV infected species were also reported in the hilly and coastal areas. Only the 28% of cases presented anatomical histopathological lesions, primary affecting the lung. Complete or nearly complete CDV consensus sequences were obtained from samples with the lowest Ct values (Ct<22) while partial and very partial consensus sequences were produced from samples with Ct range 23-30. Indeed, some sequencing attempts of CDV strains were unsuccessful likely due to the degradation of the samples and to the low viral titer evidenced by the real time RT-PCR. Molecular characterization and phylogenetic analysis are ongoing. Carcasses of wild animals obtained from passive surveillance are not always suitable for an accurate necropsy and histopathological examination because of the advanced autolytic phenomena. This aspect, along with the sensitivity of the test, able to detect animals even at the early or late stage of infection during the recovery phase, might justify the low percentage of clinical cases recorded upon real time RT-PCR positivity. Monitoring programs based on convenience sampling are widely known to be affected by selection bias, however as basic population parameters are unknown on regional scale, the implementation of conventional surveillance techniques is difficult. In any case, opportunistic surveillance can detect wildlife health events as CDV outbreaks as reported by our study and it is valuable activity to monitor the presence of a disease over time in a given area.

FIRST INSIGHTS ON ANTIMICROBIAL-RESISTANT *ESCHERICHIA COLI* IN WILD UNGULATES AND CARNIVORES FROM NORTH-WESTERN ITALY

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Introduction

The complex dynamics leading to the emergence and spread of antimicrobial resistance (AMR) require a One Health approach that integrates surveillance not only in humans and livestock, but also in the environment. Among the potential hosts of bacteria carrying AMR genes, wildlife is an excellent model for environmental surveillance because it should consist of antimicrobial-free species. However, it has been shown that free-ranging populations can act as reservoirs for these micro-organisms and contribute to the spread and/or maintenance of AMR. In particular, wild ungulates are of great interest because their population densities are increasing rapidly and the continuing expansion of their range is extending in close proximity to anthropised areas with farms and pastures. Other wild species, such as small carnivores (e.g., red fox), have adapted to human expansion and now live in urban areas in close contact with anthropogenic risk factors (e.g., the presence of human domestic waste). All these interactions may lead to health risks across the wildlife-domestic animal-human interface, such as the spread of antimicrobial resistant *Escherichia coli*. Here we present preliminary data from the first year of a wider research project (GR-2021-12374932), aimed at investigating the prevalence of extended-spectrum β-lactamases (ESBLs), other β-lactamases (AmpCs), carbapenemase-producing and *mcr*-carrying *E. coli* in wildlife from North-western Italy.

Material and Methods

A total of 424 faecal samples (wild ruminants=191, wild boar=87, canids=116, mustelids=30) were collected in 2023 and 2024 from three Italian administrative regions: Liguria (N=106), Piedmont (N=96) and Aosta Valley (N=222). The samples were analysed using phenotypical and molecular tests to assess the spread of AMR strains. In particular, for the pre-enrichment phase, 1 g of faeces was diluted in 9 mL (1:10 dilution) of buffered peptone water. After incubation, a drop of broth was cultured on both MacConkey agar supplemented with 1 mg/L cefotaxime and Chromid® Carba Smart agar. Positive growths were identified as *E. coli* and single bacterial colony DNA was extracted and screened for ESBL/AmpC ($bla_{CTX\text{-}M}$, bla_{TEM} and bla_{SHV}, bla_{CMY}), carbapenemase ($bla_{OXA\text{-}48}$, $bla_{GES\text{-}5}$, bla_{NDM}, bla_{KPC}, bla_{VIM}, bla_{IMP}) and mobile colistin resistance (*mcr* 1-10) genes.

Results and Discussion

The overall phenotypic prevalence of ESBL/AmpC *E. coli* was 3.3% (14/424). In particular, positive samples were only found in wild ruminants (red deer *Cervus elaphus*) from Aosta Valley (9.7%, 11/113) and in three wild boars, one from each of the three regions: Liguria (2.4%, 1/42), Piedmont (5.3%, 1/19) and Aosta Valley (3.8%, 1/26). No ESBL/AmpC *E. coli* was detected in canids and mustelids from any of the regions considered.

Concerning ESBL/AmpC molecular investigations, $bla_{CTX\text{-}M}$ was the most common gene (28.5%, 4/14), followed by bla_{TEM} (14%, 2/14) and bla_{SHV} (14%, 2/14). Bla_{CMY} was not present in any of the samples analysed. No sample tested positive for carbapenemase-producing *E. coli*. No *mcr* genes were found in any of the samples tested.

These data indicate a generally low prevalence of ESBL/AmpC *E. coli* in both wild ruminants and wild boar, and the absence of these bacteria in canids and mustelids in the study areas. As wild species are generally not treated with antimicrobials and may acquire AMR bacteria or genes from the environment through food and water contaminated by domestic animals or humans, this finding seems reassuring. They suggest a low level of environmental spread of these AMR bacteria within the investigated regions, with the exception of wild ruminants in the Aosta Valley, where we found a 10% prevalence. Our results were not always in agreement with previous Italian studies on wildlife, especially for wild boar. For instance, a previous study in Lombardy reported a prevalence almost five times higher for this species. Nevertheless, comparisons with other studies should be made with caution, considering the differences in analytical methods (bacteriological and molecular) and in the areas studied (anthropisation, livestock density, etc.).

The results of this study should be considered as preliminary, as the number of analysed specimens per species in each region will be increased and a more refined environmental analysis (level of anthropisation, density of farms, pastures, etc.) should be carried out to properly assess the level of AMR environmental contamination. While the absence of resistance to life-saving antimicrobials, such as carbapenems and colistin, is encouraging, AMR requires continuous surveillance to early detect the spread of these resistances in the environment and to support policy against AMR.

AVIAN TUBERCULOSIS IN A NORTHERN BALD IBIS: A CASE REPORT

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Introduction

Avian tuberculosis is a chronic debilitating infectious disease affecting all bird species, both wild and domestic. Pathology is generally caused by *Mycobacterium avium* subsp. *avium*, but other mycobacteria species can be involved, including *Mycobacterium intracellulare* as part of the *Mycobacterium avium* complex (MAC). These bacteria can affect not only birds but also many other animal species including pets, farmed animals and humans, normally with asymptomatic infections; although cases of full-blown disease have been reported, especially in predisposed subjects. Common clinical findings in birds are progressive weight loss, impairment of body functions and lethargy (chronic wasting syndrome), often leading to death.

The Northern bald ibis (*Geronticus eremita*), once widespread in several regions of Europe, Africa and Middle East, is a species with a global population currently less than 1,000 wild individuals, listed in the IUCN Red List of Threatened Species as regionally extinct (ER) in Europe and endangered (EN) globally. From 2014 to 2019, the European fund LIFE supported the first Northern bald ibis reintroduction project in Europe (LIFE+12-BIO_AT_000143), and in 2022 a second LIFE project (LIFE20 NAT/AT/000049), implemented by 10 partners in four countries, including Italy, with the aim of establishing a self-sustaining European population of Northern bald ibis. Given the very small numbers of these birds, each individual is of critical importance to the successful survival of the species in the wild.

We report a case of avian tuberculosis diagnosed in a Northern bald ibis found death in the province of Cremona, during wintering period.

Case presentation

On January 28, 2024, Cremona Provincial Police recovered a female hermit ibis carcass, found dead in the countryside of Cremona province. The animal was ringed with an immovable metal ring on its right lower limb bearing the inscription "waldrapp.eu 471" and a GPS transmitter. Thanks to these elements, it was possible to identify the animal as the subject named "Marianne," ringed and managed by the LIFE project. The animal was then delivered to the IZSLER Official Laboratories in Cremona to determine the cause of death.

The northern bald ibis named "Marianne" was a female born in the zoological park of Rosegg, Austria, on April 5th 2022. The ibis was left from Germany (Bavaria) in September 2023 for overwinter in North of Italy, in Cremona province. GPS reported bird's death on January 26th 2024.

The carcass showed a lack of the head and part of the neck. Traumatic lesions referable to predation were noted: skin laceration at the right wing; skin perforations at the pectoral area deepening into the underlying musculature resulting in hemorrhagic spreading. No hunting pellets were found. Furthermore, gross lesions referable to avian tuberculosis were observed: numerous nodules of yellowish color and firm consistency ranging in size from barely visible to 2-3 mm in diameter, scattered throughout the liver parenchyma and in the intestinal wall.

Histological examination was carried out on the portion of liver and intestine with macroscopic lesions, showing severe chronic multifocal granulomatous inflammation with centrolesional necrosis, multinucleated cells and perilesional fibrosis in both organs. Direct Ziehl-Neelsen staining showed the presence of acid-resistant bacilli. The liver sample was analyzed for *Mycobacterium* spp. by PCR with positive result, confirmed by positive PCR analyses for *Mycobacterium avium* and negative RT-PCR result for *Mycobacterium tuberculosis* complex (IS6110).

Microbiological research was also performed, but it was not possible to isolate the bacterial strain. These results confirmed the suspicion of avian tuberculosis.

Conclusions and discussion

The localization of tuberculous lesions in the case described here agrees with the oral route of infection, which is the main route of transmission of avian tuberculosis in birds, leading to subsequent dissemination of the *Mycobacterium* through the gastrointestinal tract and into the liver.

Only rare references report the presence of avian tuberculosis in Northern bald ibis, so no inferences can be drawn about the meaning of the presence of this disease in this species. However, the finding of this case highlights an additional factor that may threaten the survival of the Northern bald ibis in the wild, although illegal killing currently remains the main threat.

HEPATITIS E VIRUS SURVEILLANCE IN WILD ANIMALS FROM NORTH-WEST ITALY.

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Introduction

Hepatitis E virus (HEV) represents an emerging threat in industrialized countries, as epidemiological investigations have demonstrated an increase in the prevalence and incidence of HEV in both humans and animals and have identified pigs and wild boars as possible sources of human infection through the consumption of raw or undercooked meat, especially in regions where game meat is widely consumed. Among wild species, wild boar is considered the main reservoir of HEV and plays an important role in the transmission of the virus in Europe, especially in countries where intensive pig farming is widespread, because contact between domestic pigs and sympatric species is facilitated, thus increasing the risk of interspecies transmission. Furthermore, very limited data are currently available on the prevalence of HEV in other game species that could similarly contribute to the spread of the virus. Knowledge of the health status of wild fauna is therefore essential, not only to minimize the direct effect on human health resulting from the consumption of hunted game, but also to study the spread of zoonotic diseases in the territory. In this work, data on the presence and characterization of HEV in wild animals present in North-west Italy during the years 2023 and 2024 are reported.

Material and Methods

During 2023-2024, in the framework of an ongoing research on public health issues related to wildlife (Ricerca Finalizzata GR-2021-12374932), a total of 713 liver samples were collected from hunting and dead wild animals belonging to 8 different species (*Capreolus capreolus* n=212, *Vulpes vulpes* n=131, *Sus scrofa* n=127, *Dama dama* n=82, *Rupicapra rupicapra* n=65; *Cervus elaphus* n=41, *Meles meles* n=28 and *Canis lupus* n=27) in the regions of Piedmont, Liguria and Valle d'Aosta (Italy). For each sample, aliquots of chopped tissue (25 mg) were homogenised in 10% (w/v) sterile phosphate-buffered saline (pH 7.2) by a TissueLyser (QIAGEN, Hilden, Germany) for 2 min and then clarified by centrifugation at 14,000 g for 2 min. Nucleic acids were extracted from 200 µl of the supernatant using an IndiSpin Pathogen Kit (Indical, Bioscience), according to the manufacturer's instructions. RNA was eluted in 100 µL and tested for HEV genome by real-time RT-PCR using primers and probe as previously described (Jothikumar et al., 2006 J Virol Methods 131, 65-71).

Results and Discussion

Overall, PCR-positivity for HEV was found in the liver samples of 9/713 (1.26%) wild animals analyzed. Among the tested species, only the wild boar resulted positive for HEV RNA with a prevalence of 7.1% (9/127, 95% CI: 3.3-13.0). The regions with positivity were Liguria and Piedmont with a prevalence of 12.1% (8/66, 95% CI: 5.5-22.5) and 4.5% (1/22, 95% CI: 0.1-22.8) respectively. More in detail, six positive wild boars were hunted in Genova Province, four of which in the same area, 2 in La Spezia Province and 1 in Cuneo Province. Four animals that tested positive for HEV were female, three were male and two were unidentified, moreover all positive animals were younger than 2 years old. The obtained data showed that the HEV virus is circulating mainly in Liguria, and partly in an area of the Piedmont region that borders the Ligurian territory. Interestingly, the only species implicated in the transmission appears to be the wild boar and there does not appear to be an interspecies transmission. However, further investigations will be conducted by enlarging the sample size and the geographical area. Finally, the presence of HEV in wild boar liver samples implicates a potential zoonotic risk for hunters and consumers when handling and/or consuming raw or undercooked meat and products made from liver from this animal. To minimize the risk of virus transmission, food safety education for consumers and training on correct handling procedures for categories at high risk of exposure to HEV are recommended.

EPIDEMIOLOGY OF SARCOPTIC MANGE IN RED FOX (*VULPES VULPES*) AND GREY WOLF (*CANIS LUPUS*) POPULATIONS OF EMILIA-ROMAGNA REGION

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Introduction

Sarcoptic mange is an epizootic skin disease caused by the mite *Sarcoptes scabiei*, infesting over 100 mammalian hosts worldwide including wild and domestic canids. It is an emerging disease for certain wildlife species, potentially endangering their welfare and conservation.

Sarcoptic mange infestation in wildlife, unlike domestic animals, is characterized by high mortality and greater morbidity of the affected subjects, due to the difficulty of carrying out therapeutic interventions to limit acariasis. Furthermore, co-infections between *S. scabiei* and other ecto-endoparasites are not uncommon in the wild environment.

The aim of this study is to describe the distribution of sarcoptic mange in the Emilia-Romagna region (Northern Italy). Specifically, the results of the research carried out in 2023, with particular reference to grey wolves and red foxes, are reported.

Material and Methods

From January to December 2023, a total of 78 wolves (46 males, 29 females, 3 not reported) and 364 foxes (102 males and 143 females, 119 not reported) of different ages were conferred to the IZSLER laboratories for *post-mortem* examination and surveillance of main wildlife infectious diseases, according to the regional wildlife health surveillance plan. Part of the sampled foxes came from culling plans, others were part of passive surveillance (deceased in rescue centers or found dead). Each carcass was kept at 4°C until examination, that was carried out within 24 h.

In accordance with the WOAH (World Organisation for Animal Health) manual, Sarcoptic mange diagnosis was based on the lesions detected during the anatomopathological examination followed by the demonstration of mites presence in skin scrapings.

Microscopic examination of skin scrapings at 40× magnification, under a dissecting microscope, and at 100× and 400× under an optical microscope, was performed to check the presence of mites and eggs. Collected material was digested in boiling 10% NaOH, centrifuged and microscopically examined (at 100× and 400× magnification) according to the method described by Sréter et al., 2003. The identification of mites was based on morphological characteristics according to Anne S. Baker, 1999.

Results and Discussion

Seven wolves (9.0%) and 19 (5.2%) foxes were mangy. The most frequently identified mite species was *Sarcoptes scabiei* (Linnaeus, 1758). Infested animals were detected in all provinces of the region. The provinces with the highest number of positive specimens were Forlì-Cesena, Modena and Reggio Emilia.

The finding of sarcoptic mange in the grey wolf and red fox populations started at the beginning of January. Most cases of infected animals were reported during winter and early spring while in late spring and fall only one mangy-confirmed animal was recorded. This may be due to greater contact between animals that, in colder periods, remain in their dens for longer.

Our study provided a preliminary overview of the disease within the red fox and wolf population of the Emilia-Romagna Region. However, the results highlighted the wide distribution of this ectoparasite and highlighted the need to acquire more information to assess its possible effect on other wild species and its zoonotic potential. Therefore, surveillance should be maintained and, eventually, more information should be gathered through more extensive surveys by increasing both the number of carcasses analysed and species tested in order to provide a better understanding and characterization of sarcoptic mange in wildlife and to assess the epidemiology of this parasitic disease.

FIFTEEN-YEAR HEALTH MONITORING OF WOLVES IN THE LAZIO REGION: POST-MORTEM FINDINGS AND PRELIMINARY ANALYSIS OF RETROSPECTIVE DATA

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Introduction

The Apennine wolf (*Canis lupus italicus*) has spurred remarkable conservation efforts over the years. After disappearing from several territories in Italy, now its population trend is increasing, due to the progressive human abandonment of agricultural land, the increased prey abundance and the legal protection of the species. Post-mortem surveillance of wolves is crucial not only for conservation, but also for public health. Indeed, wolves can be sentinels and reservoirs of infectious agents that are transmissible at the human-domestic-wildlife interface. We hereby reported the results of the health monitoring conducted on the wolves found dead in the territory of the Lazio region over a fifteen-year period.

Material and Methods

Wolves found dead and notified to the competent local authorities were conferred to the laboratories of the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana (IZSLT) for post-mortem examination, in agreement with the regional authority for the environment of the Latium region. Date and area of finding, sex and morphometric data were recorded over the years. Necropsy was performed to determine the cause of death and assess the health status of the animals. Based on the macroscopic findings and on the degree of decomposition, histopathology, parasitology, microbiology and virology samples were collected. DNA typing was employed for molecular species identification (pure wolf, recent hybrid with the dog, old introgressed wolf). Toxicological analyses were performed on samples of liver and gastric content in case the anamnesis or lesions were consistent with poisoning. When suitable, samples of brain and lung were collected for the virological and molecular analysis of Rhabdoviridae and canine distemper virus (CDV). *Trichinella* spp. larvae were systematically searched for in muscle samples through artificial digestion. All *Trichinella* positive samples were forwarded to the International Trichinella Reference Center at the Istituto Superiore di Sanitá, for the species identification by a multiplex PCR. Results were recorded in the IZSLT laboratory information system (SIL) over the years. We then organized retrospective data, downloaded from SIL, in an excel file reporting for each animal date and area of finding, sex, age class, decomposition score, presence of traumatic lesions, results of species identification, histopathology, parasitology, virology, microbiology and toxicology. Based on the postmortem findings, we classified the cause of death as follows: trauma, poisoning, infectious disease or sepsis, neoplasia, degenerative, congenital, other inflammatory, undetermined. The manner of death was classified as follows: roadkill, poisoning, shotgun, other anthropogenic, natural, interaction either intraspecific or with other canids, euthanasia, undetermined.

Results and Discussion

A total of 251 wolves were found dead in Latium region in the period ranging from January 2009 and July 2024. Over 208 animals genotyped, 146 were confirmed as pure wolves, 48 were recent hybrids with the dog, and nine were introgressed wolves, showing non-recent domestic ancestry. Most of the animals were collected on the territory of the provinces of Rome (101 cases) and Rieti (81 cases). The cause of death could be determined for 229 out of 251 cases. The most frequent cause of death was trauma (205), either related to roadkill (112), shotgun (15), other anthropogenic trauma (34), or interaction with other wolves or canids (9). The second most frequent cause of death was poisoning: over 22 confirmed cases, poisoning has been determined as the main cause of death in 11 cases, and as a concomitant condition with a traumatic cause of death in the other 11 cases. Chemical analysis revealed zinc phosphide as the most used toxicant (50% of positive cases), followed by phorate (23%). Other detected toxicants were brodifacoum, metaldheyde, aldicarb and strychnine. Infectious diseases or sepsis have been determined as the cause of death in 10 cases, with death being caused by sepsis (5 cases, secondary to traumatic injuries), bacterial pneumonia (2), CDV infection (1), severe systemic angiostrongylosis (2). Nevertheless, infectious diseases were detected as concomitant condition other than the cause of death in 97 cases, with endoparasites being the most frequent etiologic agents overall (enteroparasites, cardiopulmonary nematodes, urinary nematodes and *Leishmania infantum*). Analyses for *Trichinella* spp. were performed on 230 animals and larvae were detected in 30 cases. Multiplex PCR on larvae allowed the identification of *T. britovi* species in 19 cases and of the concomitant presence of *T. spiralis* and *T. britovi* in one case of July 2024, being the first report of *T. spiralis* in a wolf in Italy. As regards the considered period, we observed a general increasing trend of case entries over the years: this could be due both to the increasing population trend of wolves, and to the improving coverage of collection of wildlife carcasses. We detected a high rate of roadkill related deaths, but at the same time, most of the recorded finding locations were along main roads. More data are needed to overcome a possible detection bias, estimate the real proportion of roadkill related deaths and its impact on wolf population mortality. Positive results for some infectious agents, such as the unprecedented detection of *T. spiralis* in the Latium territory, underline the role of the wolf as reservoir and sentinel of zoonotic pathogens and the relevance of systematic surveillance of wildlife diseases from a public health perspective.

VIROLOGICAL INVESTIGATIONS IN FREE-RANGING APENNINE WOLVES (*CANIS LUPUS ITALICUS*) IN 2022-2023

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Introduction

Until the beginning of the 1970s, the population of wolves in Italy was in sharp decline, which led to the wolf being officially recognised as an endangered species. In fact, only about a hundred individuals were counted, mostly located in the southern part of the country. Since then, the implementation of species protection laws, the establishment of new protected areas, and profound changes in the cultural perception of the wolf have enabled an increase in the population of the species. Consequently, in recent decades, a natural expansion of the wolf into more suitable habitats, with the colonisation of the entire Apennines and a large part of the Alps, occurred. Such a marked increase draws attention to the potential health implications that could arise, considering the inevitable overlap of habitats with other domestic and wild species and humans. For this reason, a network of different scientific institutions was set up to conduct studies on the wolf's health status in Italy. The IZSLER has been involved in investigating the presence of viruses of conservation and zoonotic interest to understand the role of the wolf in the epidemiology of these pathogens.

Material and Methods

In 2022-2023, a total of 163 wolf carcasses were mainly collected from different Apennines areas in Italy and were subjected to necropsy. Samples from the lungs, liver, spleen, kidneys, and intestine were collected and homogenised. Several PCR protocols were adopted to detect Influenza A virus (IAV), Canine Distemper virus (CDV), Coronavirus, Mammalian Orthoreovirus (MRV), Canine Adenovirus (CAdV-1 and -2), Carnivore ProtoParvovirus 1 (CPPV-1), Hepatitis E virus (HEV). In addition, out of 163 wolves, 43 brains collected in Lombardy and Emilia-Romagna regions were analysed for the Aujeszky Disease virus (ADV). When possible, positive samples were sequenced for viral confirmation and typing.

Results and Discussion

The anatomical-pathological examination showed that most wolves were in good body condition, with no signs or lesions attributable to specific infectious diseases. About 70% of animals died as a result of collisions with motor vehicles, but in nine cases, signs of poisoning were detected, and nine wolves died from a gunshot wound. Signs referable to sarcoptic mange were detected in 16 wolves. CDV was detected in four (2.4%) animals from Emilia-Romagna, Marche, Lazio and Umbria regions. Sequencing of the H gene made it possible to identify these strains as belonging to the European Wildlife lineage, previously recognised in foxes from the same geographical areas. Four other wolves (2.4%) from Emilia-Romagna, Marche, Umbria and Calabria tested positive for MRV, but we were unable to type the viral strains. Twelve wolves (7.3%) tested positive for Canine Adenovirus type 1 (CAdV-1), whereas all samples were negative for CAdV-2. A total of 92 animals (56.4%) tested positive for CPPV-1 (84 for Canine Parvovirus type 2 and eight for Feline Parvovirus, respectively). Indeed, detecting CAdV-1 and CPPV-1 is quite common in wild carnivores, often showing high prevalence. The absence of clinical signs would suggest that these pathogens occur predominantly asymptomatically or subclinically. The coinfection with CDV, CAdV-1 and CPPV-1 was detected in one poisoned wolf, which presented not optimal body condition and was also affected by sarcoptic mange.

All animals resulted negative for ADV, IAV and HEV. These latter results, combined with the low prevalences found for CDV, Coronavirus and MRV, would suggest that the wolf, despite its susceptibility to infection, does not play a predominant role in the maintenance and spread of these infections.

These preliminary results allow us to provide a broader and more in-depth view of the health status of the wolves spread along the Apennines in Italy, at least from a virological point of view. Deepening this knowledge is particularly important, especially in relation to the constant population growth observed in recent years and the potential overlap with other sensitive domestic and wild species that could be involved in the spread and circulation of viral agents.

FLEA DIVERSITY AND HOST-ASSOCIATIONS IN WILD CARNIVORES FROM NORTHERN ITALY

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Introduction

Fleas (Insecta: Siphonaptera) are obligate blood-feeding insects that parasitize birds and mammals, and are found worldwide.

Fleas of medical importance have been documented on different carnivore species, including canids, felids and mustelids. In Italy, a wide range of wild carnivores are present including red foxes (*Vulpes vulpes*), badgers (*Meles meles*), martens (*Martes foina*), and the spontaneous recolonization of the European grey wolf (*Canis lupus italicus*) in Northern Italy has been observed since the late 90's. Nonetheless, no comprehensive studies have investigated the diversity of fleas in wild carnivores in Northern Italy.

The aim of this study is to report flea species recovered with flea species as a proxy of diversity, and host-associations of fleas in wild carnivores from Piedmont, Northwestern Italy.

Material and Methods

Over the years 2023 and 2024, carcasses of wild carnivores from different locations in Piedmont were opportunistically collected in the frame of regional passive surveillance.

The carcasses were frozen at -20° after the death of the animal until examination to prevent postmortem loss of ectoparasites. The whole body of the animals was carefully inspected for the detection of fleas. Fleas were collected from each animal in individual 2 ml labelled tubes with 70% ethanol at -20°. Morphological identification has been done following taxonomical keys (Whitaker AP. Fleas: Siphonaptera. Field Studies Council. 2007). Photographs were taken using an Olympus BX61 microscope.

Results and Discussion

Overall, the carcasses of 181 wild carnivores were collected. A total of 213 fleas were harvested from 70 (49%) animals, of which 52 red foxes, 13 badgers, 3 wolves, 2 stone martens. All over the whole sample, *Pulex irritans* was the most prevalent flea species (73%), followed by *Paraceras melis* (14%), *Chaetopsylla* spp (14%), *Ctenocephalides felis* (8%), *Ctenocephalides canis* (4%), *Ceratophyllus* (*Monopsyllus*) *sciurorum* (3%), *Dasypsyllus gallinulae gallinulae* (3%), *Nosopsyllus fasciatus* (3%).

Coinfection with up to three different flea species occurred in 16 animals (23%), and foxes presented the highest degree of flea diversity/species (flea species n= 8). Flea abundance and diversity are influenced by several factors including host behavior, environmental factors, their interactions and sample size. The use of shared habitats, such as dens by the red fox and badger, or rendezvous sites by wolves can provide humid, dark, and warm environments with suitable conditions for fleas, thereby influencing their abundance. *Pulex irritans* was confirmed as the most abundant flea in wild carnivores in Europe, as already documented by previous studies (Perez et al., 2022; Deak et al., 2024). Rodent and bird species-specific flea species (*C. sciurorum, N. fasciatus,* and *D. gallinulae gallinulae* respectively*)* were found in three foxes as single or mixed infestation with *P. irritans*, suggesting that foxes might have preyed on infested small rodents (squirrel, rats) or birds. To the best of the authors' knowledge, this is the first large-scale study investigating the diversity of flea species infesting wild carnivores in Italy. Future investigations on flea-borne pathogens are warranted to understand the vectorial potential of these insects of medical importance. This study was supported by the projects "Ricerca corrente" (grant numbers: IZSPLV RC 09/24) funded by the Italian Ministry of Health.

FIRST OSTEOBLASTIC OSTEOSARCOMA IN A FREE-RANGING EURASIAN OTTER: A CASE REPORT

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Introduction

The Eurasian otter (*Lutra lutra*) is a semiaquatic mammal classified as Near Threatened at European and global levels and it is considered as vulnerable in Italy. Once widespread throughout the country, in recent years it has been confined to the southern regions of the Italian peninsula, although a re-expansion of its range is underway. A post-mortem study was conducted on this species in Italy between 2009 and 2017 and found that road traffic collisions were the major cause of death, followed by other causes such as blunt chest trauma of uncertain origin, dog and conspecific attacks or more rarely diseases. Neoplastic diseases are not frequently reported in Eurasian otters, and only single cases have been described in animals kept in captivity. To the best of our knowledge, this is the first reported case of osteoblastic osteosarcoma in a dead free-ranging Eurasian otter, found in Tuscany 35 years after its extinction in this region.

Material and Methods

A free-ranging Eurasian otter of sex male, adult, was found dead in the Magra river near Massa Carrara (Tuscany) and it was taken to the laboratory of Istituto Zooprofilattico Sperimentale del Lazio e della Toscana for a post-mortem examination. The external examination, skinning and internal examination were accurately conducted by forensic pathologists following the veterinary forensic guidelines. Necropsy was performed and tissue samples, organs and bones were collected for supplementary laboratory exams. Virological and bacteriological exams were performed on target organs, and samples of faeces were analyzed searching for parasites. The entomofauna found was subjected to entomological evaluation for identification of species useful for dating the death of the animal. Samples of thigh muscle and femoral bone with macroscopic lesions were collected, fixed in 10% buffered formalin, decalcified with a commercial mixture of hydrochloric acid and EDTA for 48 hours and routinely processed for histopathological examination.

Results and Discussion

The animal was found in an advanced state of decomposition. Poor body condition associated with generalized muscular atrophy and evident exposure of bones prominences was detected. During the external examination, a severe swelling on the left knee and thigh was noticed. In the same area, the subcutis revealed extensive bruise. The left knee and thigh muscles were intensively hemorrhagic (mostly affecting the dorsal part of the bundles) and softened with loss of the entire macro architecture of the muscles. Numerous bone fragments (various shapes and sizes) were attached to the hemorrhagic and softened muscles, especially in the area of the left coxo-femoral joint. In fact, the left femur showed an increase in size and a rough consistency of the periosteum, which appeared proliferated and infiltrated. The diagnostic exams revealed pathogenic bacteria (*Muellerella wisconsensis*, *Gemella haemolysans* and *Aerococcus viridans*) in the lungs, kidneys, liver and brain. No parasites and viruses were detected. Histologically, the tissues were altered by diffuse autolytic changes probably due to the late discovery of the body after death. Microscopical examination of bone revealed the presence of neoplastic tissue with cellular and nuclear pleomorphism, frequently associated with bizarre mitosis, and markedly hyperchromatic chromatin. The tumoral osseous matrix was detected in almost all samples investigated with a pattern of small islands. In all samples of neoplastic tissues clear signs of prevalent osteoblastic differentiation have been detected in focal path and frequently mixed with foci of collagen. An admixture of two prevailing elements represents in different proportions the common histologic feature of osteosarcoma. Pleomorphism and atypia involved nuclei and cytoplasm detected represented fundamental diagnostic elements. Osteosarcomatous cells were characterized by hyperchromatic nuclei, frequently central or eccentric in the cell soma. Mitotic bizarre figures and prominent nucleoli were not very common. On the basis of the described anatomo-histopathological findings, also compatible with the artifacts present related to the poor conditions of the carcass, a very plausible diagnosis of primary osteoblastic osteosarcoma in Eurasian otter was made. Starvation was the cause of death, due to the characteristics of the bone tumor, which made it impossible for the animal to provide itself with food. In addition, the animal's debilitation made it more susceptible to a secondary infection, which may have contributed to its death.

TOXOPLASMA GONDII PREVALENCE IN HUNTED WILD BOARS FROM CENTRAL ITALY: AN UPDATE 10 YEARS APART

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Introduction

Toxoplasma gondii (Phylum Apicomplexa) is a cyst-forming parasite of primary public health concern, that is distributed worldwide. Wild and farmed game meat consumption, especially those of wild boar (*Sus scrofa*) and roe deer (*Capreolus capreolus*) are considered an important risk factor for *T. gondii* infection in humans and thus European Food Safety Authority (EFSA) includes this pathogen in the list of zoonotic agents to be subject to epidemiological monitoring, in wild animals too. Wild boar represents the most popular large game animal species in Umbria region (Central Italy), with a marked increase of the population density observed over the last 40 years) and may represent a key-sentinel of environmental contamination with *T. gondii*, due to their capacity for adaptation to different habitats, wide geographical distribution and high reproductive rates; thus a careful evaluation of the prevalence of *T. gondii* infection in hunted animals is needed to protect public health.

In 2009-2011 a survey on the seroprevalence of *T. gondii* infections in hunted wild boar from Umbria, was conducted and antibodies against *T. gondii* were detected in 13.7% of the analysed serum samples, with a rate similar to that detected in farming swine of the region (16.9%) highlighting the significant role that wild boar may play in maintaining a sylvatic cycle of the parasite. Aim of the present work was given an update prevalence on the occurrence of *T. gondii* infection in wild boars hunted in central Italy, after 10 years from the previous investigation.

Material and Methods

One hundred wild boars shot in the central areas of Umbria during three subsequent hunting seasons (2021–2023), were included in the present epidemiological survey. Blood samples were collected directly by hunters from each animal by cardiac punctures, placed into sterile tubes without anticoagulant and centrifuged to obtain serum. The sera obtained were screened by Immunofluorescent Antibody Test (IFAT) (Diagnostik Megacore, Horbranz, Austria) for the detection of anti-*T. gondii* specific IgG at a screening dilution of 1/40 (cut off). Sera of a *T. gondii*-free and a naturally infected wild boar were included in each reaction as positive and negative controls. The seroprevalence for *T. gondii* was calculated and compared through Chi-square test with those detected 10 years before. Statistical analyses were performed using the WINPEPI (PEPI-for Windows) freeware epidemiological software with the *p*-value set at 0.05.

Results and Discussion

No clinical signs of toxoplasmosis were reported in the wild boars of the present study; eleven out of the 100 (12%, 95% CI: 9–15%) examined animals were found to be positive for *T. gondii* antibodies. No statistical difference among the seroprevalence rates detected 10 years apart in the same sampling areas of the Umbria region were found ($p<0.05$). The prevalence detected are in line with the pooled worldwide seroprevalence of toxoplasmosis in wild boars reported in a systematic review 23% (95% CI: 19-27%) but lower if compared to that detected by biomolecular methods in other studies conducted in Southern Italy (39.6% and 44%). The present finding supports the stable maintenance of the sylvatic cycle of the parasite through wild boars and thus the constant risk for this animal species to be source of infection for human beings, in fact the hunted wild boars are usually utilized to produce products supplied to the local market without specific control for the detection of the parasite.

Since the percentage of seropositive people is decreasing, the increasing risk that people will first be exposed to the parasite as adults (e.g., pregnant women or immunocompromised people) is arised. A systematic evaluation of the prevalence of infection with *T. gondii* in hunted animals should be performed to guide the development of appropriate public health interventions by regulatory agencies, that should be include: i) public education (e.g., training courses focusing on appropriate application of safe hygiene practices among hunters and proper meat cooking and processing for consumers); ii) implementation of specific regulatory legislations to adopt methods of wild boar density reduction (e.g., through controlled hunting or fertility control programs); iii) monitoring of the whole food chain. The Authors would like to thank IZSUM since the data used for the present work are included within the results obtained by the financed project RCIZSUM 09/21 Selv.Ind. Ep.

PARAPHIMOSIS ASSOCIATED WITH DIFFUSE SUPPURATIVE-HAEMORRAGIC AND ULCERATIVE BALANITIS IN A RED DEER (*CERVUS ELAPHUS*) AFTER THE RUTTING SEASON IN VENETIAN ALPS (ITALY): A CASE REPORT

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Introduction

An adult male red deer (*Cervus elaphus*) was first reported with sign of illness in December 2023 in Auronzo di Cadore (Belluno, Veneto), in the framework of the passive surveillance carried out by official and unofficial stakeholders. In a video taken by a local hunter on the 21st of December, the deer showed a peculiar elongated inguinal lesion that was referred to a not bleeding mass. During January 2024 the animal was noted several times suffering in poor body condition and the service of Corpo di Polizia Provinciale of Belluno decided to euthanize it in February.

Material and Methods

Immediately after death the animal underwent to post-mortem examination that was conducted on-site.

Samples of the inguinal lesion were collected and submitted to the diagnostic unit of the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe) in Belluno for macroscopic evaluation. Furthermore, samples of the mass were fixed in 10% neutral buffered formalin and routinely processed for histological examination.

The dental development was evaluated in order to establish the age of the animal.

Results and Discussion

The animal was estimated to be about 6-7 years old and the body showed no notable external injuries other than the previously reported mass in the inguinal region, which was confirmed to be the dramatical enlargement of the penis. Macroscopically the organ was strangulated by the prepuce, with a marked difference between proximal healthy tissue and distal pathological one, which appeared ulcerated in its entire length and was extremely compact to palpation and cutting. On cross-section the mass was entirely composed of firm to hard grey tissue intermingled with fibrous bands. Since there were no notable lesions affecting the tissues proximal to the lesion, only mass samples were submitted for histological examination.

Histologically all the samples were characterized by abundant fibrovascular tissue featured by large-calibre vessels, mainly arteriosus, with an associated well differentiated proliferation of fibroconnective tissue, somewhat disorganised and affected by moderate interstitial oedema. The external margins of the sections showed diffuse ulceration, with no intact lining epithelium and an intense diffuse suppurative haemorrhagic inflammation, with presence of necrotic debris and disseminated aggregates of bacteria, mainly spherical-shaped, with rare aggregates of rod-shaped ones.

Physiologically, male cervids have a fibroelastic rod shaped penis with a urethra covered by the corpus spongiosum. When not erect, the penis maintains an S-shaped bend (sigmoid flexure) thanks to the retractor penis muscles and the prepuce entirely protects it. On the other hand, when erect, penis is largely exposed out of the prepuce, especially during the rutting season, characterized by marking behaviours, violent and ritualized fights to define hierarchies and also contact with the external ambient increases the chance of infection. In such situations paraphimosis is more likely to occur. This might be due to several, single or concurrent causes such as trauma, hematoma, bacterial (e.g. staphylococcosis, streptococcosis, corynebacteriosis, colibacillosis, necrobacillosis), mycotic (e.g. candidiasis) and viral (e.g. infectious pustular balanoposthitis) infections, foreign bodies or neoplasia: a penis harm does not allow an easy retraction and the active hyperaemia and progressive venous congestion for the proximal restriction, in addition to the drying out of the mucosal surface, get the penis to become oedematous and further exacerbate paraphimosis, with possible subsequent secondary lesions due to pain suffering.

The breeding season of the deer in Italy usually occurs between mid-September and mid-October, but it can vary from year to year based on temperatures and geographical area. The maximum activity in the area of Belluno occurs during early October, hence, we can assume that the penis enlargement of the present case, maybe less pronounced and still with no bleeding, was evident in about one month and a half. Based on the animal signalment and the macroscopic and histological findings the lesion was suggestive of a paraphimosis likely connected with mating season and complicated by fibroplasia and secondary infection. Unfortunately, it is not possible to comment on possible concomitant or prior involvement of viral agents, as the lining epithelium could not be examined microscopically due to extensive ulceration, and collateral further investigations were not performed.

BLOOD METABOLIC PROFILES IN BLOOD FROM CAVERNOUS SINUS AS MARKER OF STARVATION IN RED DEER

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Introduction

Blood profiles are an objective and reproducible way to assess the metabolic status of animal populations but its use in wildlife is weakened by several issues including the necessity of high-quality fresh blood that is often difficult to obtain in field conditions. Blood from cavernous sinus (CS) has been proposed as an alternative to that from major vessels since it provides a high volume of sample with minimal hemodilution. This type of sampling was considered suitable for investigating serology of infectious diseases and, recently, it has been proposed also for evaluating metabolic profiles, with a good concordance with results obtained with classical sampling, although with some caution. Hematochemical results from CS are in agreement with those of major vessels for many variables (urea, NEFA, BOHB, and phosphorus) while for total protein, albumin, cholesterol, and calcium, CS showed lower concentrations likely due to unpredictable levels of water dilution. However, results in the two matrices appear correlated, thus suggesting that CS could be used as a valid alternative for metabolic profiles as long as adequate reference values are calculated. Aims of the present research are: 1) to calculate reference values for main metabolic variables in CS from red deer of different gender and age, and 2) to evaluate if metabolic profiles from CS correlate with classical variables used for evaluating starvation in red deer.

Material and Methods

Plasma samples from CS blood were collected from 104 red deer of different sex and age classes during a culling plan conducted in the Stelvio National Park; samples with hemolytic index of equal or less than the value of 4 (corresponding to 100 mg/dL hemoglobin) were considered suitable for analysis (no 72 = 69%). Metabolic profiles, including total protein, albumin, total cholesterol, urea, total calcium, inorganic phosphorus, triglycerides, NEFA, and beta hydroxibutyrate, were evaluated using an automated chemical analyzer. Data on nutrition status (Kidney Fat Index – KFI- percentage of fat in bone marrow -%BM-, eviscerated body weight) were recorded for each deer at the control center. Hematochemical results were described with data on gender and age and compared with KFI, %BM, and weight using a Generalized Linear Mixed Models (GLMM). We fit different models, with hematochemical parameters as the dependent variables and the biometric data as the independent ones. In order to investigate the effect of age we differentiate juveniles (< 0.5 years) and subadults-adults (>1.5 years).

Results and Discussion

Protein/albumin ratios decrease with the body mass increase (*p 0.01*) and triglycerides increase with the increase of %BM (*p 0.04*). Total cholesterol and Ca/P ratio were statistically higher in females (*p 0.01* and *p 0.04* respectively). Juveniles showed higher cholesterol/protein ratio (*p 0.01*) and lower Ca/P and protein/albumin ratios (*p 0.01* and *p 0.02* respectively).

Samples from CS are effective for evaluating metabolic profiles in culled red deer providing objective and higher reproducible additional information. For albumin, total calcium and cholesterol the normalization for the concentration of proteins allowed to reduce the unpredictable effect of water dilution in CS providing values comparable to those of major vessels. Although the values for CS may slightly differ from those from major vessels, the use of specific reference values for CS and the normalization of some variables may overcome the issues related to unpredictable water dilution.

ANALYSIS OF ALOPECIC LESIONS IN THE ALPINE MARMOT (*MARMOTA MARMOTA*)

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Introduction

A high prevalence of alopecic lesions has been observed and recorded during the capture of Alpine marmots (*Marmota marmota*).

This study aims to investigate the possible influences of biotic and abiotic factors on the occurrence of these lesions.

Material and Methods

In the three-year period 2021 to 2023, 531 marmots were captured in the municipality of Livigno (SO), Northern Italy, for translocation. The presence, location and form of alopecia manifested by 506 individuals was recorded. Samples were collected for the detection of dermatophytes by the culture method from a subsample of the animals with lesions. Certain factors were considered and analyzed as they were suspected to have an influence on the probability of manifesting the observed lesions.

Results and Discussion

298 individuals (58.89%) showed alopecic lesions. Sex and age group showed no correlation with the occurrence of alopecic lesions. On the other hand, factors such as location, belonging to certain metapopulations and year of capture were significant. individuals with greater body length and weight, for adult males and subadults, and body length, for adult females, showed a significant higher probability to show sign of alopecia.

The frequent occurrence of this type of lesion has been reported in multiple papers, but, to date, the etiology remains unclear. The suspicion is that the origin lies in a complex and multifactorial pathogenesis involving population density, social stress, trophic sources, meteorological influences and the impact of anthropic presence (including livestock facilities). The present work provides a consistent historical series of observations and lays the groundwork for future investigations.

SHIGA TOXIN-PRODUCING AND AMPC-PRODUCING *ESCHERICHIA COLI* IN ENVIRONMENTAL FECES OF RED DEER AND BOVINE FROM ALPINE PASTURES

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Introduction

Red deer (*Cervus elaphus*) has been identified as potential reservoir of Shiga toxin-producing *Escherichia coli* (STEC) associated with human diseases, including haemolityic uremic syndrome. *E. coli* isolates have also been used as indicator bacteria of extended-spectrum beta-lactamase (ESBL) and AmpC production, in antimicrobial resistance (AMR) studies. The aim of this study was to investigate the presence of STEC and ESBL/AmpC/carbapenemase (CP)-producing *E. coli* in the environmental feces of red deer and cattle sharing alpine pastures, to further investigate the potential interspecies transmission of bacteria and the epidemiological role of animal species.

Material and Methods

Fresh environmental feces of both species were collected in Stelvio National Park (Lombardy sector) from three pasture areas grazed by red deer only, bovine only, and both species in September 2023. Samples were enriched in buffered peptone water (BPW) and screened for *stx1* and *stx2* genes by qPCR. Positive samples were further analyzed and *E. coli* colonies were identified using matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) and tested by qPCR for *stx* genes. One STEC isolate/sample was further analyzed by PCR targeting the *eaeA* gene. BPW was also inoculated on MacConkey agar supplemented with 1 mg/L cefotaxime and one colony grown in the selective media/sample was subjected to species identification by MALDI-TOF MS. One *E. coli* isolate for each sample was used for ESBL, AmpC and CP phenotypes confirmation and phenotypically characterized isolates were subjected to PCR analysis for major ESBL (*bla*$_{\mathrm{CTX\text{-}M}}$, *bla*$_{\mathrm{TEM}}$, *bla*$_{\mathrm{SHV}}$), plasmid-mediated AmpC (*bla*$_{\mathrm{CMY\text{-}2}}$), chromosomal-mediated ampC or CP encoding genes.

Results and Discussion

In total, 64 fecal samples were analyzed. Results on STEC analysis showed the presence of 15 (23.4%) positive samples. STEC isolates were identified in red deer (10/28, 35.7%) and bovine feces (5/36, 13.9%) from pasture with the presence of both species. All STEC isolates possessed the *stx2* gene and one isolate from red deer possessed also the *stx1* gene. The *eae* gene was detected in one STEC isolate from bovine, whereas all other isolates were *eae*-negative. AmpC-producing *E. coli* was detected in one (1.6%) fecal sample from bovine collected from the area of pasture with the presence of both red-deer and cattle, showing a 2.8% (1/36) presence in bovine samples. The AmpC-producing *E. coli* harbored changes in the promoter/attenuator region typically associated with chromosomal AmpC overproduction, was resistant to more than three antibiotic classes and was defined as multidrug resistant (MDR). ESBL- and CP-producing *E. coli* were not detected.

The presence of STEC isolates in red deer and bovine supports previous reports on the role of red deer as STEC reservoir. The absence of the *eae* gene strengthen the hypothesis that especially red deer may represent a carrier for LEE-negative STEC strains. The apparently similar genetic characteristics of the majority of STEC isolates from red deer and cattle, harboring the *stx2* gene in the absence of the *eae* gene, and the presence of few isolates with different genetic features in cattle compared to red deer require further characterization of the virulence genes asset to define the possible interspecies transmission of bacteria and the zoonotic potential of wildlife-associated STEC, also considering human consumption of game meat. Despite circulation of AMR in red deer was not observed, further investigations are needed to confirm this finding. Our results strengthen the circulation of AmpC-producing *E. coli* in environmental cattle feces also in alpine pastures, highlighting the need of AMR monitoring to identify the most relevant animal species responsible for fecal shedding.

PREVALENCE AND GENETIC CHARACTERIZATION OF ESBL-PRODUCING *ESCHERICHIA COLI* IN EUROPEAN HEDGEHOGS FROM URBANIZED AREAS OF NORTHERN ITALY

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Introduction

Antimicrobial resistance (AMR) is a significant threat to global health, with Extended-Spectrum β-Lactamase (ESBL)-producing *Escherichia coli* being particularly concerning due to their resistance to critical β-lactam antibiotics. Wild animals, especially omnivorous and synanthropic species, have increasingly been recognized for their role in the spread of antibiotic-resistant bacteria. The European hedgehog (*Erinaceus europaeus*), which thrives in urbanized areas and interacts with both human and animal populations, is a promising sentinel species for monitoring AMR pollution in the environment. This study aimed to investigate the prevalence of ESBL-producing *E. coli* and the presence of genes coding ESBLs in wild hedgehogs from urban areas in the Lombardy region of Northern Italy.

Materials and methods

Intestinal content samples from 90 hedgehogs, that had died in a Wildlife Rescue Centre in Lombardy, were collected. Only animals without prior exposure to antibiotics were included to ensure that the results reflected environmental contamination. Samples were first enriched in buffered peptone water and incubated overnight at 37°C. They were then plated onto CHROMagar™ ESBL, a selective medium for the isolation of ESBL-producing *E. coli*. All the suspected colonies were confirmed as *E. coli* using the MALDI Biotyper® system (Bruker). A multiplex PCR targeting the *bla*$_{\text{CTX-M}}$, *bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$, and *bla*$_{\text{CMY-2}}$ genes was then performed on the isolated ESBL-producing *E. coli* strains.

Results and conclusions

ESBL-producing *E. coli* were isolated from 33 out of 90 hedgehog carcasses (37%). Molecular analysis revealed that *bla*$_{\text{TEM}}$ was the most common detected gene, present in 58% (n=19) of the isolates, followed by *bla*$_{\text{SHV}}$ (39%, n=13) and *bla*$_{\text{CTX-M}}$ (24%, n=8). Eleven strains carried two ESBL genes: 8 strains showed the *bla*$_{\text{TEM}}$ / *bla*$_{\text{SHV}}$ combination, while 3 harbored *bla*$_{\text{TEM}}$ / *bla*$_{\text{CTX-M}}$. None of the strains tested positive for the *bla*$_{\text{CMY-2}}$ gene. The results of this study provide good evidence of the ESBL-producing *E. coli* presence in wild hedgehogs in Northern Italy. Our findings suggest the potential role of hedgehogs as efficient bioindicators for monitoring the spread of antibiotic resistance genes in the environment. Given their permanent residence in areas with frequent human and animal interaction, hedgehogs may reflect these activities and their effect on the environment. This study highlights the importance of wildlife surveillance to understand and monitor the dynamics of AMR and calls for further research to track the dissemination of resistance genes in the different ecosystems.

PRELIMINARY HISTOPATHOLOGICAL ANALYSIS OF LYMPH NODES FROM WILD BOARS (*SUS SCROFA*) WITH LYMPHADENOMEGALY: INVESTIGATIONS AT A GAME-HANDLING ESTABLISHMENT

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Introduction

The contact between wildlife and livestock, especially if free range farmed, has garnered more attention in recent times because of the spread of diseases between domestic and wild animal populations. In order to stop disease spillover between them, it is imperative to gain a deeper understanding of the wildlife/livestock interface. Precise evaluation of the risk associated with infections that could affect the health of humans and livestock requires detailed information regarding the prevalence and abundance of wild species and their diseases in specific territories. In central Italy, wild boar could be a good candidate for these purposes throughout disease screening at Game Handling Establishment level.

The aim of this study was to conduct preliminary histological and bacteriological investigations to evaluate the pathological state of lymph nodes of wild boars found to be enlarged at slaughter.

Material and Methods

A total of 40 lymph nodes were collected at a game-handling establishment from 23 wild boars hunted in the north-east areas of the Umbria region, Italy. Fresh samples were submitted to the laboratory services of the Veterinary Teaching Hospital at the Department of Veterinary Medicine of Perugia. Each lymph node was divided in 2 halves, one half was partly used for standard bacteriological examination and partly frozen (-80°C); the other half was fixed in 10% neutral buffered formalin. Routine histological investigations were performed on the fixed samples. The fixed samples were embedded in paraffin, 3 µm slides were cut using a microtome, stained with Hematoxylin and Eosin (H&E), and observed under an optic light microscope.

For selected cases, histochemical investigations were carried out using PAS, Congo Red, and Perls-Van Gieson stains, along with immunohistochemical investigations using anti-IBA1 antibody to detect macrophages.

Results and Discussion

No macroscopic alterations of the organs were observed at the post-mortem inspection at the slaughterhouse, except for widespread lymph adenomegaly. Cervical and inguinal lymph nodes were collected from the animals involved. Histological examination showed variable, but rather repetitive, patterns in the different lymph node sections examined.

The range of histological findings varied from hyperplastic lesions with follicular germinal center enlargement and/or paracortical zone hyperplasia to germinal center depletion with atrophy and rare instances of karyorrhectic nuclei. These findings were almost consistently associated with marked histiocytosis of the subcapsular and medullary sinuses/macrophage hyperplasia (cells immuno-positive for IBA1); sometimes, the macrophages contained brownish-black pigment consistent with hemosiderin pigment (Perls-positive staining).

A variable amount of eosinophilic granulocytes and weakly eosinophilic extra-cellular amorphous material, PAS-negative and Van Gieson-positive, interpreted as hyalinosis, was also observed. Multifocal hemorrhagic foci were present in some lymph nodes.

The standard bacteriological examination was negative for the presence of common aerobic non-fastidious bacterial pathogens.

Wild boars (*Sus scrofa*) are a significant wildlife species that can be subject to various diseases and health disorders. Evaluating the health status of their lymph nodes can provide insights into their overall health and disease prevalence. The main lesions observed are referable to variable degrees of follicular and paracortical reactive hyperplasia, atrophy with karyoretic necrosis, sinus histiocytosis and hyalinosis. These features are not attributable to a specific disease but can be the result of various noxa that directly or indirectly can involve the lymphatic system. The investigations carried out so far have excluded fungal diseases and some bacterial diseases. The next steps will include the investigation of the most common viral agents that wild boars can share with pigs (i.e. porcine circoviruses).

Wild boars can serve as reservoirs of infections that can then be transmitted to domestic pigs, making it important to monitor and manage wild boar populations to prevent the spread of diseases.

PARASITE INFECTIONS IN GOLDEN JACKALS: A PRELIMINARY COMPARATIVE ANALYSIS OF DIAGNOSTIC TECHNIQUES

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Introduction

The golden jackal (*Canis aureus*, Linnaeus, 1758) has recently expanded into northeastern Italy, establishing itself in the regions of Friuli-Venezia Giulia (FVG), Veneto, and Trentino-Alto Adige, while maintaining connections with the larger Balkan population. Golden jackals thrive in a wide range of habitats, from coastal to suburban areas, where they exploit available resources. Their opportunistic diet and extensive home range increase their exposure to various pathogens, including viruses, bacteria, and parasites. As a result, golden jackals are known to harbor a variety of infectious agents, many of which are significant for both veterinary and public health. Health monitoring of this species has been implemented, primarily through necropsies performed on animals found dead. Unfortunately, necropsy-based approaches are time-consuming and often linked to culling programs or passive surveillance efforts. In contrast, copromicroscopy offers a less invasive, lower-cost alternative, enabling the examination of larger numbers of animals with less time and effort. Given the potential for expanding the use of copromicroscopy in golden jackal health surveillance, this study aimed to compare the performance of two commonly used copromicroscopy techniques as standard flotation and Mini-FLOTAC in this species, using the scraping, filtration, and counting technique (SFCT) and lungs examination as gold standard.

Material and Methods

This study investigates intestinal and pulmonary helminths in 23 golden jackals, primarily deceased due to human-related causes in the FVG region. For safety reasons, the specimens were preserved at -80°C for at least 72 h before necropsies. The SFCT was applied to small and large intestines, using sieves of 1000 μm, 500 μm, 250 μm, and 75 μm for the isolation of intestinal helminths. The tracheobronchial tree, along with the pulmonary arteries and their branches, was opened; subsequently, the lung parenchyma was submerged in water, gently squeezed and soaked in water for 24 hours. Identified parasites were classified to genus or species level based on morphometric keys. Faecal material was collected from the rectum during necropsy and subsequentially analysed using standard flotation (FT) and Mini-FLOTAC (MF) on 2 g of faeces, both performed with a Zinc sulfate solution (s.g. 1.350). Eggs and oocysts were identified based on morphology to the lowest taxonomic level possible.

The performances of FT and MF, their concordance and sensitivity (by the Clopper-Pearson exact method) were computed for each detected parasite taxa, using SFCT as gold standard.

Results and Discussion

Overall, SFCT detected at least one parasitic taxon from 17 samples out of 23. In particular, four nematodes (*Toxocara canis*, *Uncinaria stenocephala*, *Trichuris vulpis, Molineus* spp.), two cestodes (*Mesocestoides* spp. and *Taenia* spp.) and one trematode (*Metagonimus yokogawai*) were retrieved. Considering the lungs, a total of three nematode species (*Eucoleus aerophilus*, *Crenosoma vulpis* and *Angiostrongylus vasorum*) were detected.

MF analysis was carried out in all specimens, whereas FT could not be performed for 3 samples, due to lack of material. Copromicroscopic analysis allowed the isolation of eggs of intestinal helminths, including Ancylostomatidae, *Molineus* spp., *T. vulpis*, Taeniidae, oocysts of *Sarcocystis* spp., and eggs of pulmonary nematode *E. aerophilus*.

Considering the Ancylostomatidae family, the concordance between the SFCT and both copromicroscopic tests was comparable (82.6% for MF and 85.0% for FT). Sensitivity for both methods was also similar, with MF at 50.0% (95% CI: 15.7-84.3) and FT at 57.1% (95% CI: 18.4-90.1). Similarly, in the case of *Molineus* spp., concordance was similar for MF and FT (86.9% and 80.0%, respectively), but the sensitivity was very low for both (0.0%). For *T. vulpis*, concordance was 95.6% for MF, and 95.0% for FT. Sensitivity for both methods was 100% (95% CI: 2.5-100). Considering Taeniids, concordance between SFCT and MF was slightly higher at 65.2% than FT (45.0%). Sensitivity of MF was 20.0% (95% CI: 2.5-55.6), whereas FT did not detect any taeniid egg. Regarding *E. aerophilus*, concordance with detection of worms in the airways was 65.2% for MF and 55.0% for FT. Sensitivity was low for MF [20.0% (95% CI: 0.5-71.6)], whereas FT had a value of 0% (95% CI: 0.0-33.6).

Notwithstanding the limited sample size, this study confirmed previous studies on other species that reported the low sensitivity of both copromicroscopic methods applied, limiting their effectiveness. Effects of freezing at -80°C should not be ruled out. Their inclusion in surveillance programs should only be considered if careful attention is given to determining the appropriate sample size, accounting for the limitations in sensitivity of these imperfect tests into the estimation of parasite prevalence.

A NOVEL PARVOVIRUS AS A POSSIBLE ETIOLOGICAL AGENT OF FATAL ENTERITIS IN THE EUROPEAN HEDGEHOG, (*ERINACEUS EUROPAEUS*).

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Introduction

In recent years, anthropic activities and reduction of wild territories have been strongly impacting European hedgehog populations. Wildlife Rescue Centres play a vital role in their conservation by providing veterinary care and supporting reintroduction efforts. However, many factors, such as the simultaneous presence of a considerable number of juvenile hedgehogs during the breeding season, the stress of captivity, or the restricted spaces available in Rescue Centers, may facilitate the exchange and spread of pathogens among the animals.

This study describes an outbreak of fatal enteritis that occurred in 2022 at the Apulian Regional Wildlife Rescue Center and the evidence of a novel parvovirus never described before in European hedgehogs.

The outbreak

At the beginning of June 2022, three juvenile subjects, hospitalized in the pre-release acclimatization enclosure with seven other hedgehogs, died simultaneously during the night apparently without overt clinical signs. All subjects, aged between 1 and 3 months, were hospitalized due to orphanhood. They completed the weaning process and exhibited a favorable general health condition at the time of hospitalization. After the initial case, all the juvenile hedgehogs were individually housed in rabbit cages under quarantine, and the biosafety measures were increased. Deep cleaning and disinfection of the enclosures with sodium hypochlorite were carried out routinely. In the following weeks, other hedgehogs, admitted after the first case occurred, exhibited signs of enteric disease.

They were all hospitalized for at least 7 days before the onset of clinical signs and initially presented a slight decrease in appetite, evolving into complete anorexia within a few days. The treatment protocol for anorexic subjects included forced feeding and subcutaneous rehydration. At the same time, gastrointestinal signs appeared with the production of semi-solid, dark red, fetid feces. Some subjects developed respiratory symptoms, including sneezing and a mild nasal discharge. When the first symptoms appeared, a broad-spectrum antibiotic therapy with injectable enrofloxacin (10 mg/kg bid) was performed but there was no response. Death occurred within 4–6 days of the onset of clinical signs in all the animals with a case fatality rate of 100%.

Overall, the outbreak involved 19 immature hedgehogs, raising the annual mortality rate in this time span in immature hedgehogs from 16% (7/44) in 2021 to 53% (19/36) in 2022. The outbreak died out with the end of hedgehog breeding season. After mid-August, the number of new immature subjects admitted to the rescue center drastically decreased.

Post-mortem examination demonstrated a uniform pathological profile in both the initial case hedgehogs and those that died subsequently. Pathological findings included enteritis, confined to the small intestine, characterized by thickened intestinal walls and congested mucosa. All intestinal segments were excessively distended due to the accumulation of gas. Congestion of the liver, spleen, and lung were also observed.

Parasitological and bacteriological analyses were carried out on the intestinal content and the organs. In addition, a sequence-independent amplification (SISPA) protocol was applied to pooled samples of intestinal content and the organs of the died hedgehogs. The obtained libraries were sequenced using Minion (Oxford Nanopore Technology, ONT) platform. FASTq data were analysed using WIMP tool on the cloud-based analysis platform EPI2ME (ONT)

Results and Discussion

Parasitological and bacteriological tests were negative or inconsistent. Massive sequencing after SISPA showed that about 85% of the reads were of bacterial origin, and 64% of them were attributed to *Bacteroides fragilis*, a common commensal species. Less than 3% of the reads were of viral origin. Metaviromic analysis identified a novel parvovirus in all fecal samples and in some internal organs. The virus showed 90.4% nucleotide identity to a Chaphamaparvovirus found in Amur hedgehogs *(Erinaceus amurensis)*. Chaphamaparvoviruses have been associated with inclusion bodies nephropathies and kidney fibrosis in mice, diarrhea and emesis in cats, and respiratory or neurological symptoms in bearded dragons. Additionally, the detection of the parvoviruses in the internal organs is suggestive of a systemic infection.

These results allow us to suppose a role of these parvoviruses in the etiology of the outbreak described. Further research will be necessary to confirm this hypothesis and verify the actual virulence of this virus, to develop strategies for its control in rescue centers and in the wild environment.

EMERGENZA PSA GIS DASHBOARD: A GEOGRAPHIC INFORMATION SYSTEM TO VISUALISE AFRICAN SWINE FEVER SURVEILLANCE ACTIVITIES IN WILD BOAR POPULATION ACROSS ITALY

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Introduction

African Swine Fever (ASF) is a fatal disease, affecting exclusively domestic and wild suid, such as *Sus scrofa* in Eurasia and some African wild species, and ticks of the genus *Ornithodoros* limited to Africa. ASF is a serious threat to pig production considering also there are no vaccine and effective treatment available.

In 2022 in mainland Italy, the genotype II of the ASF virus has been detected within the wild boar population. After the virus introduction, one of the principal Italian surveillance strategies put in place was to the strengthened passive surveillance in wild boars. Under these circumstances, standardise collection of wild boar data accompanying samples is critical to better understand the efficacy of control strategies and to analyse the responses of affected populations. This process was ensured by a standardized nationwide collection of all metadata on recovered wild boars (such as sex, age class, geographic coordinates of the recovery site, carcass preservation state, and context of sampling). Data collection is also finalised to solve Italian informative debt through European Food Safety Authorities, European Commission and World Organisation for Animal Health. Accordingly, to national laws, in Italy, all ASF data collection lies down through national information systems SIMAN (Animal Disease Information System) for wild cases and domestic outbreaks and SINVSA (National Veterinary Information System for Food Safety) for samples features and results. Given the emergency state, the Istituto Zooprofilattico dell'Abruzzo e del Molise "G. Caporale" developed, along with National Reference Centre for the Study of Pestivirus and Asfivirus Diseases (CEREP) and Italian Ministry of Health, a GIS dashboard ("Emergenza PSA GIS Dashboard") to permit the dynamic visualisation to veterinary services of the ASF zoning measures, disease spread through national territories and also to monitor the surveillance activities conducted to support decision makers.

Material and Methods

To effectively monitor data and trends related to the ASF emergency, an advanced GIS Dashboard has been developed that aggregates information on sampling, outbreaks, and zoning. The data, updated daily through automated Python pipelines running on a local Windows Server, is made available to users via web, through the GIS Dashboard built with Esri Software as a Service (SaaS) technology in a private cloud environment (ArcGIS Online). With its interactive maps, charts, tables and KPIs, this tool simplifies data analysis, speeds up decision-making processes, and provides valuable support in planning the necessary actions to counteract the spread of the disease. Specimens and outbreaks data are supplied by the Experimental Zooprophylactic Institutes staff and Regional and Local Veterinary Services through the national information systems SIMAN and SINVSA. The restriction zone data are updated after amendments made to the Annex I of Commission Regulation (EU) 2023/594.

Results and Discussion

"Emergenza PSA GIS Dashboard" facilitates real-time visualization of African Swine Fever cases, outbreaks and sample data, by using interactive thematic map, graphs, filters, and tables. It is the first system for the collection and visualization of surveillance data at livestock-wildlife interface implemented in Italy. In dashboard interface are available tables with cases/outbreaks and samples metadata, counters for the number of cases/outbreaks and sampling both detailed for wild and domestic animals, itemised ring and bar charts. Additionally, samples, strengthened passive surveillance activities, wildlife cases and outbreaks are geographically located and visualised through the interactive map. By clicking on specific elements on the map, it is possible to acquire information and by zooming in visualise the active restriction zone. The "Emergenza PSA GIS Dashboard" allow the users to join samples and cases/outbreaks features with restrictions in terms of official zoning. Considering the large amount of data collected for the wild boar population during the two years of the Italian ASF emergency, the standardisation and harmonisation implemented is a valuable example of how wildlife data can be made accessible to decision-makers to implement control measures for the disease.

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