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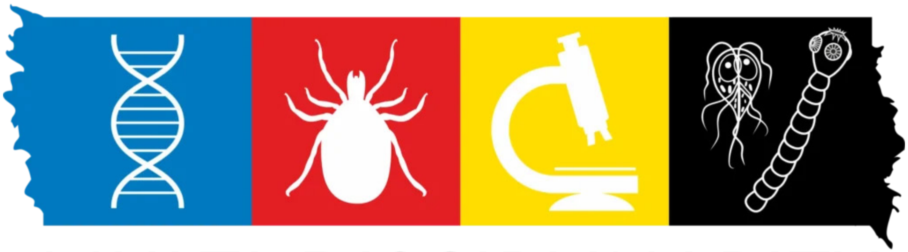

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Abstracts

[PS1]
Plenary session I

[PS2]
Plenary session II

Session: [PS1] Plenary session

The quest for parasitological knowledge: breadth, depth, or both?

Robert Poulin

Department of Zoology, University of Otago, Dunedin, New Zealand

Abstract

Because of limited time and resources, it is not possible for any research group to simultaneously increase both the breadth and depth of parasitological knowledge. This leads to a trade-off between breadth and depth of knowledge, with most parasitologists working at one or the other end of the spectrum. I will illustrate this phenomenon by examining our knowledge of parasite species. On the one hand, we have discovered many parasite species (great breadth), but we know almost nothing about them (little depth). I will show that most parasite species that have been discovered and given a Latin name are never studied again or mentioned again the scientific literature. On the other hand, we know a great deal (great depth) about only a few parasite species (little breadth), the so-called model species. I will highlight the great contributions these have made to our understanding of multiple facets of host-parasite interactions, but also point out the limitations associated with a strict focus on few model species. Finally, I will argue for a better balance between breadth and depth of knowledge if we are to move our field forward in coming decades.

OS – oral session

Session: [PS1] Plenary session

Multi-actor perspectives in parasite diagnosis and epidemiology

Laura Rinaldi

University of Napoli Federico II

Abstract

The multi-disciplinary and inter-disciplinary nature of parasitology has been well recognised for many years at the international level in various areas of basic and applied research. The need for a multi-disciplinary approach and inter-sectoral collaboration in parasitology has even increased in recent decades, in line with the revolution in technologies for the diagnosis of parasite infections in animals and humans, as well as for epidemiological studies and field applications.

Parasitology fosters synergies between public health and other disciplines (e.g., ecology, geoinformatics, information technology, social sciences, economic sciences, etc.) that link parasites and hosts for a more holistic assessment of parasitism, enabling prioritisation of interventions across public health boundaries.

Artificial intelligence (AI) and machine learning (ML) technologies are transforming the field of parasitology, offering new and innovative solutions to some of the most pressing challenges facing researchers, health professionals and citizens. For example, AI- and ML-powered tools are improving the accuracy and efficiency of parasite diagnosis and improving the surveillance and control of parasitic diseases by enabling more accurate disease modeling. Parasitology can even be considered a "meta-discipline" as it combines information from different research areas to gain insights and perspectives that cannot be achieved with a single research area alone. From the analysis of archaeological material to the remote use of drones…...paleoparasitology, geoparasitology, digital parasitology, socio-parasitology, eco-parasitology, citizen parasitology, AI parasitology, IT parasitology are just a few topics that show that parasitology is a dynamic discipline meaning that the tools and methods of parasitology are evolving as much as the discipline itself.

Keywords: parasitology, epidemiology, diagnosis, multidiscipinarity

OS – oral session

Session: [PS1] Plenary session

Modern methods of data analysis in nematode infections

Michael Stear[1], David Piedrafita[2], Sarah Preston[2], Katarzyna Donskow-Łysoniewska[3]

1 La Trobe University, Melbourne, Australia
2 School of Science, Psychology and Sport, Federation University Australia
3 Department of Experimental Immunotherapy, Faculty of Medicine, Lazarski University, Warsaw, Poland

Abstract

Many data in biology are normally distributed and t-tests, analysis of variance, regression and correlation were developed to analyse these types of data. However, many parasitological data do not follow a normal distribution, including egg counts, worm burdens, antibody responses and histological counts of eosinophil and mast cell responses. Consequently, traditional experimental designs can be underpowered while traditional methods of analysis can give misleading results. Suitable methods of analysis include generalised linear models, generalised linear models, mixture models and Monte Carlo Markov Chain (MCMC) procedures. Fortunately programs that can carry out these analyses are widely and freely available. The use of these programs will be illustrated with data from natural and deliberate infections.

The aim of my research has been to understand the mechanisms underlying variation among animals in resistance to infection and to use this understanding to develop better methods of controlling disease and the effects of subclinical infection. I have examined genetic variation in immune responses to parasites of cattle, sheep and fish. My research has included epidemiology, genetics, immunology, pathology, host-parasite coevolution and mathematical modelling.

Keywords: immunology, host-parasite coevolution, modeling

OS – oral session

Session: [PS1] Plenary session

Genetic signals of selection by ACT drug treatment in the genome of *Plasmodium falciparum* isolates from Africa

Colin Sutherland

London School of Hygiene & Tropical Medicine, London, UK

Abstract

Clinical management of uncomplicated malaria caused by *Plasmodium falciparum* has been reliant on the effectiveness of artemisinin-based combination therapy (ACT) since the mid 2000s. New parasite genotypes encoding variants of the pfk13 gene are now emerging in Africa, and these are less susceptible to the artemisinin component drugs of ACT. This then poses a risk of resistance-selection against ACT partner drugs. Parasites of African origin isolated from UK travellers with documented ACT treatment failure and adapted to long-term culture in 2022 and 2023 show evidence that lumefantrine is also failing in some patients. Thus, the major ACT artemether-lumefantrine (AL) may now be at risk. This presentation will consider evidence for selection in the recent past by ACT against parasite genotypes conferring chloroquine resistance, and discuss approaches to identify new genotypes of concern. Future drug strategies for more effective malaria chemotherapy in Africa will be proposed in the light of these findings. Finally, we will consider the wider public health implications of a potential emergence of AL-tolerant *P. falciparum* in Africa.

Keywords: malaria, drug resistance, artemisinin combination therapy

OS – oral session

Session: [PS1] Plenary session

Bacteriophages: predators or parasites?

Grzegorz Węgrzyn

University of Gdansk

Abstract

Bacteriophages are viruses infecting bacteria, and propagating in bacterial cells. For decades they played crucial roles as models in genetics and molecular biology, and as tools in genetic engineering and biotechnology. Currently, their huge roles in the natural environment and their importance in human health and disease are widely recognized. However, despite our understanding of mechanisms of development of bacteriophages, in the literature, these viruses are described either as predators or parasites. This might seem surprising as in biological sciences, especially in ecology, there are fundamental differences between predators and parasites. Therefore, I asked whether bacteriophages should be classified as former or latter biological entities. Analysis of the literature and biological definitions led me to the conclusion that bacteriophages are parasites rather than predators, and they should be classified and described as such. If even more precise ecological classification is needed, some bacteriophages, specifically those which can develop only according to the lytic mode, could be included into the group of parasitoids. However, phages which can lysogenize host cells and those which develop according to the permanent infection mode (also called the chronic cycle), like filamentous phages, reveal features of classical parasites.

Keywords: Plenary lecture, bacteriophages, life cycles, parasite-host interactions, predator vs. parasite

OS – oral session

Session: [PS2] Food and Waterborne Parasitology (Journal of the EFP) Plenary Session

Detection of protozoa in water and food by metagenomics: approaches, results and challenges

Simone Mario Caccio

Istituto Supeiore di Sanita, Rome, Italy

Abstract

Contamination of water and food with infective stages of helminths and protozoa plays a significant role in the transmission of these pathogens to their hosts. Detection of these stages in environmental matrices remains difficult due to low contamination levels, and molecular methods are often used as they offer high specificity and sensitivity. In situations where detection of one or a few parasites is required, targeted methods, such as qPCR, are appropriate. However, untargeted methodologies, such as shotgun or amplicon-based Next Generation Sequencing, allow describing the entire microbial community present in a matrix.

Here, I describe the application of these two NGS approaches to detect protozoan pathogens in faecal samples, raw and treated water samples and other environmental matrices. Food samples spiked with known amounts of specific protozoa were also analysed in an effort to identify crucial parameters affecting the sensitivity and specificity of detection.

I will discuss the advantages and limitations of both methods, and introduce a novel analytical workflow developed to guard against false positives.

Keywords: Parasite detection, food, water, metagenomics

OS – oral session

Session: [PS2] Food and Waterborne Parasitology (Journal of the EFP) Plenary Session

Community risk of environmental-borne cystic echinococcosis: lessons learnt from PERITAS project in South America

Adriano Casulli

ISTITUTO SUPERIORE DI SANITA (Italy)

Abstract

Cystic (CE) and alveolar (AE) echinococcosis are zoonotic diseases caused by infection with the larval stages (metacestodes) of *Echinococcus granulosus sensu lato* (*s.l.*) and *Echinococcus multilocularis*, respectively. However, because of the long latent period of months or years between the event of infection and the eventual appearance of symptoms, it is almost impossible to track back the source of infection and identify what pathway and contaminated matrices.

In this context, we present multicentre studies from: i) PERITAS project, aiming to elucidate the pathways of transmission of *E. granulosus* in South America and ii) MEME project, aiming to identify both *E. granulosus* and *E. multilocularis* DNA in fresh vegetables and fruits in Europe and beyond.

In PERITAS project, A total of 4,512 people was screened by ultrasound (US), in Chile, Argentina and Peru to identify high endemic villages to conduct environmental sampling. In these villages, 21% (117/557) of environmental matrices tested positive to *E. granulosus s.l.* DNA. The percentage of faeces contaminated by *E. granulosus s.l.* DNA was found to increase from CE-negative (3%) to CE-positive (17%) households/backyards at US, up to public areas (91%).

In MEME project, 1,597 lettuce and berry samples from 12 countries were analysed. *E. multilocularis* DNA was detected in 1.2% of lettuces, 5.4% of strawberries and 7.3% of blueberries in Europe. *E. granulosus* DNA detected in 1.3% of lettuces, 1.5% of strawberries and 1.3% of blueberries in Europe. Whether DNA may be related to the presence of infective eggs, therefore a risk of infection for humans, this is currently unknown.

Such results suggest shifts: i) from individual risk to community risk in endemic areas, ii) from mainly “food-borne” to more broadly “environmental-borne” disease.

Keywords: *Echinococcus multilocularis*, *Echinococcus granulosus* sensu lato, environmental contamination, pathways of transmission

OS – oral session

Session: [PS2] Food and Waterborne Parasitology (Journal of the EFP) Plenary Session

Cryptosporidium outbreaks linked to food and water; what have we learnt?

Rachel Chalmers

Public Health Wales, UK

Abstract

Cryptosporidiosis is often described as a waterborne disease, and outbreaks in many countries have been linked to drinking water supplies and recreational waters. Outbreaks can have a high impact due to strict public health interventions such as improvement notices or closure of premises such as swimming pools, or notices to boil drinking water. Outbreaks also present a high risk of onward community transmission. In England and Wales, *Cryptosporidium* outbreaks linked to swimming pools far outnumber those linked to drinking water supplies. Until May 2024 it was over ten years since the last mains water-related *Cryptosporidium* outbreak, but recent events have led to such an outbreak. In contrast, awareness of *Cryptosporidium* as a foodborne parasite is lower, outbreaks are fewer in number and may be overlooked. However, there is the potential for large, widespread outbreaks

linked to nationally (or even internationally) distributed fresh produce as well as more localised outbreaks, for example those linked to farm gate milk vending machines. This paper will explore the drivers behind foodborne and waterborne outbreaks, and the factors influencing their detection as well as the lessons learnt.

Acknowledgements: Zoonoses and Gastrointestinal Disease teams at the Public Health Wales Communicable Disease Surveillance Centre and the UK Health Security Agency, and the members of the Outbreak Control Teams.

Keywords: *Cryptosporidium*, food, water, outbreak,

OS – oral session

Session: [PS2] Food and Waterborne Parasitology (Journal of the EFP) Plenary Session

Fish-borne parasites of importance for EU: status on identification, detection and treatment in fish products

Ivona Mladineo

University of Tasmania

Abstract

According to EFSA Scientific Opinion, the most important fish-borne parasites of public health importance in EU that occur in the marine fish are the nematodes *A. simplex* (*sensu stricto*), *A. pegreffii*, *Phocanema decipiens* sensu lato and *Contracaecum osculatum* sensu lato, and the trematode *Cryptocotyle lingua*. The most important zoonotic parasites from freshwater fish are trematodes *Metorchis* spp., *Opisthorchis felineus*, *Paracoenogonimus ovatus*, *Pseudamphistomum truncatum*, and the cestode *Dibothriocephalus* spp. The research on their identification, detection and treatments in fish products significantly improved during the past decade. Fish-borne parasites are usually identified by molecular techniques; immunoassays, proteomics, and conventional and quantitative PCR, the latter valuable if relying on nuclear and mitochondrial markers. Detection, visualisation and isolation techniques (UV-press method, artificial digestion) have been also optimised, while innovative tools, optical hyperspectral sensing and the use of AI need further development and validation. Lastly, treatments for inactivating parasites in fishery products, such as heating, high-pressure processing, pulsed electric fields, drying, ultrasounds, salting, marinating and the use of natural products have been compared for efficiency, resulting in some of these to be dismissed as ineffective, such as ultrasound treatment or different marinade combinations.

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez- Ordóñez A, Bover-Cid S, Chemaly M, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Nonno R, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Buchmann K, Careche M,... Bolton D (2024) Re-evaluation of certain aspects of the EFSA Scientific Opinion of April 2010 on risk assessment of parasites in fishery products, based on new scientific data. Part 1: ToRs1–3. EFSA Journal, 22(4), e8719.

Keywords: Anisakids, EFSA Scientific Opinion, fish-borne parasites

OS – oral session

Session: [PS2] Food and Waterborne Parasitology (Journal of the EFP) Plenary Session

Transmission of parasites to people via food and water: where are we now and should we care?

Lucy Robertson

Norwegian University of Life Sciences, Norway

Abstract

It is a given fact that many parasites can be transmitted via food and/or water, either as contaminants (e.g., eggs, oocyst, cysts shed in faeces), or via particular infectious stages occurring in food animals (e.g., larvae) or in water (e.g., cercariae, trophozoites). Efforts to combat these transmission routes have been of varying success. For example, in many industrialised countries, awareness of waterborne parasites combined with introduction of ultra-violet treatment of drinking water has likely contributed to reducing the occurrence of outbreaks of waterborne giardiasis and cryptosporidiosis. Similarly, efforts to eliminate dracunculiasis have had a major impact on its occurrence – but have not yet reached elimination status.

Different national and international bodies are gradually realising the importance of food and waterborne transmission of parasite as their impact is quantified and compared. With a basis in foodborne/waterborne parasites that are usually transmitted orally, the intention of this presentation is twofold. Firstly, to provide some insights into where we are now based on the assessments that have been conducted and are in progress. Secondly, to address which ambitions we might have, what is realistic, and if we wish to reach total interruption of some life cycles (and thus elimination), what obstacles lie ahead and how might we address them.

This is an exciting period in food and waterborne parasitology, and we should capitalise on the current interest in this important field.

Keywords: Burden, control, foodborne transmission, lifecycles, waterborne transmission

OS – oral session

Session: [PS2] Food and Waterborne Parasitology (Journal of the EFP) Plenary Session

Identification of genetic determinants for *Toxoplasma gondii* oocyst environmental resistance

David Arranz-Solis[1], David Warschkau[2], Benedikt Fabian[2], Frank Seeber[2], Jeroen Saeij[3]

1 Faculty of Veterinary Science, Complutense University of Madrid, Spain
2 Mycotic and Parasitic Agents and Mycobacteria (FG16), Robert Koch Institute, Berlin, Germany
3 Pathology, Microbiology and Immunology department, School of Veterinary Medicine, University of California Davis, Davis, CA, 95616, USA

Abstract

Toxoplasma gondii oocysts exhibit remarkable environmental stability, withstanding numerous inactivation methods while remaining highly infectious. This crucial transmission stage of Toxoplasma is under-explored, because they are not cultivatable *in vitro* and difficult to access *in vivo*. Consequently, it is vital to uncover the genetic factors contributing to the robust environmental resistance of sporozoites. Our research aimed to determine if specific sporozoite proteins confer *Toxoplasma* oocysts resistance to environmental stresses. We focused on Late Embryogenesis Abundant (LEA)-related proteins, known for their protective roles against drought, salinity, and freezing in various organisms. Toxoplasma possesses four LEA genes located in a cluster (LEAc) on chromosome XII, all displaying traits of intrinsically disordered proteins. In vitro biochemical assays using recombinant proteins demonstrated that all four LEAs provided cryoprotection for Toxoplasma lactate dehydrogenase, and their overexpression in E. coli enhanced freezing resistance. Utilizing CRISPR/Cas9 technology, we created a LEAc knockout in the ME49 strain, compatible with feline hosts, and evaluated the sensitivity of these oocysts to environmental stresses relative to wild-type parasites. Our findings reveal, for the first time, the critical role of LEA proteins in oocyst resistance to high salinity, freezing, and desiccation. Future research involving individual or combined LEA gene knockouts is essential to pinpoint the specific LEAs necessary for this resistance.

Keywords: *Toxoplasma*, Oocysts, Sporozoite, Environmental resistance, Late Embryogenesis Abundant proteins

OS – oral session

Session: [PS2] Food and Waterborne Parasitology (Journal of the EFP) Plenary Session

Freshwater ecosystems - an underexplored reservoir of zoonotic parasites

Nikola Betić[1], Katarina Breka[2], Dragana Miličić[2], Aleksandra Uzelac[3], Vladimir Ćirković[3], Vesna Djordjević[1], Ivana Klun[3]

1 Institute of Meat Hygiene and Technology, Serbia
2 Faculty of Biology, University of Belgrade, Serbia
3 Institute for Medical Research, University of Belgrade, Serbia

Abstract

Freshwater ecosystems are understudied reservoirs of zoonotic parasites. Previously, *Pelophylax esculentus* complex frogs were revealed to be carriers of a dematiaceous hyphomycete - *Fonsecaea* sp., the causative agent of chromoblastomycosis and phaeohyphomycosis, neglected diseases in humans, and a rare basidiomycete - *Quambalaria cyanescens*, an opportunistic pathogen. To gain insight into the role of freshwater ecosystems in the transmission of zoonotic parasites to terrestrial hosts, copepods and branchiopods and surface water from the Sava and Danube rivers and the floodplains around Belgrade were sampled and screened for the presence of *Toxoplasma gondii* and *Giardia* spp. gDNA by PCR. Surface water (10 L) was concentrated by filtration through a 1.2 µm polycarbonate filter. Crustaceans were collected using nets. Total DNA was extracted from the water pellet and crustaceans using Trizol reagent and the 529bp RE and β-giardin were amplified using specific primers. *T. gondii* was detected in 6/8 freshwater samples, and in 1/4 crustacean samples (*Cyclops* sp.). *Giardia* spp. gDNA was not detected. All samples originated from freshwater ecosystems from areas which are heavily used for commercial and recreational purposes around Belgrade and are habitats for wildlife, thus providing opportunities for parasite exchange. Surface water in some areas is contaminated with *T. gondii* oocysts while copepods may be an underappreciated link in their transmission. As frogs carry pathogenic fungi, they could be important as vectors for zoonotic parasites due to their biphasic life cycle, and an investigation of frogs in the transmission of *Spirometr*a spp. is underway.

This research was supported by the Science Fund of the Republic of Serbia, #2424, Environmental Monitoring of Food and Waterborne Parasites - PARASITE_HUNTER.

Keywords: Freshwater ecosystems, zoonotic parasites, *Toxoplasma gondii*, *Giardia* spp., reservoirs

PS – poster session

Session: [PS2] Food and Waterborne Parasitology (Journal of the EFP) Plenary Session

Unusual food poisoning due to massive infestation with *Anisakis simplex* sensus lato

Manon Robert[1], Louise Durand[1], Carelle Koudougou[2], Florent Morio[3], Fakhri Jeddi[3], Patrice Le Pape[3], Rose- Anne Lavergne[3]

1 Laboratoire de Parasitologie et Mycologie, CHU de Nantes, F-44000 Nantes, France
2 Cabinet de gastro-entérologie, Clinique Jules Verne, 44300 Nantes, France
3 Nantes Université, CHU de Nantes, Cibles et médicaments des infections et de l'immunité, IICiMed, UR1155, F-44000 Nantes, France

Abstract

Anisakidosis is a food-borne parasitic disease due to the consumption of raw or undercooked fish or shellfish. This disease can manifest either as acute gastric forms, late intestinal forms, or allergic manifestations. Most infections are self-limiting in humans. We report two cases of food poisoning caused by *Anisakis simplex* (s.l.) following the consumption of homemade European hake (*Merluccius merluccius*) ceviche in a single family in 2021. Massive infestation in one of the two patients led to the removal of more than forty larvae by gastroscopy, performed two days after the consumption of contaminated meal, illustrating the singularity of this case. Based on morphological characteristics, larvae were identified as Anisakidae belonging to *A. simplex* (s.l.) using mitochondrial DNA cytochrome c oxidase 2 sequencing. The European hake, widely traded in commercial markets, is also known for its high prevalence of *Anisakis* spp. larvae infestation. Epidemiological data about human anisakidosis are still scarce in France, but 37 cases have been reported in a national survey conducted between 2010 and 2014 in Parasitology laboratories of university hospitals and collective food poisoning is probably underrated. These cases underline the importance of raising awareness of this disease in the general population and reminding the importance of cooking/freezing fish before raw consumption in case of homemade dishes.

Keywords: *Anisakis*, Anisakidosis, foodborne parasitic disease

PS – poster session

Session: [PS2] Food and Waterborne Parasitology (Journal of the EFP) Plenary Session

Synanthropic birds and rodents as indicators for *Toxoplasma gondii* oocyst contamination in urban and suburban environments

Aleksandra Penezić[1], Ilija Pantelić[1], Aleksandra Uzelac[2], Neda Bogdanović[1], Duško Ćirović[1], Ivana Klun[2]

1 University of Belgrade, Faculty of Biology, Serbia
2 University of Belgrade, Institute for Medical Research, Serbia

Abstract

Synanthropic rodent species are known reservoirs of *Toxoplasma gondii* and due to their diet may serve as indicators of environmental contamination with *T. gondii* oocysts. Although hooded crows (*Corvus cornix*) and rooks (*Corvus frugilegus*) are common resident omnivorous bird species of urban and suburban environments, that frequently feed off the ground, Their role as reservoirs and potential indicators of environmental contamination with *T. gondii* oocysts has been poorly investigated.

For this study, hearts were collected from 27 legally hunted synanthropic birds (n = 17 *C. cornix*, n = 10 *C. frugilegus*) and from 15 rodents obtained from rodent control in two suburban areas of Belgrade. Most birds (n = 19) and all rodents originated from an area near the Sava River with fewer human habitats, while the remaining samples originated from a residential area further away from the river. Total DNA was extracted using Trizol reagent and the presence of *T. gondii* gDNA was ascertained by amplification of the 529 bp repeat element. *Toxoplasma gondii* was detected in 12/15 (80%) rodents and 10/27 (37%) birds. In the area closer to the river with less human habitats, 6/17 hooded crows and 1/2 rooks harboured *T. gondii*, as did 3/8 rooks from the residential area. The results show that 40% of rooks and 35.3% of hooded crows are infected with *T. gondii*, indicating significant exposure to the parasite which suggests environmental contamination with oocysts. Preliminary investigation of surface water in the area confirmed the presence of *T. gondii* gDNA while studies in soil are currently underway. The results of these studies will show whether synanthropic birds can serve as indicator species for environmental contamination with *T. gondii* oocysts.

This research was supported by the Science Fund of the Republic of Serbia, #2424, Environmental Monitoring of Food and Waterborne Parasites- PARASITE_HUNTER.

Keywords: synanthropic birds, *Toxoplasma gondii*, oocysts, environment, indicators

PS – poster session

Session: [PS2] Food and Waterborne Parasitology (Journal of the EFP) Plenary Session

Trichinella infection in Southeastern Europe

Sasa Vasilev[1], Ivana Mitic[1], Laurentiu Mihai Ciupescu[2], Nikolay Lalkovski[3], Davor Balic[4], Jovana Stefanovska[5], Ani Vodica[6], Darko Despotovic[7], Budimir Plavsic[8], Gianluca Marucci[9]

1 University of Belgrade, Institute for the Application of Nuclear Energy – INEP, Banatska 31b, Belgrade, Serbia
2 Institute for Hygiene and Veterinary Public Health, Campul Mosilor 5, Bucuresti, Romania
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4 Croatian veterinary institute, Veterinary department Vinkovci, Croatia
5 Ss. Cyril and Methodius University, Faculty of veterinary medicine-Skopje, Macedonia
6 Food Safety and Veterinary Insitute, Tirana, Albania
7 Veterinary Institute of the Republic of Srpska "Dr. Vaso Butozan" Banja Luka, Republic of Srpska, Bosnia and Herzegovina
8 World Organization for Animal Health (OIE)
9 European Union Reference Laboratory for Parasite; Istituto Superiore di Sanità, Rome, Italy

Abstract

In several Southeastern European (SEE) countries, trichinellosis stands out as a significant foodborne zoonotic disease. New epidemiological data for 2019-2023 suggests a notable decrease in both human cases and the incidence of infected domestic pigs within SEE countries. Typically, untreated pork from backyard settings serves as a common source of human trichinellosis in Serbia, Bulgaria, and Romania. Untested wild boars constitute a crucial source of *Trichinella spiralis* infection in SEE, with three additional species, namely *T. britovi*, *T. nativa*, and *T. pseudospiralis*, also reported in these countries. The prevalence of *Trichinella*-positive wild boars remains consistent over the years, emphasizing the importance of educating hunters and consumers of wild game meat about the risks associated with consuming untested meat.

Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Grant No. 451-03-66/2024-03/200019

Keywords: *Trichinella* infection, Trichinellosis, Prevalence, SEE

PS – poster session

Session: [PS2] Food and Waterborne Parasitology (Journal of the EFP) Plenary Session

Parasitological contamination of arable soil in selected regions of Poland – preliminary research

Jolanta Zdybel[1], Jacek Karamon[1], Jacek Sroka[1], Ewa Bilska-Zając[1], Angelina Wójcik-Fatla[2], Piotr Skowron[3], Tamara Jadczyszyn[3], Jan Jadczyszyn[4], Tomasz Cencek[1]

1 Department of Parasitology and Invasive Diseases,National Veterinary Research Institute-State, 7 Partyzantów Avenue, 24-100 Puławy, Poland
2 Department of Health Biohazards and Parasitology, Institute of Rural Health, Lublin, Poland
3 Department of Plant Nutrition and Fertilization, Institute of Soil Science and Plant Cultivation State Research Institute, Puławy, Poland
4 Department of Soil Science Erosion and Land Protection, Institute of Soil Science and Plant Cultivation State Research Institute, Puławy, Poland

Abstract

The hygienic status of arable soils in developed countries has been unknown. In the presented study, preliminary investigation was undertaken to determine the contamination of arable fields in Poland with eggs of parasitic nematodes.

The study was conducted from autumn 2021 to spring 2022 in 5 provinces: Dolnośląskie (DS), Mazowickie (MZ), Podlaskie (PL), Pomorskie (PM) and Zachodniopomorskie (ZP). A total of 133 soil samples were collected. Parasitological examination of soil samples was carried out using the PN-Z-19006 method, with confirmed high sensitivity. Parasite eggs were found in a total of 67 samples, of which 56 samples contained eggs of roundworms of the genus *Ascaris* (an average of 3.29 eggs/100g of soil), 23 contained eggs of whipworms (an average of 1.22 eggs/100g), and 3 contained eggs of *Toxocara* (1 egg/100 g). The percentage of positive samples collected in autumn (53.57%) was higher than the percentage of positive samples collected in spring (48.05%), and this difference was statistically significant (Chi2 test p=0.002). Similarly, the average number of eggs of all three parasite species together in positive samples collected in autumn (3.43 eggs/100 g) was higher than the average number of eggs in samples collected in spring (2.90 eggs/100 g). This difference was statistically significant (Mann-Whitney test p=0.003834). Differences in the percentage of positive samples were also found depending on the region of origin of the samples. The highest percentage of positive samples was found in the PM (85.19%), which is characterized by a 2-5 times higher pig population per 100 ha of agricultural land than in other provinces. The differences in the percentage of positive samples between the PM and other provinces were statistically significant.

The conducted research confirms relatively frequent contamination of arable soils with eggs of parasitic intestinal nematodes, although the concentration of these eggs in the soil is generally low.

Keywords: parasite eggs, soil analysis, *Ascaris* spp., *Trichuris* spp., *Toxocara* spp.

PS – poster session

[SE1]

Alien and invasive host species and parasites

Session: [SE1] Alien and invasive host species and parasites

Rhynchophorus ferrugineus in Portugal: invasive pathways, phoretic mites, and genetic diversity

Inês Matos[1], Luis Filipe Rangel[2], Duarte Frade[3], Miguel Andrade[4], Paula Ramos[5], Maria João Santos[6], Camilo Ayra-Pardo[2]

1 CIIMAR / Portugal
2 CIIMAR/ Portugal
3 CCMAR-Algarve/ University of Algarve / Portugal
4 University of Coimbra / Portugal
5 IPMA/ Portugal
6 Porto University/ CIIMAR/ Portugal

Abstract

Rhynchophorus ferrugineus (RPW), a weevil originally from Southeast Asia (SeA), a protelean parasite, has spread through trade in infested palms and has become a global parasitic pest. This study investigates its invasion in Portugal using phylogenetic relationships and haplotype networks and examines its associated mites and morphological variation in the new areas. Between 2021 and 2023, 348 adult RPW were sampled in seven districts of Portugal (Braga, Viana do Castelo, Porto, Aveiro, Lisboa, Algarve, Madeira). These samples were measured, sexed, and inspected for mites, and DNA was extracted for genetic analysis. The sex ratio was 2.2:1 (female: male), except in Lisbon, where it was 4.1:1. Seven mite morphotypes were identified, with higher mite diversity in the northern districts than in the southern and insular districts. The study found a greater diversity of mite species in Portugal compared to previous studies. Genetic analyzes showed that the Portuguese RPW samples clustered with those from Europe, the Middle East, SeA, and South America, while a separate branch included samples from South Asia, East Asia, and SeA. The Madeira samples' branching from the Middle East suggests a pathway influenced by trade routes rather than natural migration. The presence of the widespread mite genus *Centrouropoda*, found on other *Rhynchophorus* species worldwide, suggests that the mites either migrate with the weevil or are already present in the new areas. The study concludes that the RPW populations in Portugal probably arrived through the Middle East–North Africa pathway. Further studies of mite communities could provide deeper insights into the establishment and spread of the species.

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Keywords: Red palm weevil, protelean parasite, invasive species, phoretic mites

OS – oral session

Session: [SE1] Alien and invasive host species and parasites

The impact of global change on parasites, vectors and vector-borne pathogens.

Hans-Peter Fuehrer

Institute of Parasitology, Department of Pathobiology, University of Veterinary Medicine Vienna, Austria

Abstract

Natural or human-driven environmental changes affect the ecological balance of hosts, vectors, and pathogens. Climate change will increase the impact of parasites and vector-borne diseases on human and animal health around the world by direct effects on the biology of the vectors and transmission cycles, or indirectly via socio-economic mechanisms affecting the sensitivity of humans and animals to these diseases and our capacity to prevent and control them.

Global Change Biology not only includes climate change and alien/invasive species but also landscape changes, loss in biodiversity and biological adaptions to changed environments. Therefore, an overview on this topic will be given, including invasive host species (like racoons) and parasites (like *Baylisascaris procyonis*), and alien vectors (like tiger mosquitoes).

Keywords: global change, alien species, invasive species

OS – oral session

Session: [SE1] Alien and invasive host species and parasites

Symbiotic species diversity can explain invasion success and stability of the host-parasite system

Volodimir Sarabeev[1,3], Juan Antonio Balbuena[2], Mikuláš Oros[3], Mykola Ovcharenko[4]

1 Department of Biology, Zaporizhzhia National University, Zhukovskogo 66, 69063 Zaporizhzhia, Ukraine
2 Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, PO Box 22 085, 46071 Valencia, Spain
3 Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, 04001 Košice, Slovak Republic
4 Institute of Biology and Science of Earth, Pomeranian University in Słupsk, Arciszewskiego 22b, 76200 Słupsk, Poland

Abstract

Using a macroecological framework, we aim to examine patterns of symbiotic biodiversity of native and invasive hosts to understand the mechanisms underlying the stability of the host-parasite system. For this purpose, we used our published dataset comprising 60 symbiotic species from 612 host individuals of 7 gammarid species, two native and five invasive, in the Baltic region of Poland. Our results suggest that both locality and host species are critical factors in determining the composition and abundance of symbiotic communities in gammarids. Locality appears to be a stronger determinant of the symbiotic community of gammarids than host species, whereas habitat conditions were a stronger determinant of the symbiotic communities than geographic distance. Comparative analysis of the diversity indexes showed that rich symbiotic communities of native gammarids paradoxically had low diversity indexes. We hypothesize that the rich and unequal symbiotic communities keep the host-parasite system of native gammarids in equilibrium. Strongly dominated symbiotic species with a high load per host individual may control the host population growth and stabilise a host-parasite system. Simplified symbiotic communities of the invasive hosts showed a relatively even distribution of species with moderate loads per individual, with heavily infected gammarids being rare in the population. It can be assumed that the survival rate of invasive gammarids is relatively high, facilitating their population growth and further spread. Complex communities of native gammarids, composed of many symbiotic species, can prevent explosive growth of host populations, while such mechanisms may be completely or partially reduced in invasive hosts.

The work is funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under project #09I03-03-V01-00017 and partially supported by the Slovak Research and Development Agency (APVV SK-CZ-RD-21-0078).

Keywords: *Gammarus pulex*, *Gammarus zaddachi*, *Gammarus roeselii*, *Gammarus tigrinus*, *Dikerogammarus villosus*

OS – oral session

Session: [SE1] Alien and invasive host species and parasites

Occurrence and initial distribution of *Baylisascaris procyonis* in wild raccoons in France

Gérald Umhang[1], Alain C. Frantz[2], Hubert Ferté[3], Christine Fournier Chambrillon[4], Manon Gautrelet[5], Jean- Marc Boucher[1], Christophe Caillot[1], Franck Boué[1], Céline Richomme[1]

1 *ANSES, Nancy laboratory for rabies and wildlife, Malzéville, France.*
2 *Musée National d'Histoire Naturelle, Luxembourg, Luxembourg*
3 *Université de Reims Champagne–Ardenne, Reims, France*
4 *GREGE, Villandraut, France*
5 *Université de Reims Champagne–Ardenne, Reims, France; GREGE, Villandraut, France*

Abstract

Baylisascaris procyonis is the etiological agent of larva migrans in humans (neural, ocular, visceral or asymptomatic), which can lead to severe neurological sequelae and rarely fatal cases. The raccoon (*Procyon lotor*) is the primary definitive host of the nematode Transmission to paratenic hosts (such as rodents, birds and rabbits) or to humans occurs through accidental ingestion of embryonated eggs. The presence of *B. procyonis* in wild raccoons has been reported in several countries in Europe.

In France, raccoons are currently established in three separate and expanding populations, in the north-east, central (Auvergne) and south-west of the country as the result of three independent voluntary or accidental releases of a few individuals since the 1960s, the 1990s and 2007, respectively. Recently, we found no evidence of *B. procyonis* in 92 raccoons in the south-west population. In the north-east population, among 206 only a single infected animal was identified at the edge close to Belgian and Luxemburg borders. Genetic analyses confirmed that this raccoon originated from the local genetic population, while the same three approaches showed that the worms were genetically distinct from the two nearest known populations in Germany and the Netherlands. Investigations in Auvergne started in 2023 are still ongoing. For now, 44 raccoons have already been caught by trappers, frozen and sent to the laboratory. Necropsy and opening of their intestines revealed the presence of *B. procyonis* in 26 individuals (59.1%) from 20 different municipalities in the region. Genetic analyses of both raccoons and worms will be realized.

Investigations of the three French raccoon populations for the presence of *B. procyonis* has revealed three different epidemiological situations. Continuation of these studies, combining parasitological and genetic analyses, is necessary to gain a better understanding of the epidemiological situations and to prevent potential human cases.

Keywords: *Baylisacaris procyonis*, wild raccoons, larva migrans, invasive host species

OS – oral session

Session: [SE1] Alien and invasive host species and parasites

Molluscs alien to Europe as hosts of trematodes

Elżbieta Żbikowska, Anna Stanicka, Anna Cichy

Nicolaus Copernicus University in Toruń

Abstract

Molluscs play a crucial role in the transmission of trematodes. Snails serve as the site for producing thousands of cercariae – larvae that actively infect vertebrates and invertebrates. Additionally, snails and bivalves serve as hosts, accumulating metacercariae that are transported through the food chain to the final hosts, including farm animals and humans. Relatively rare, molluscs may also serve as sites for the sexual reproduction of parasites. The critical role of molluscs in the life cycle of Digenea results from the high specificity between the parasite and the first intermediate host. This fact means that the spread of host molluscs determines the extent of parasite species. Interest in the interaction between alien molluscs and parasites in the invaded areas from a short-term perspective does not produce fruitful results. However, the detailed search for traces of new associations allows a better understanding of the long-term effects on the ecosystem. We have been studying natural and experimental invasion of trematodes to aliens in Europe molluscan species: *Potamopyrgus antipodarum, Sinanodonta woodiana*, *Dreissena polymorpha*, *Dreissena bugensis* and *Corbicula fluminea*. We checked whether individuals of these species collected in Europe (outside the limits of their natural range) may be infected with some trematodes. We studied both natural and experimental invasion. All tested species were invaded by Aspidogastrea or Digenea forms. Infected molluscs were first or second intermediate hosts and, in some cases, were infected with pre-adult forms of parasites. Some experiments with parasite invasion have succeeded. Only the use of echinocercariae has resulted in an effective invasion. The results show a potential and real possibility of building associations between alien species of molluscs and native parasite fauna. Knowing new associations' long-term effects requires including the parasitic thread in environmental monitoring studies.

Keywords: molluscs, Digenea, alien species, new association

OS – oral session

Session: [SE1] Alien and invasive host species and parasites

Alien to Europe *Potamopyrgus antipodarum* (Gray, 1843) snails reduce the infection of *Echinoparyphium aconiatum* (Trematoda: Digenea) metacercariae in native lymnaeids.

Anna Cichy, Anna Stanicka, Elżbieta Żbikowska

Nicolaus Copernicus University in Toruń

Abstract

Potamopyrgus antipodarum (Gray, 1843), is one of Europe's most effective colonizers of inland waters. The wide expansion of this species outside the native range is due to its large tolerance to environmental factors and the lack of natural enemies. The invasive status of *P. antipodarum* is still debatable, although most studies indicate its negative impact on the native free-living fauna, including molluscs. However, this is not so obvious if we include parasites in the interactions between alien and native snails. Our experimental study aimed to check (i) whether the presence of *P. antipodarum* reduces the infection of the digenean trematode, *Echinoparyphium aconiatum*, in native *Radix* spp. snails, and (ii) whether the water temperature and the biomass of the alien host impact the expected 'dilution effect'. According to this hypothesis, an increase in biodiversity may promote lower prevalence in hosts differing in susceptibility to infection. We found that the survival of *E. aconiatum* cercariae decreased with increasing temperature, while cercarial infectivity increased with temperature. There were no significant differences between the ability of *E. aconiatum* cercariae to enter both the native host and alien host and transform into metacercariae. Both snail species had similar susceptibility to infection with *E. aconiatum* cercariae; however, the number of echinostome metacercariae in *Radix* spp. decreased with an increase in the biomass variants of *P. antipodarum*. Our results indicate that in multispecies systems, including hosts and parasites, the presence of the New Zealand mud snails may positively impact native *Radix* spp. communities by reducing the prevalence of *E. aconiatum*. Additional research is necessary to determine the further fate of *E. aconiatum* metacercariae in the snail - whether and how long echinosotme metacercariae survive inside *P. antipodarum* and whether they remain invasive to the final host in the life cycle of this parasite (waterfowl).

Keywords: alien species, *Potamopyrgus*, Digenea, dilution effect, metacercariae

PS – poster session

Session: [SE1] Alien and invasive host species and parasites

The role of raccoon latrines in the transmission of the nematode *Baylisascaris procyonis* to native vertebrate species

Marcin Popiołek, Katarzyna Buńkowska-Gawlik, Marlena Zawiślak, Lilla Marcickiewicz, Natalia Kolenda

Department of Parasitology, University of Wrocław

Abstract

The raccoon roundworm, *Baylisascaris procyonis*, is one of the most significant and dangerous parasites transmitted by the invasive raccoon to the recently colonised territory. This nematode, while having raccoon as a definitive host, is also associated with larva migrans in a number of mammal and bird species, causing rapidly progressing clinical conditions, most often neurological in nature. Invasive eggs of *B. procyonis* are deposited with faeces in latrines, which may represent important transmission hotspots for this parasite to native smaller vertebrate fauna. It has been hypothesised that granivorous mammals (e.g. rodents) would be at increased risk of *B. procyonis* infection when foraging from the ground or in litter, as their migration routes routinely include areas where raccoons locate latrines. Similarly, grain-eating birds may be attracted to these latrines by the presence of undigested seeds in the faeces. Surveys on the presence of *B. procyonis* eggs in raccoon faeces were conducted from March 2022 to March 2024 in forested polders on the Oder River near Kostrzyn nad Odrą (western Poland). A total of 275 faecal samples were collected and analysed. In addition, 11 selected latrines were continuously monitored by fototraps from November 2022 to March 2024. Eggs of *B. procyonis* were found in 72 samples (26.2%) with an average of 19.8 (range: 1-143) per sample. The analysis of images and videos captured using fototraps revealed a high level of foraging activity in latrines, with both small mammals (mainly rodents) and birds exhibiting frequent visits to these sites. The presence of these animals at these sites may result in infection with *B. procyonis*. These infections may have a long-term impact on the populations of native mammals and birds and promote the spread of this parasitosis.

Keywords: raccoon, parasites, latrines, transmission

PS – poster session

[SE2]

Biology and taxonomy of helminths

Session: [SE2] Biology and taxonomy of helminths

Characterization of extracellular vesicles from three zoonotic nematodes: *Anisakis, Ascaris* and *Dirofilaria*

Serena Cavallero[1], Ilaria Bellini[1], Simona Gabrielli[1], Marco Pombi[1], Luigi Venco[2], Antonio Varcasia[3], Ettore Napoli[4], Emanuele Brianti[4], Claudia Chiovoloni[1], Silvia Rondón[1], Agostina Pietrantoni[5], Claudia Tamponi[3], Anna Kashkanova[6], Federica Rinaldi[7], Maria Gioia Fabiano[7], Eleonora D'Intini[7], Stefano D'Amelio[1]

1 Department of Public health and infectious diseases, Sapienza University of Rome, Italy
2 Ospedale veterinario Città di Pavia, Pavia, Italy
3 Department of Veterinary Medicine, University of Sassari, Sassari, Italy
4 Department of Veterinary Sciences, University of Messina, Messina, Italy
5 Core facilities, Istituto Superiore di Sanità, Rome, Italy
6 Max Planck Institute for the Science of Light, Erlangen, Germany
7 Department of Drug Chemistry and Technology, Sapienza University of Rome, Rome, Italy

Abstract

The role of helminths extracellular vesicles (EVs) in the pathogenesis of parasitoses is a topic of great interest and applicative potentiality [Drurey et al., 2021. Mol Immunol, 137:124-133], being nanoparticles transporting bioactive molecules. However, hurdles remain to describe specific physical properties, heterogenicity and functions due to challenges in EVs isolation, characterization of specific markers and functional studies. Here, we compared precipitation and size exclusion chromatography isolation methods for efficacy in separating and recovering EVs released by *Ascaris suum, Anisakis* and *Dirofilaria* to infer useful aspects for downstream applications (i.e., dose/response in functional studies,). EVs were characterized using classic Nanoparticle Tracking Analyses (NTA), new Interferometric Nanoparticle Analysis (iNTA) and Dynamic Light Scattering (DLS). Morphology was evaluated by Scanning and Transmission Electron Microscopy (SEM, TEM). Adults of *A. suum*, *Anisakis* L3 and adults of *D. immitis* were collected from pigs, fish and dog, respectively, and cultured to collected media every 24h. EVs were isolated and characterized in terms of size, concentration, inner water content, zeta potential and protein amount. Thus, to assess the potential influence of the protein corona, EVs were treated with Proteinase K. The two isolation methods provided similar particles and protein concentrations, but a very different volume of recovered fractions. Precipitation presented the higher degree of protein amount which impeded the use of nanopore based methods. Interestingly, *Ascaris* EVs were bigger than *Anisakis* EVs, probably due to a larger protein corona, according to TEM, SEM, and the refractive index. The iNTA revealed 5-20% of non-water content, suggesting EVs biological cargo. The methodology to select when approaching to EVs is increasingly important to plan future research lines, especially in parasitology, where the knowledge is very limited.

Keywords: *Anisakis*, *Ascaris*, *Dirofilaria*, extracellular vesicles, protein corona

OS – oral session

Session: [SE2] Biology and taxonomy of helminths

Morphological and phylogenetic analysis of *Raillietina*, the most common cestode in indigenous chickens (*Gallus gallus domesticus*) in Bangladesh

Tanbin Rubaiya Siddiqui, Md. Razibul Hoque, Babul Chandra Roy, Anisuzzaman Anisuzzaman, Mohammad Zahangir Alam, Mst. Sawda Khatun, Anita Rani Dey

Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Abstract

Raillietina spp. (Cestoda: Davaineidae), the most common cestodes in indigenous chickens, cause a substantial production loss in poultry industry in Bangladesh. Here, we estimate the prevalence, confirm the species and determine the genetic pattern of species of *Raillietina* using molecular tools isolating the parasites from indigenous chicken reared in scavenging system in Bangladesh. We collected and examined 375 chickens from different household and isolated and identified the worms. For the molecular study, DNA was extracted from adult parasites, amplified *ITS-2* and *ND-1* genes, sequenced and analyzed. Out of 375 samples, 270 (70.0%) were found to be infected with one or more species of *Raillietina* with an average burden of 10.46±0.56. Three species of *Raillietina*, namely, *R. echinobothrida* (41.1%), *R. tetragona* (52.8%) and *R. cesticillus* (37.9%) were identified microscopically on the basis of their morphological features. Grossly, *R. echinobothrida* (15.58±1.01 cm), *R. tetragona* (18.39± 0.7 cm) were larger than *R. cesticillus* (3.96± 0.13cm). The length and width of scolex, sucker and rostellum were also measured. Among different factors, age, farming nature and flock size were significantly ($p<0.05$) associated with *Raillietina* infections. We confirmed three species such as *R. echinobothrida, R. tetragona* and *R. cesticillus* by amplifying *ITS-2* and *ND-1* genes. To further validate our data, the sequences of *R. echinobothrida*, *R. tetragona* and *R. cesticillus* searched and the studied sequences were 99.63% - 100% identical with the reference sequences of *ITS-2* gene. The phylogenetic analysis of *ITS-2* and *ND-1* gene from this study formed unique cluster with other *R. echinobothrida*, *R. tetragona* and *R. cesticillus* ascertaining the conformation of the species. This is the first confirmation of species of *Raillietina* along with their factors governing the prevalence of the species, which will be helpful for the formulation of a control strategy.

Keywords: *Raillietina*, ITS-2, ND-1, phylogeny

OS – oral session

Session: [SE2] Biology and taxonomy of helminths

The tapestry of the Middle East: diversity and phylogeny of *Dactylogyrus* reveal the evolutionary pattern of parasites and their cyprinoid fish hosts

Farshad Nejat[1], Michal Benovics[1], Cunyet Kaya[2], Sadi Aksu[3], Asghar Abdoli[4], Ali Serhan Tarkan[5], Andrea Šimková[1]

1 Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic
2 Faculty of Fisheries, Recep Tayyip Erdogan University, 53100 Rize, Turkey
3 Vocational School of Health Services, Eskişehir Osmangazi University, Büyükdere Meşelik Yerleşkesi, 26040 Eskişehir, Turkey
4 Department of Biodiversity and Ecosystem Management, Environmental Science Research Institute, Shahid Beheshti University, Shahid Shahriari Sq. Velenjak, 1983969411 Tehran, Iran
5 Department of Basic Sciences, Faculty of Fisheries, Muğla Sıtkı Koçman University, 48000 Menteşe, Muğla, Turkey

Abstract

Several recent studies explored the diversity and phylogeny of host-specific *Dactylogyrus* (Monogenea), gill parasites of cyprinoid fish, within the peri-Mediterranean region. However, the diversity and phylogeny of *Dactylogyrus* species in the Middle East are neglected. To reveal historical dispersion of cyprinoid fish through the phylogenetic relationships of their associated *Dactylogyrus* species, we aimed to reconstruct the phylogeny of *Dactylogyrus* spp. including the species collected in the Middle East. Several field trips in Iran, Iraq, and Turkey were conducted, and the gills of 89 cyprinoid species were examined for the presence of *Dactylogyrus* species. Seventy-two *Dactylogyrus* species were identified. *Dactylogyrus vistulae*, with 24 host species, had the widest host range in the Middle East. Concerning *Dactylogyrus* diversity at individual host species, *Squalius* spp. harbored the most *Dactylogyrus* species on them. Our sampling revealed overall sixteen potentially new *Dactylogyrus* species for science. The partial fragments of 18S rDNA and 28S rDNA, and the complete ITS1 region were used for phylogenetic reconstruction. Several *Dactylogyrus* species exhibited high genetic intraspecific variabilities. Phylogenetic reconstruction using Middle Eastern *Dactylogyrus* species and those corresponding to different geographical regions retrieved from GenBank revealed eight major clades. Middle Eastern species were present in five clades alongside European, North African, and East Asian species, thus confirming the significant role of Middle East in *Dactylogyrus* diversification. Mapping morphological characters of haptor onto the phylogeny revealed the evolution of parasite haptor adaptation to their host historical dispersal route.

This study was supported Czech Science Foundation, project no. GA20-13539S.

Keywords: host specificity, historical dispersion, diversification, cyprinoid fish

OS – oral session

Session: [SE2] Biology and taxonomy of helminths

Environmental and anthropogenic drivers of parasite distribution throughout Holocene: a meta-analysis

Karina Vanadzina, Kenneth De Baets

University of Warsaw

Abstract

Parasitism is among the most prevalent survival strategies on Earth, with up to 40% of all species classified as parasitic. While parasites are integral to the healthy functioning of modern ecosystems, broad-scale investigations of parasite occurrence and life-history traits using historical data remain rare. Understanding how parasite distributions have shifted in response to past changes in climate conditions and availability of hosts could improve our ability to predict future spread of parasites and related diseases. Here, we compiled information from paleoparasitological and palynological studies that report resistant parasite finds throughout the Holocene at high spatial and temporal resolution, but have seldom been used in an evolutionary context. The dataset comprises over 2000 parasite occurrences primarily derived from sediment samples and coprolites found in human settlements and burials. It encompasses a wide range of localities, cultures, and time periods, with parasite eggs being the most common type of evidence for the presence of parasites. Our preliminary analysis shows that parasite finds are concentrated in the late Holocene (4200 yBP onwards), with a steep increase at the start of the Middle Ages. It is dominated by nematodes (genera *Ascaris* and *Trichuris*) and flatworms (genera *Fasciola* and *Taenia*), likely owing to the favourable preservation conditions of their eggs. Using a variety of modelling approaches, we identify human population density and temperature as significant drivers of distribution of the most common genera of intestinal helminths. The project additionally aims to provide a comprehensive and up-to-date database of parasite finds throughout Holocene for the wider scientific community.

Keywords: Holocene, parasite occurrence, anthropogenic change, climate change, historical record

OS – oral session

Session: [SE2] Biology and taxonomy of helminths

Update on systematics of deer large lungworms: the last missing piece of puzzle in discussion on validity of *Dictyocaulus eckerti*

Anna Maria Pyziel

Department of Food Hygiene and Public Health Protection, Institute of Veterinary Medicine, Warsaw University of Life Sciences – SGGW, Warsaw, Poland

Abstract

Nematodes of the genus *Dictyocaulus* locate in the respiratory tract of artiodactylids leading to severe lung pathology of their hosts. Although, dictyocaulosis is very common in various deer species, the lungworms systematics is confusing. Since the first systematic revision of the genus, *D. eckerti* Skrjabin, 1931 (described from a reindeer) has been maintained as a collective species for cervids. This arrangement was actual until *D. capreolus* Gibbons and Höglund, 2002 from roe deer and moose, as well as *D. cervi* Pyziel et al, 2017, and *D. skrjabini* Pyziel et al., 2023 from red deer were described and separated from *D. eckerti*. Both, *D. cervi* and *D. skrjabini* were described using morphological and molecular approach based on ITS2, SSU ribosomal RNA and mitochondrial cox1 and cytB genes. Our phylogenetic reconstruction revealed clear distinctions between *D. cervi*, *D. skrjabini* and *D. eckerti*. Up to date, *D. cervi* was found in red deer in Sweden and Italian Alps, as well as in a Rocky Mountain deer in the USA, moose in Poland and Sweden, and fallow deer in Australia; whereas *D. skrjabini* was diagnosed in fallow deer in Sweden and Australia. Moreover, the mixed infections of both the species were observed for the first time in 2 fallow deer in Australia. The lungworms of nucleotide sequences corresponding to *D. cervi* were also found in Hungary, and those of *D. skrjabini* in Italy. According to the authors from Australia, the previous reports on *D. eckerti* in red deer in New Zealand and other cervids in Europe were most likely also *D. cervi*. A fatal case of dictyocaulosis caused by *D. cervi* was observed in a Rocky Mountain deer, whereas infected red deer from Poland manifested the signs of interstitial pneumonia, bronchitis and bronchiolitis. With regard to the above the need arises to verify the large lungworms infecting reindeer as the *D. eckerti* was described basing on the specimens isolated from reindeer.

Keywords: large lungworms, cervids, systematics of *Dictyocaulus*

OS – oral session

Session: [SE2] Biology and taxonomy of helminths

Molecular identification and high genetic diversity of *Haemonchus* spp. in domestic ruminants of Greece

Konstantinos Arsenopoulos[1], Styliani Minoudi[2], Isaia Symeonidou[1], Alexandros Triantafyllidis[2], George Fthenakis[3], Elias Papadopoulos[1]

1 Laboratory of Parasitology and Parasitic Diseases, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
2 Laboratory of Genetics, Development and Molecular Biology, School of Biology, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
3 Veterinary Faculty, University of Thessaly, 43100 Karditsa, Greece

Abstract

The aim of the present study was to identify the species of the sympatric populations of the genus *Haemonchus*, a nematode parasite which infects ruminants (sheep, goats, cattle, buffaloes) from different regions of Greece (continental and insular) by molecular methods. At the same time, an attempt was made to identify the possible subpopulations of *Haemonchus* spp. in Greece, to investigate their phylogenetic relationships as well as to determine the genetic diversity of each population. Two hundred eighty-eight worms of the genus *Haemonchus* were processed by molecular methods; of which 96 were collected from sheep, 96 from goats, 48 from cattle and finally, 48 from buffaloes. A fragment of 321 base pairs of the second internal transcribed spacer gene of nuclear DNA was amplified for species identification which, after Blast analysis, revealed that they

belonged to *H. contortus*. A fragment of 820 base pairs of the subunit 4 of the nicotinamide dehydrogenase gene of mitochondrial DNA was amplified for genetic diversity analysis. and compared it to 169 available *H. contortus* sequences. Our mitochondrial subunit 4 of the nicotinamide dehydrogenase sequences of *H. contortus* were classified into 140 haplotypes and the values of the average nucleotide and haplotype diversity were lower compared to the respective values derived from Italy, Malaysia, USA and China. Phylogenetic analysis of the subunit 4 of the nicotinamide dehydrogenase gene revealed a clear grouping of the Greek haplotypes when compared with Asian ones and, at the same time, no profound grouping of the same haplotypes regarding to their different hosts and geographical origin within different regions of Greece. The forementioned findings confirmed that *H. contortus* prevails in our country and can infect all species of ruminants, without geographical boundaries, when the right conditions (i.e., common grazing) are created.

Keywords: *Haemonchus contortus*, genetic diversity, phylogenetic relationship, ITS2 sequence, ND4 gene

PS – poster session

Session: [SE2] Biology and taxonomy of helminths

Molecular screening of tapeworms with COI and NADH genes from Croatian wildlife

Ema Gagović[1], Ana Kajmić[2], Daria Jurković[1], Šimun Naletilić[1], Tibor Andreanszky[1], Krunoslav Pintur[3], Relja Beck[1]

1 Croatian Veterinary Institute, Croatia
2 Faculty of Veterinary Medicine Zagreb, Croatia
3 Karlovac University of Applied Science, Croatia

Abstract

Cyclophyllidean and Diphyllobothroid tapeworms obligatorily parasitize numerous mammalian species, including herbivores, domestic animals and humans. Many of the potential definitive hosts have been increasing in number in the last decades. Despite their importance only few studies have been performed in Croatia so far and in genaral data are scarce. In the current study tapeworms from intestines of red foxes, golden jackals, brown bears, europen badgers, stone martens, europen pine martens, hedgehogs, dogs and cats were morphologicaly identified and from each further genotyping was performed by sequencing of portion of COI and NADH genes. In total 162 tapeworms and larval stages were succesfully amplified and sequencing. After BLAST anlysis at least 15 species were confirmed including *Mesocestoides litteratus*, *M. lineatus*, *Dipylidium caninum*, *Echinococcus canadensis*, *E. granulosus*, *E. multilocularis* and sevaral *Taenia* species including *T. arctos*, *T. crassiceps*, *T. hydatigena*, *T. krabbei*, *T. martis*, *Hydatigera kamiyai*,*T. polyacantha*. In intestines of two golden jackals and stone marten the plerocercoids were found, which sequnces of COI reveled 98% similarity to *Spirometra mansoni* and 96.38% with *S. erinaceieuropaei*. From cats, stone marten and golden jackal three haplotypes of *Joyeuxiella pasqualei* were recorded showing similarity from 87–99%. In wild boars larval stages of *E. canadensis*, *T. hydatigena* and *E. multilocularis* were present, while in roe deer *T. hydatigena* and *T. krabbei*. Given that more sequences showed a similarity of 75% to 85% with known sequences additional research is needed to clarify the real number of species in Croatia. With the present research, we have shown the real diversity of tapeworms, which in a way was to be expected considering the geographical divergence and different ecosystems of Croatia.

Keywords: molecular analysis, tapeworms, wildlife, Croatia

OS – oral session

Session: [SE2] Biology and taxonomy of helminths

The role of temperature and salinity in hatching success of *Contracaecum rudolphii* A and B (Nematoda: Anisakidae)

Marialetizia Palomba[1], Beatrice Belli[2], Gianpasquale Chiatante[1], Marta Favero[1], Giuseppe Nascetti[1], Daniele Canestrelli[1], Simonetta Mattiucci[2]

1 Department of Ecological and Biological Sciences, Tuscia University / Viterbo, Italy
2 Department of Public Health and Infectious Diseases, Section of Parasitology, Sapienza University of Rome / Rome, Italy

Abstract

Egg hatching in parasitic nematodes is complex and poorly understood process despite its significance. Indeed, eggs act as protective barriers and vehicles for the first larval stage to initiate infection (Mkandawire et al., 2022). Abiotic factors, such as water salinity and temperature, are crucial cues for anisakid egg hatching. Currently, there is no knowledge about the hatching success of anisakids *Contracaecum rudolphii* A and *C. rudolphii* B eggs. Previous studies suggest these species have life cycles respectively adapted to brackish and freshwater environments. They show significant different proportions in both their definitive host, i.e. the great cormorant *Phalacrocorax carbo sinensis* and intermediate fish hosts from those different ecosystems (Mattiucci et al., 2020). This study investigates how temperature and salinity influence the hatching success of *C. rudolphii* A and B eggs, shedding light on their life cycles in the two different aquatic ecosystems.
Experiments were performed *in vitro* using eggs from genetically identified female of both species. Eggs were placed in water with salinity from 0 to 70 psu and temperatures of 5°C, 13°C, 20°C to simulate natural conditions and 32°C to mimic a heatwave scenario.
Eggs from both species successfully hatched in freshwater at 13°C, 20°C, 32°C, but not at 5°C. The hatching duration varied, with the shortest at 32°C (3 days) and the longest at 13°C (14 days). Significant differences in salinity tolerance were observed: *C. rudolphii* A hatched successfully at salinities from 0 to 70 psu, while *C. rudolphii* B hatched only within 0 to 20 psu. These findings suggest distinct adaptive strategies of *C. rudolphii* A and B to saline and freshwater environments, respectively. These adaptations likely result from evolutionary pressures shaping their physiological traits, underlying their differential distribution in fish and cormorants from these aquatic ecosystems.

Keywords: *Contracaecum rudolphii* (s.l.), eggs hatching, salinity, temperature

PS – poster session

Session: [SE2] Biology and taxonomy of helminths

Molecular diversity of the genus *Ichthyocotylurus* (Digenea, Strigeidae) from gulls (Laridae) in central Europe

Julia Gabrysiak[1], Gerard Kanarek[2], Grzegorz Zaleśny[3], Joanna Hildebrand[1]

1 Department of Parasitology, Faculty of Biological Sciences, University of Wrocław, Poland
2 Ornithological Station, Museum and Institute of Zoology, Polish Academy of Sciences, Gdańsk, Poland
3 Department of Systematic and Ecology of Invertebrates, Wrocław University of Environmental and Life Sciences, Poland

Abstract

Ichthyocotylurus Odening, 1969 is a small group of holarctic digeneans in the family Strigeidae, which adults develop within piscivorous birds mainly gulls. The current consensus recognizes four species: *I. pileatus* (Rudolphi, 1802), *I. erraticus* (Rudolphi, 1809), *I. platycephalus* (Creplin, 1825) and *I. variegatus* (Creplin, 1825). *Ichthyocotylurus* is closely related to the genus *Cotylurus* Szidat 1928, which was originally a part of. The main distinguishing feature of both genera, except cercariae morphology is their specificity to the second intermediate hosts, which for *Ichthyocotylurus* are fish, and for *Cotylurus* are leeches or snails. However, for some *Cotylurus* species, such as *C. raabei* Bezubik, 1958, the status of the second intermediate host is still unknown. Given that *C. raabei* is molecularly the most distinct from the remaining members of the genus, and some morphological and ecological features are similar to those observed in *Ichthyocotylurus*, it may turn out to exhibit a life-cycle closure strategy that has not yet been discovered in *Cotylurus*.

We examine adults of *Ichthyocotylurus* found in 19 individuals of five gull species (*Larus cachinnans, L. argentatus, L. canus, L. michahellis, L. marinus*). In total we isolated 335 strigeid flukes, which morphologically have been classified to the genus. DNA analysis was performed on 23 selected individuals. Analysis using sequences available in Genebank confirmed that they all group within the genus. The variability of the obtained CO1 sequences is 9.4%. In conclusion, the Polish fauna of *Ichthyocotylurus* is represented here by three taxa.

This preliminary study provides extensive data on molecular diversity of *Ichthyocotylurus* species derived from gulls in Poland and will allow the initiation of research on the species diversity of Strigeidae metacercaria in fish, as well as the continuation of research and consideration on the actual affiliation of *Cotylurus raabei*.

Keywords: Digenea, Strigeidae, *Ichthyocotylurus*, gulls

PS – poster session

Session: [SE2] Biology and taxonomy of helminths

Freshwater parasite diversity along a gradient of habitat degradation: a case study from Cambodia, South-Est Asia.

Simona Georgieva[1], Claudia Bommarito[2], Marine Combe[2], Rodolphe Elie Gozlan[2]

1 Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
2 ISEM, University of Montpellier, CNRS, IRD, Montpellier, France

Abstract

The tropics are biodiversity hotspots hosting an extraordinary number of species. Compared to temperate regions, they are believed to be more resistant to stressors, including global warming, habitat alteration, pollution, and species invasion. A higher native biodiversity results in increased competition, predation, and parasitism. Despite their importance as disease agents for a significant part of the parasite species, our understanding of their natural diversity, host associations, and life cycle strategies is still far from complete. As part of a comprehensive survey on the "dilution effect", i.e., biodiversity's role in reducing the emergence of infectious diseases, we characterized the larval trematode diversity in diverse freshwater snail hosts across a gradient of anthropogenic habitat modification in Cambodia. Larval trematodes recovered were genetically characterised with multiple markers, further used to infer species identities and conduct family-wide phylogenies. We recovered a total of 13 trematode species. Matching sequence data led to the elucidation of four life cycles. A higher overall prevalence of infection was detected in moderately disturbed habitats. Our study provides the first characterisation of the larval digenean diversity across an anthropisation gradient in a biodiversity hot spot region. It comprises an essential contribution to understanding disease outbreaks that would help prevent various zoonotic disease emergence.

This study was supported by The University Agency of the Francophonie (AUF), the H2020 Biodiversity Conservation to Mitigate the risks of emerging infectious diseases (BCOMING HORIZON-CL6-2021-BIODIV-01-11) and PREZODE (CZZ3222AfriCam) projects.

Keywords: Trematoda, diversity, phylogeny, anthropisation, Cambodia

PS – poster session

Session: [SE2] Biology and taxonomy of helminths

Phylogenetic inference of *Echinococcus multilocularis* by whole mitochondrial sequencing

Severine Lallemand[1], Jorge Oyhenart[2], Benoit Valot[1], Romain Borne[1], Louis Bohard[3], Gerald Umhang[4], Jacek Karamon[5], Sergey Konyaev[6], Caroline Ronnberg[7], Bruno Gottstein[8], Delphine Weil-Verhoeven[9], Carine Richou[9], Solange Bresson-Hadni[10], Laurence Millon[10], Anne Pauline Bellanger Clerget[10], Jenny Knapp[10]

1 UMR CNRS 6249 Chrono-Environnement – University of Franche-Comté – 16 Route de Gray, 25030 Besançon cedex, France
2 Instituto de Ciencias de la Tierra y Ambientales de La Pampa, Consejo Nacional de Investigaciones Científicas y Técnicas – Facultad de Ciencias Naturales Universidad Nacional de La Pampa, La Pampa, Argentina
3 Department of Infectiology, University Hospital of Besançon, 25030 Besançon, France
4 INTERFAS Unit, National Reference Laboratory for Echinococcus spp., Rabies and Wildlife Laboratory, ANSES, Malzéville, France
5 Department of Parasitology and Invasive Diseases, National Veterinary Research Institute, Puławy, Poland
6 Institute of Systematics and Ecology of Animals, SB RAS, Novosibirsk, Russia
7 Public Health Agency of Sweden, Department of Microbiology, Unit for Parasitology, Solna, Sweden
8 Institute of Infectious Diseases, Faculty of Medicine, University of Bern, CH-3012, Bern, Switzerland
9 Department of Hepatology and Intensive Cares, University Hospital of Besançon, 25030 Besançon, France
10 Department of Parasitology-Mycology, Reference National Centre for Echinococcoses, University Hospital of Besançon, 25030 Besançon, France

Abstract

Alveolar echinococcosis (AE) is a rare but severe disease that affects more than 18,000 people per year through the Northern hemisphere, with an epidemiology that highly contrasts among areas within dispersed endemic foci. The complete sequencing of the mitochondrial genome of the etiological agent of AE, *Echinococcus multilocularis,* has made it possible to study the genetic diversity of the parasite and its spatial and temporal evolution. A collection was established with 113 samples from Europe, Asia, and North America with human and intermediate and definitive host animal parasites. The whole mitochondrial genome was obtained by one uniplex and two triplex PCR to cover the 13,738 bp of the mitogenome and then sequenced the amplicons with Illumina technology. Three major haplogroups were found: HG1, which clustered samples from Saint-Lawrence Island (Alaska), Yakutia (Russia) and Svalbard, HG2, with samples from Asia, North-America and Europe, and HG3, subdivided into three micro-haplogroups. HG3a included samples from North America and Europe, whereas HG3b and HG3c only include samples from Europe. In France, HG3a included samples from patients more recently diagnosed in a region outside the historical endemic area. A fourth putative haplogroup, HG4, with a single sample from the Lake Baikal region was also found. The increased discriminatory power of the complete sequencing of the *E. multilocularis* mitogenome has made it possible to highlight four haplogroups, in line with the clusters found in the literature, but also three micro-haplogroups in France.

Keywords: Echinococcus multilocularis, whole genome sequencing, phylogenetic inference, haplogroup, mitogenome

PS – poster session

Session: [SE2] Biology and taxonomy of helminths

First record of *Flamingolepis liguloides* in *Artemia parthenogenetica* from a Mediterranean extreme environment, Le Saline dei Monaci (Apulia Region, Italy)

Marta Favero[1], Gianpasquale Chiatante[1], Eleonora Di Basilio[1], Simonetta Mattiucci[2], Daniele Canestrelli[1], Marialetizia Palomba[1]

1 Department of Ecological and Biological Sciences, Tuscia University, Largo dell'Università s.n.c. 00100 Viterbo, Italy
2 Department of public health and infectious diseases, Sapienza-university of Rome piazzale aldo moro, 5 00185 Rome, Italy

Abstract

Hypersaline environments are valuable natural resources with significant economic and ecological importance. Le Saline dei Monaci, along the Salento coastline (Southeast Italy), is one of Europe's most pristine hypersaline habitats, characterized by extreme salinity and high biodiversity. The brine shrimp, *Artemia parthenogenetica*, a critical species in this ecosystem, plays a pivotal role in the local food web, serving as the primary food source for shorebirds. Despite the habitat's ecological significance, parasitic interactions involving brine shrimps remain largely unexplored. Understanding these interactions is essential for elucidating their roles in ecosystem dynamics, including host population regulation, food web stability and community structure (Lafferty et al., 2008).

This study aims to investigate the occurrence of the cestode *Flamingolepis liguloides* parasitizing *Artemia parthenogenetica* in Le Saline dei Monaci and assess their infection levels.

In October 2023, 100 *Artemia parthenogenetica* individuals were collected from Le Saline dei Monaci (Apulia Region, Italy) using a plankton hand net. The collected specimens were transported to the laboratory for parasitological and molecular analyses.

Infection levels showed a prevalence of 91% and a mean abundance of 10 (± 13.9) cysticercoids per brine shrimp. The majority of *F. liguloides* (55.3%) were located in the thorax, followed by the abdomen (22.2%), ovisac (15.2%) and head (7.4%). A significant positive correlation was observed between brine shrimp length and parasite abundance. Molecular identification of the collected parasites was carried out by 18S and 28S rRNA sequences.

This study represents the first record of F. liguloides in the studied area. These findings enhance the understanding of ecological processes in Le Saline dei Monaci, emphasizing parasitic interactions' role in maintaining hypersaline habitats' health and stability.

Keywords: *Flamingolepis liguloides*, *Artemia parthenogenetica*, hypersaline habitat, molecular identification

PS – poster session

Session: [SE2] Biology and taxonomy of helminths

Phylogenetic and taxonomic study of the genus *Dilepis* (Cestoda, Dilepididae)

Rusłan Sałamatin[1,2], Jiljí Sitko[3], Pablo Oyarzún-Ruiz[4], Alan Fecchio[5], Izabella Rząd[6], Adam Kaczmarek[1], Vadim Kornyushin[7], Vasyl V. Tkach[8]

1 Department of Microbiology and Parasitology, Faculty of Medicine. Collegium Medicum, Cardinal Stefan Wyszyński University in Warsaw, Warsaw, Poland
2 Department of General Biology and Parasitology, Medical University of Warsaw, Warsaw, Poland
3 Comenius Museum in Přerov, Přerov, Czech Republic
4 Universidad de Concepción, Chillán, Chile
5 Universidade Federal da Bahia, Salvador, Brazil
6 Institute of Marine and Environmental Sciences, University of Szczecin, Szczecin, Poland
7 I. I. Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, Kyiv, Ukraine
8 University of North Dakota, Grand Forks, North Dakota, USA

Dilepis is the central genus in the large cestodes family Dilepididae. Its members are distributed on all continents other than Australia and are primarily parasitic in songbirds, although infections were reported from a variety of other hosts, including mammals such as moles, shrews and foxes. *Dilepis* spp. with known life cycles utilize earthworms as intermediate hosts, therefore these cestodes are commonly found in thrushes Turdidae on different continents. The type species of the genus, *Dilepis undula* (Schrank, 1788) was originally described from the carrion crow *Corvus corone* in Europe and subsequently reported from a number of birds, primarily *Turdus* spp., on different continents. In many similar cases, the use of DNA sequences has revealed the presence of more than one morphologically similar ("cryptic") species. *Dilepis undula*, with its extremely broad distribution, was an obvious candidate for a molecular systematic analysis. In this study, we compared DNA sequences of specimens preliminarily identified morphologically as *D. undula*, from several bird species (including the type host) in Europe, North and South America. Newly generated sequence data from the nuclear ribosomal 28S gene and mitochondrial *nad1* and *cox1* genes were used for comparison and phylogenetic analyses. The results have revealed demonstrated the presence of at least 7 genetically distinct species-level lineages within the "*Dilepis undula*" group with the lineages from Europe and the Americas forming two major clades. The morphological analysis of these lineages will follow.

This study was supported in part by the Kosciuszko Foundation (New York, USA) fellowship to Rusłan Sałamatin.

Keywords: *Dilepis undula*, Dilepididae, Cestoda, cryptic species, birds

PS – poster session

Session: [SE2] Biology and taxonomy of helminths

Effect of arsenic exposure on rat tapeworm *Hymenolepis diminuta* in *in vitro* model with intestinal cells

Zuzana Čadková[1], Lukáš Praus[1], Jaroslav Vadlejch[1], Ivo Doskočil[1], Ondřej Vosála[2], Petra Matoušková[2], Barbora Szotaková[2], Jiřina Száková[1]

1 University of Life Sciences in Prague, Prague, Czech Republic
2 Charles University, Hradec Králové, Czech Republic

Abstract

Adult helminths in gastrointestinal tract are directly exposed to environmental contaminants from host digesta. Since they are able to survive and to develop successfully under these conditions, one may conclude that they possess mechanisms protecting them from the destructive effects of various xenobiotics such as risk elements or organic compounds. However, information concerning interactions between helminths and above-mentioned pollutants yet to be elucidated in detail. Unlike other risk elements, arsenic can be not only accumulated but also metabolized by the helminth organism. Therefore, we chose this element for our experiment. To study cestode reaction to arsenic exposure in the host gut we cultivate *ex vivo* viable adults of *H. diminuta* in As-enriched medium (concentration 20 µg/mL) with/without intestinal cells line HT29. Within 24 hours, we observed a gradual increase in As concentrations in the tapeworm strobila from 0.14 µg/g through 80 µg/g after 6 hours and 96 µg/g after 12 hours up to 185 µg/g in the end of 24-hour period. At the same time specimens incubated together with intestinal cells showed significantly lower As concentrations (from 68 through 76 up to 106 µg/g). In order to ex-

plain the fate of As within tapeworm organism, tissue samples are subjected to speciation analysis using HPLC-ICP-MS. This analysis allows to determine the As species present in the *H. diminuta* for the very first time and confirm whether tapeworms are able to actively metabolize this element or adsorb it just passively on the tegument surface. Subsequent determination of the activity of xenobiotics-metabolizing enzymes such as catalase, glutathione peroxidase, glutathione-S-transferase, and carbonyl reductase in both tapeworm tissues and excretory-secretory products enable us to understand biochemical mechanisms which help tapeworms to cope with risk element exposure.

Keywords: risk element exposure, Cestoda, As-species, antioxidative defense mechanisms, xenobiotics-metabolising enzymes

PS – poster session

Session: [SE2] Biology and taxonomy of helminths

Why we should we care about extinct parasites! Constraints on the evolution and extinction of helminths with fossil evidence and modelling approaches

Kenneth De Baets, Weronika Łaska, Aleksandra Skawina, Karina Vanadzina

1 *University of Warsaw, Poland*

Abstract

Parasitism is one of the most common modes of life. Despite the large diversity and importance of helminths today, their evolutionary history, including extinction, remains poorly constrained. This has been largely related to their poor fossil record, which hampers our understanding of the impact of extinction and host radiations on their evolution. Recent data by the PARADIVE group underline that the fossil record of parasitism is patchy but more diverse than commonly assumed. Recent analyses of helminths with preservable structures or characteristic host pathologies show an amplification of parasitism with biodiversity and dilution with extinction. These analyses also provide evidence for obvious host switches or extinctions, which cautions against models of pure co-divergence. The reliance on host calibrations to constrain divergence time therefore needs to be approached with caution. Novel cophylogenetic approaches and historical data can be used to forecast the impact of host shifts and planetary health on diversity more accurately, particularly when extinct taxa can meaningfully be integrated. Moreover, a comprehensive understanding of historical transitions can serve as a foundation for predicting future shifts in their diversity and distribution.

Keywords: evolutionary history, extinction, fossil record, cophylogenetic approaches

PS – poster session

[SE3]

Emerging parasitoses and zoonoses in Europe – questions about the future

Session: [SE3] Emerging parasitoses and zoonoses in Europe – questions about the future

Trich-tracker for tracing inbred parasite outbreaks

Ewa Bilska-Zając[1], Peter Thompson[2], Benjamin Rosenthal[2]

1 National Veterinary Research Institute, Department of Parasitology and Invasive Diseases

2 USDA-Agricultural Research Service, Animal Parasitic Diseases Lab

Abstract

When a *Trichinella* infection is found in pigs, it is particularly important to identify the source of the infection for the animals and stop further transmission of the parasite to next pigs and into surrounding areas. For this, an epidemiological investigation is used, which is a complex procedure including few stages: epidemiological interview, serological investigtions, species identification of discovered *Trichinella* larvae, and differentiation of isolates of the same species. The last step is practically not applicable due to the lack of available methods to distinguish isolates of *T. spiralis* larvae. And thus, in most cases, it was not possible to obtain an answer as to what could have caused the infection of pigs in a given outbreak of trichinellosis.

Here, we propose a method based on ddRADseq and bioinformatics analysis called - Trich-tracker. The methodology applies the ddRADseq technique during which DNA libraries are created using restriction enzymes. Such DNA libraries contain the sequences of multiple random loci distributed throughout the genome under analysis. The obtained in NGS raw sequence data is used for finding single nucleotide polymorphisms (SNPs) and then for phylogenetic analysis or genetic structure analysis. Both of the analyses are useful to distinguish isolates one from another, what is the most important during epidemiological investigation in *Trichinella* outbreaks on pigs' farms. The discriminating power of this tool is tunable and scalable, allowing application in a variety of epidemiological contexts. The simplicity of the entire procedure, and the timeliness and cost effectiveness of Trich-tracker ensure the usefulness of its practical application in ongoing *Trichinella* outbreaks. Furthermore, Trich-tracker may be also used to track origin of cured meat containing *T. spiralis* which was a source of human infection. Additionally, this tool may be adapted to distinguishing other species of *Trichinella* and of any other parasites.

Keywords: *Trichinella*, molecular epidemiology, outbreak, Trich-tracker

OS – oral session

Session: [SE3] Emerging parasitoses and zoonoses in Europe – questions about the future

Estimating the risk of zoonotic trematodes through the impact of bioecological determinants on the population distribution of freshwater snails in the Alqueva Dam lake, Alentejo region, Portugal

Maria Teresa Bispo[1], Isabel L. Maurício[1], Pedro M. Ferreira[1], Silvana Belo[1], Manuela Calado[1]

1 Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Rua da Junqueira 100, 1349-008 Lisboa, Portugal

Abstract

Understanding the dynamics of freshwater snail species, intermediate hosts of trematodes, can help estimate risk of emerging waterborne diseases caused by these parasites, such as cercarial dermatitis and fascioliasis, for example, which have birds and cattle, respectively, as definitive hosts. By examining the interplay between environmental and biotic factors, we can determine the distribution of these snails and implement effective control measures. The study aimed to investigate the influence of environmental and biotic factors on the occurrence and abundance of intermediate hosts of trematodes in areas frequently visited by humans. A malacological and environmental survey were carried out in 25 shore locations around Alqueva, a large artificial lake in the Alentejo region, Portugal, that is used for recreation, fishing, local household use, and is drunk by livestock. At these locations, we sampled populations of freshwater snails and recorded water quality parameters, habitat characteristics, human activity, and the presence of vertebrate reservoirs of zoonotic trematodes. We collected 3915 snails between May 2023 and April 2024 and as expected, the number of snails were influenced by water temperature, water velocity and pH levels, with higher alkalinity (pH>8). Invertebrate abundance and bird sightings also varied between collection months, with greater invertebrate numbers and fewer bird sightings in the summer. The collected snails were morphometric identified as *Physella* sp., *Radix* sp., *Gyraulus* sp. Four populations of *Physella* sp. and two of *Radix* sp. collected in the summer released cercaria upon exposure to artificial light. Concluding, several bioecological variables can directly impact the presence and number of intermediate snail hosts and, thus, the emergence of zoonotic diseases caused by trematodes in the region.

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Keywords: zoonotic trematodes, snail-borne diseases, Alqueva, Portugal

OS – oral session

Session: [SE3] Emerging parasitoses and zoonoses in Europe – questions about the future

Shedding light of the helminths infections in "hungry valleys" in Slovakia

Lukáš Ihnacik[1], Ingrid Papajová[1], Júlia Šmigová[1], Carmen Anthonj[2]

1 The Institute of Parasitology of the Slovak Academy of Sciences, Košice, Slovak Republic

2 Faculty of Geo-Information Science and Earth Observation, ITC, University of Twente, Enschede, the Netherlands

Abstract

Intestinal helminthiases remain a serious concern even in developed countries, yet they often go overlooked. While their prevalence is generally low in Slovakia, there are areas inhabited by marginalized communities, living in unsatisfactory conditions with poor hygiene habits, where their number is increased. This can pose a health hazard for the whole society. Therefore, understanding the epidemiological situation, as well as disease transmission risk factors and areas, are crucial for implementing prevention strategies. In this study, we focused on the region known as „hungry valleys" in Slovakia. Overall, 3 816 human stool samples were collected for parasitological analysis. The helminth eggs (HE) were detected in approximately 5% of the samples. The eggs of *Ascaris lumbricoides* were the most common. Statistical analysis revealed a significantly higher overall occurrence of HE in the Roma population, with this population having nearly 23 times the likelihood of infection compared to the majority population. HE were significantly more present in stool samples from rural than in urban areas. Significantly more HE in stool samples were found in age groups from 2 to 66 y/o. Correlation analysis showed that the density of people and some WASH conditions significantly influenced the number of positive samples, while regression analysis showed that the WASH conditions did not have a significant impact. Spatial analysis in the QGIS environment along with factors weighting using analytic hierarchy process resulted in creation of partial maps, than connected into final risk maps of helminths spread in this area, where areas with a higher risk of human infection with helminths are easily observable. Based on these maps, it will be possible to predict the incidence of helminthiasis in the surveyed districts and improve the health status of the population.

Study was financially supported by projects APVV-18-0351 and VEGA 2/0138/21.

Keywords: helminths, eggs, marginalised population, transmission factors, risk maps

OS – oral session

Session: [SE3] Emerging parasitoses and zoonoses in Europe – questions about the future

Last years emerging parasites in Europe - a challenge on how to anticipate

Grégory Karadjian[1]

1 Anses - Animal Health Laboratory

Abstract

According to WHA, emerging diseases are new infections caused by the evolution or modification of an existing pathogen, such as a parasite, due to a change of host, vector, pathogenicity or strain. These diseases could become more numerous in the context of global warming, which could alter the range of certain parasites and/or their potential vectors. With regard to emerging parasites, the concept of emergence is associated either with a new parasite described by science or with a parasite whose area of incidence or host range is expanding. Very often, the mechanism of the emergence consists of two steps: first, the introduction of a new parasitic agent and second, its spread to a more susceptible or immunologically unadapted population.

The emergence of parasites is often linked to their reservoir hosts, often found in wildlife (e. g. foxes in the case of *Echinococcus multilocularis*). More recently, some parasite introductions or expansion into new areas have been associated with an increase in vectors such as the recent increase of *Dirofilaria repens,* which emerged recently in Northern European countries.

Regarding vectors, people think about biting arthropods. What about gastropods? They host many parasites, including some emerging ones. The recent emergence of *Angiostrongylus cantonensis* in Spain, first in Tenerife, then in Majorca and finally in mainland Spain in and around Valencia, is evidence of their importance. In addition to Human diseases, an important point is the increase in parasitic diseases also in domestic animals such as pulmonary infections in cats due to *Aelurostrongylus abstrusus, Troglostrongylus brevior* … Moreover, the increase and spread of *Angiostrongylus chabaudi*, *A. vasorum*, as well as the recent cases caused by *A. dujardini* show the importance of taking into account terrestrial gastropods. The impact of these parasites/hosts on Global Health highlights the need for a European network, which we are currently building.

Keywords: emergence, anticipation, increase, Europe

OS – oral session

Session: [SE3] Emerging parasitoses and zoonoses in Europe – questions about the future

A 40-year collection of epidemiological and clinical data on 906 patients: The French alveolar echinococcosis registry

Jenny Knapp

Reference National Center of Echinococcoses, Besançon University Hospital, France

Abstract

In France, surveillance of alveolar echinococcosis (AE) is performed by the National Reference Centre for Echinococcoses (NRC-E), mostly through active investigation from the NRC-E and a voluntary approach from physicians. Epidemiological and clinical information were collected on 906 patients with an AE diagnosis made in France over three periods of times, 1982-1999 (P1), 2000-2010 (P2) and 2011-2021 (P3). Patients' mean age at diagnosis was 58.6. Residence at diagnosis was located in the at-risk regions in France in 85% of cases, in a rural area in 53.3%. and 26.9% of AE cases have agricultural activities. Picking activities was declared by 82.4% of patients, gardening by 77.1%, owning a pet by 76.7%, fox contact by 38.1% and hunting activity by 14.8%. Asymptomatic patients at diagnosis markedly increased over time (19.1% to 56.2%). Albendazole therapy was administered to 91% of patients. The number of interventions significantly decreased over the time, to reach 30.2% in P3. Curative surgery was increasingly practiced, to reach 87.8% of the interventions in the P3 period. Liver involvement represents 98.6% of AE cases in P3, neighboring organ extension was 17.2%, parasite distant metastases 4.7% and primary extra-hepatic localization 2.2% of patients. Immunosuppressed (IS) patients, represented 25.1% in P3. In total, 72% of P1 patients were alive in 2000 versus 78% of P2-P3 patients in 2021. From this 40-years back investigation, an increasing number of asymptomatic patients at diagnosis, a large administration of albendazole therapy, an increase of curative surgeries and an increasing number of IS AE patients was observed in France from 1982 to 2021.

Keywords: alveolar echinococcosis, registry, France, medical and epidemiological data, immunosuppressed patients

OS – oral session

Session: [SE3] Emerging parasitoses and zoonoses in Europe – questions about the future

Molecular identification of *Giardia duodenalis* and *Cryptosporidium* spp. from immigrants in Greece.

Anastasios Ligdas[1], Sofia Makka[2], Panagiota Ligda[1], Eirini Fragkiadaki[2], Manolis Angelakis[2], Smaragda Sotiraki[1]

1 Laboratory of Parasitology, Veterinary Research Institute, Hellenic Agricultural Organization (ELGO) – DIMITRA, Campus ELGO, 57001 Thermi Thessaloniki, Greece
2 Hellenic Pasteur Institute, Athens, Greece

Abstract

Humans are susceptible to infection with numerous species of parasites that colonise the intestinal tract. Poor hygiene and sanitation conditions, as well as exposure to animals are factors that affect parasite transmission. The zoonotic protozoa *Giardia duodenalis* and *Cryptosporidium* spp. are considered of great importance, having a significant public health impact, because of the high prevalence and disease burden, often causing major disease outbreaks and emergency responses. In Greece zoonotic and potentially zoonotic species/assemblages of both protozoa have been described. However, as the population and the environment changes (e.g., tourism, migration, etc.), the profile of parasitism may also change. The aim of this study was to update the available information concerning the infection epidemiology of *Giardia* and *Cryptosporidium* in Greece. For this, the target population was the immigrants living over 20 different Reception and Identification Centers (RICs) all over the country. Over a 2-year period, faecal samples were randomly collected from immigrants coming in Greece. In total 178 faecal samples were collected and molecularly analyzed for the identification and genetic characterization of *Giardia* and *Cryptosporidium*, targeting the β-giardin and the 18S ribosomal DNA genes, respectively. PCR positive samples were further processed with sequencing analyses on both strands. Thirty-nine samples (21.9%) were positive for *Giardia duodena-*

lis, while only 1 was positive for *Cryptosporidium*. All *Giardia* positive samples were identified as sub-assemblage AII, which is primarily a human-adapted assemblage that caused many outbreaks in the past, however genotyping of the *Cryptosporidium* positive sample was not possible. To conclude it is shown that those two protozoa are circulating among individuals in RICs and may trigger an outbreak if sanitary systems are under pressure, which under the circumstances is not unlikely. Monitoring is essential to prevent disease.

Keywords: *Giardia*, *Cryptosporidium*, molecular identification, epidemiology

OS – oral session

Session: [SE3] Emerging parasitoses and zoonoses in Europe – questions about the future

Cystic echinococcosis in children and adults from Western Romania: 2007-2022

Ana Alexandra Paduraru[1], Maria Alina Lupu[1], Ciprian Duta[2], Gabriel Veniamin Cozma[3], Bogdan Dan Totolici[4], Eugen Sorin Boia[5], Marius Octavian Cretu[3], Tudor Rares Olariu[1]

1 Discipline of Parasitology, Department of Infectious Diseases, Victor Babes University of Medicine and Pharmacy, 300041 Timisoara, Romania
2 Discipline of Surgery II, Department of Surgery II, Victor Babes University of Medicine and Pharmacy, 300041 Timisoara, Romania
3 Discipline of Surgical Semiology I and Thoracic Surgery, Department of Surgery I, Victor Babes University of Medicine and Pharmacy, 300041 Timisoara, Romania
4 Department of General Surgery, Vasile Goldis Western University of Medicine and Pharmacy, 310025 Arad, Romania
5 Discipline of Pediatric Surgery and Orthopedics, Department of Pediatrics, Victor Babes University of Medicine and Pharmacy, 300041 Timisoara, Romania

Abstract

Cystic echinococcosis (CE) represents a concerning health problem in many parts of the world including Romania, due to the high rates of infection in humans and animals. The aim of this study was to retrospectively assess the epidemiological characteristics of the disease in patients hospitalized in Western Romania.

The study included individuals admitted to four major teaching hospitals from Arad and Timis counties, between 1 January 2007-1 September 2022. Epidemiological, clinical and paraclinical data were collected from the patients' medical records.

A total of 426 patients with CE were included in this study (3-90 years, mean=41.9): 60 (14.1%) children and 366 (85.9%) adults. Of the 60 children, 36 (60%) were boys and 41 (68.3%) were from rural area while of the 366 adults, 194 (53%) were females and 225 (61.5%) rural inhabitants. During the studied period, a descending trend in the number of cases was observed, from 47 cases in 2007 to 11 in 2022 ($p= 0.004$). The majority of cases (393/426, 92.3%) had one organ involvement. The prevalence of multiple organ involvement was higher in children (10/60, 16.7%) compared to adults (23/366, 6.3%)($p=0.005$). The liver was the most affected organ (345/426, 81%). In lung involvement, the rate was higher in children (15/60, 25%) compared to adults (48/366, 13.1%) ($p=0.02$). Most of the patients had one hospital presentation (319/426, 74.9%). The rate of multiple hospitalizations was higher in children (24/60, 40%) compared to adults (83/366, 22.7%, $p= 0.004$).

CE is a serious zoonotic disease affecting all ages. Although the number of cases has decreased, patients diagnosed with CE need hospitalization and special medical care, indicating that this parasitosis is still a public health problem in Western Romania. Public health interventions that tackle risk factors and control programs should be optimized to limit the transmission of the parasite and maintain the decreasing trend of CE.

Keywords: *Echinococcus granulosus*, parasitic disease, epidemiology, zoonosis, hydatid disease

OS – oral session

Session: [SE3] Emerging parasitoses and zoonoses in Europe – questions about the future

Echinococcus multilocularis in Europe – still emerging?

Adriano Casulli[1], Thomas Romig[2]

1 Istituto Superiore di Sanita, Rome, Italy
2 University of Hohenheim, Stuttgart, Germany

Abstract

Alveolar echinococcosis (AE), caused by the fox tapeworm *Echinococcus multilocularis*, is considered as an emerging zoonosis in Europe. This is mainly based on the drastic and well documented increase of its abundance in animal hosts which was observed in the 1990s and early 2000s, when a large number of surveys in various countries demonstrated both an increase of prevalence in red foxes and an increase of fox densities. For human AE, the increase was always less clear due to the lack of standardized and reliable case reporting, but existing data suggest a corresponding emergence of case numbers with an approximate 10-year lag after the increase of *E. multilocularis* in foxes. Following the early 2000s, there is an unfortunate paucity of European prevalence data in animals, but the few surveys that were done suggest a consolidation of parasite abundance in foxes and intermediate hosts at a high level. Concerning human AE, a recent study identified around 4,000 human AE cases during the period 1997-2023 from 40 selected European countries. Here we briefly review available information guided by the question whether the parasite's 'emergence' is till observable, or whether the epidemiological status has entered a phase of stable and high endemicity in wild mammals, with stable sporadic spill-over rates to humans. If so, this apparent stability may be deceptive in view of climatic and ecological changes that are already obvious. Modeling of habitat suitability in Europe under different change scenarios until 2050 indicates a future shift to more northern latitudes and higher altitudes in the alpine region, and a marked decrease of suitable habitats in today's high endemicity areas of central Europe north of the Alps. In addition to the evaluation of frequency and endemicity data, an update is provided on potentially new host animals and animal behaviour that facilitates transmission of the parasite.

Keywords: *Echinococcus multilocularis*, alveolar echinococcosis, Europe

OS – oral session

Session: [SE3] Emerging parasitoses and zoonoses in Europe – questions about the future

Spatial distribution and genetic diversity of *Echinococcus multilocularis* in foxes in central France, a focus of human cases in the 1980's

Gérald Umhang[1], Vanessa Bastid[1], Christophe Caillot[1], Jean-marc Boucher[1], Franck Boué[1]

1 ANSES, Nancy laboratory for rabies and wildlife, Malzéville, France

Abstract

E. multilocularis is the etiological agent of alveolar echinococcosis, a chronic disease which is often life-threatening due to tumour-like progression. The lifecycle in Western Europe is based on predation of voles by red foxes, which are responsible for almost all the environmental contamination by eggs. In France, the parasite is historically present in the eastern areas belonging to the European historical focus in the Alps. Over the past two decades, the distribution of the parasite in foxes has been significantly expanded, reaching the northern half of the country: from Brittany to the border of Italy via central France. This region of central France was not investigated since the 1980s following the identification of a focus of human cases in 3 departments. From June 2019 to July 2023, 591 foxes from 8 departments were collected. The presence of *E. multilocularis* was identified in 7 departments by copro-real-time PCR, with no detection in the south-western department of Lot. The prevalence in the known endemic departments is 10.4% in Puy-de-Dôme, 14.7% in Cantal, and 46.4% in Creuse. In the 4 other departments where its presence was not previously investigated, the prevalence ranged from 4.8% to 8.7%. Microsatellites EmsB analysis of 215 worms from 50 foxes in six departments yielded 11 different EmsB profiles, including one dominant grouping 84% of the worms and present in all 6 departments. Additionally, this profile was identified in all the 3 human cases from the same areas. The low genetic diversity observed argued as the presence of the parasite in the region is much more recent compared to the eastern historical focus. The high prevalence in Creuse was unexpected, given that it is surrounded by departments with very low prevalence. The unknown endemic status of most neighboring departments from the investigated areas requires further investigations to ascertain the current distribution of *E. multilocularis* in France and communicate prevention measures.

Keywords: *Echinococcus multilocularis*, France, red foxes, endemic areas, genetic diversity

OS – oral session

Session: [SE3] Emerging parasitoses and zoonoses in Europe – questions about the future

Trichinella infection in Serbia: advancements, risks, and the path to safe consumption

Sasa Vasilev[1], Dragana Plavsa[2], Milorad Mirilovic[3], Tamara Boskovic[4], Jasna Kureljusic[5], Ana Vasic[6], Dragan Vasilev[3], Budimir Plavsic[7], Ljiljana Sabljic[1], Ivana Mitic[1]

1 University of Belgrade, Institute for the Application of Nuclear Energy – INEP, Belgrade, Serbia
2 Institute of Public Health of Serbia - Dr Milan Jovanovic Batut, Belgrade, Serbia
3 University of Belgrade, Faculty of Veterinary Medicine, Belgrade, Serbia
4 Ministry of Agriculture, Forestry and Water Management, Veterinary Directorate, Belgrade, Serbia
5 Scientific Veterinary Institute of Serbia, Belgrade, Serbia
6 5Scientific Veterinary Institute of Serbia, Belgrade, Serbia
7 World Organization for Animal Health (OIE)

Abstract

In Serbia, trichinellosis remains one of the most important foodborne zoonotic diseases. Advances in pork production systems, control measures, the artificial digestion method and *Trichinella* proficiency tests have effectively eliminated farm pork as a source of trichinellosis. Epidemiological data from the last decades show notable decrease in both human cases and infected animals. Over the years, pork has been the primary source of trichinellosis in Serbia, often associated with family outbreaks. The main risk of infection now arises from consumption of untested backyard pork. When pigs are raised without compliance with hygienic standards and veterinary testing are lacking it increases the likelihood that meat and meat products are potential sources of *Trichinella* infection. In most numbers of outbreaks in Serbia *T. spiralis* were the etiological agent of infection. According to epidemiological data it is important that consumers of backyard pigs and wild game meat should be educated about the risk associated with consumption of untested meat. Control of Trichinella testing QA system in veterinary subjects and regular participation in proficiency tests are essential for ensuring safe food for consumers. Furthermore, full integration of veterinary and public health efforts, following the One Health concept, is imperative for effective control measures.

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Keywords: *Trichinella*, risk, safe food, Serbia

OS – oral session

Session: [SE3] Emerging parasitoses and zoonoses in Europe – questions about the future

Molecular detection of cercariae of *Posthodiplostomum* sp., etiological agent of black spot disease in fish, released from *Physella acuta* collected in the Alqueva Dam lake, Alentejo region, Portugal.

Maria Teresa Bispo, Isabel L. Maurício, Pedro M. Ferreira, Silvana Belo, Manuela Calado

Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Rua da Junqueira 100, 1349-008 Lisboa, Portugal

Abstract

In the Alentejo region, Portugal, the Alqueva Dam vast artificial lake, is a hub for recreational water activities and fishing. It has altered migratory bird routes, as well as local flora and fauna. Coupled with climate change, these factors increase the risk of zoonotic diseases, including those caused by trematodes, in the region, given that freshwater snails are obligatory intermediate hosts for many trematodes. The aim of the study was to detect and identify trematodes in freshwater snails in the Alqueva Dam Lake. The malacological survey was conducted in 25 shore locations around the Alqueva basin between May 2023 and April 2024. Snails were exposed to artificial light to induce cercariae elimination and were morphometric identified as *Physella acuta*. The ITS2 region amplified from cercariae from one location had 90% sequence homology, upon a BLAST search, with sequences of the genus *Posthodiplostomum* sp. and phylogenetic analyses suggest a closer relationship to lineage II, but may belong to a previously unidentified lineage. Cercariae of this genus encyst in plants or fish muscles, inducing visible changes such as black spots on the body or eyes, and leading to weight loss and delayed development, although the impact on human health remains unknown. Species such as *Posthodiplostomum cuticola*, *P. centrarchi*, and *P. brevicaudatum* have been detected in some European countries, with homology to North American strains, suggesting the invasive potential of these parasites via bird dispersal. The widespread *Physella acuta* has, thus, been confirmed as intermediate host of *Postodiplostomum* sp. in the Alqueva Dam lake, likely posing a risk at other locations. While preliminary, our findings offer insights into water-borne parasites within the Alqueva ecosystem.

We acknowledge the Portuguese Foundation for Science and Technology for funds to GHTM—UID/04413/2020 and LA-REAL—LA/P/0117/2020 and Project PTDC-2022.01349.

Keywords: *Physella acuta*, snail-borne diseases, avian parasites, Alqueva, Portugal

PS – poster session

Session: [SE3] Emerging parasitoses and zoonoses in Europe – questions about the future

Echinococcus multilocularis genetic diversity based on samples from pigs – confirmation of the characteristic haplotypes distribution and the presence of Asian-like haplotype in Poland

Jacek Karamon, Małgorzata Samorek-Pieróg, Ewa Bilska-Zajac, Weronika Korpysa-Dzirba, Jacek Sroka, Aneta Bełcik, Jolanta Zdybel

National Veterinary Research Institute, Pulawy, Poland

Abstract

Alveolar echinococcosis is a zoonosis dangerous to human health and life, caused by larval forms of *Echinococcus multilocularis*. A typical definitive host is the red fox and typical intermediate hosts – rodents. However, other animal species (e.g. pigs) can also act as aberrant intermediate hosts. Poland's geographical location is an interesting site for genetic analysis of this tapeworm, showing a specific mixing of European and probably Asian genetic pools. The aim of the study was to determine the genetic diversity of *E. multilocularis* in pigs in highly endemic areas in Poland, as well as to attempt to confirm the occurrence and geographical distribution of haplotypes characteristic for these areas, which were previously described on the basis of examination of adult tapeworms isolated from foxes. Twenty samples of *E. multilocularis* larval forms were obtained from pigs' livers in four provinces of Poland. Genetic analyses were conducted on sequences of two mitochondrial genes: cox1 and nad2. Results: Seven haplotypes were found for the cox1 gene (OQ874673–OQ874679) and four haplotypes for nad2 (OQ884981–OQ884984). They corresponded to the haplotypes described earlier in foxes in Poland (some of them differing only in one nucleotide). The analysis showed the presence of the Asian-like haplotype in both the cox1 and nad2 genes. The remaining haplotypes were grouped in the European clade. The geographical distribution of haplotypes identified in the pig samples was noticed to bear a similarity to the distribution of haplotypes previously isolated from foxes in the same regions.

The characteristic geographical distribution of *E. multilocularis* haplotypes in Poland (including Asian-like haplotype) previously described in population of definitive hosts has now been confirmed by analysis of samples from non-specific intermediate hosts (pigs).

Keywords: *Echinococcus multilocularis*, genetic diversity, pigs, Poland

PS – poster session

Session: [SE3] Emerging parasitoses and zoonoses in Europe – questions about the future

Echinococcus multilocularis in surface water samples from endemic region of QTP, China

Xueyong Zhang[1], Yingna Jian[1], Zhi Li[1], Yong Fu[1], Yijuan Ma[1], Xiuying Shen[1], Xiuping Li[1], Hong Duo[1], Zhihong Guo[1], Anna Lass[2]

1 Qinghai Academy of Animal Sciences and Veterinary Medicine, Qinghai University; Qinghai Provincial Key Laboratory of Pathogen Diagnosis for Animal Disease and Green Technical Research for Prevention and Control, Xining 810016, P. R. China

2 Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine in Gdynia, Medical University of Gdansk, 9b Powstania Styczniowego Str., 81-519 Gdynia, Poland

Abstract

Water, among other risk factors, has been indicated as one of the possible routes of transmission of parasites to humans. *Echinococcus multilocularis* is a parasite causing dangerous zoonosis, alveolar echinococcosis (AE) with over 90% of cases recorded in China. However, data on distribution of this parasite in water is very limited. The current research was undertaken in highly endemic echinococcosis area of the Qinghai-Tibet Plateau (QTP), Western China to determine the occurrence of *E. multilocularis* in different types of water supplies.

In total, 326 water samples of 40 L in volume were collected from streams (n=216) and shallow waterholes (n=110), that serve as sources of drinking water consumed unprocessed by humans and animals, including both domesticated and wild animals. The material was filtered and subsequently analysed with molecular detection methods including real-time PCR, nested PCR and Sanger sequencing. Of the samples examined, *E. multilocularis* DNA was found in 3 (0.9 %) samples; all of them collected from streams. Sequencing of positive samples confirmed that the PCR products were fragments of the *E. multilocularis* mitochondrial 12S rRNA gene.

We, therefore, confirmed presence of the tapeworm DNA in surface waters in QTP, China. The results indicate that water should be considered a source of *E. multilocularis* infections in humans and animals in endemic areas and more studies are needed in this field.

Results indicate the potential role of surface water in dissemination of *E. multilocularis* in the QTP area and contamination of drinking water sources. This raise a question about the risk for humans and animals echinococcosis living in at-risk regions.

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Keywords: *Echinococcus multilocularis*, water, PCR, China

PS – poster session

Session: [SE3] Emerging parasitoses and zoonoses in Europe – questions about the future

Investigating the host-parasite genetic architecture and immune response in human anisakiasis in Italy caused by *Anisakis pegreffii*

Simonetta Mattiucci[1], Beatrice Belli[2], Veronica Rodriguez Fernandez[2], Paola Gesuiti[3], Ignazio Brusca[4], Fabrizio Bruschi[5], Paolo Fazii[6], Maria Teresa Ventura[7], Saverio Nenna[8], Walter Mazzucco[9], Maria Paola Perrone[3], Marialetizia Palomba[10]

1 Department of Public Health and Infectious Diseases, Section of Parasitology, Sapienza-University of Rome, University Hospital "Policlinico Umberto I", 00185 Rome, Italy

2 Department of Public Health and Infectious Diseases, Section of Parasitology, Sapienza-University of Rome

3 Immunohematology and Transfusion Medicine Unit, Policlinico Umberto 1, "Sapienza" University, Rome, Italy

4 U.O.C of Clinical Pathology Buccheri La Ferla Hospital FBF, 90123 Palermo, Italy

5 Department of Translational Research, N.T.M.S., Pisa University, Pisa, Italy

6 "S. Spirito" Hospital, Pescara, Italy

7 Department of Internal Medicine, Immunology and Infectious Diseases, University of Bari

8 Local Health Authority of Barletta & Bonomo Hospital, Andria, Puglia

9 U.O.C. of Clinical Epidemiology with Cancer Registry, Azienda Ospedaliera Universitaria Policlinico di Palermo, 90127 Palermo, Italy

10 Department of Ecological and Biological Sciences, Tuscia University, Largo dell'Università s.n.c. 00100 Viterbo, Italy

Abstract

Anisakiasis is the most reported seafood-borne zoonosis in Europe, primarily caused by *Anisakis pegreffii* and *A. simplex* (s.s.) (EFSA, 2024). A survey was carried out on past and newly

detected cases of human anisakiasis in Italy (years 2013–2023), with the following aims: *i)* genotyping the parasite from different clinical cases of anisakiasis (i.e. gastric GA, intestinal IA and gastro-allergic anisakiasis GAA); *ii)* genotyping the human HLA; *iii)* investigating patients IgE immune response. The study included patients with larval detection during endoscopy or post-surgical removal of granuloma (N=17), as well as those with chronic urticaria (CU) and other allergic disorders after fish consumption (N=50). For genetic/molecular identification, DNA was extracted from each larva removed during endoscopy and/or from granulomas. Genetic analysis was carried out by sequence analysis of the mtDNA *cox2* gene and genotyping at nuclear polymorphic microsatellite loci (SSRs DNA). HLA-DRB1, HLA-DQA1 and HLA-DQB1 of the patients were genotyped by ImmunoCOR kit. Sera were tested by IgE response in WB and ELISA assays. Genetic findings revealed that all the GA, IA and GAA cases were due to *A. pegreffii*. The larvae causing GA showed a particular heterozygote genotype at one DNA-SSR locus and they were homozygous at a specific allele of another polymorphic locus. No association was found between a certain genotype and IA cases. Finally, a certain IgE-WB hypersitization to *A. pegreffii* was detected in the sera of patients, who showed specific alleles at the HLA loci, compared to negative control patients. Investigating the genetic architecture of both the parasite species and the human host in anisakiasis can provide valuable insights into host-parasite evolutionary adaptation. This knowledge may play a role in understanding the pathogenesis of this fish-borne zoonosis.

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Keywords: anisakiasis, *Anisakis pegreffii*, genotyping, immune response

PS – poster session

Session: [SE3] Emerging parasitoses and zoonoses in Europe – questions about the future

Comparison of the prevalence of *Echinococcus multilocularis* in dogs living in shelters from two regions – Pomeranian and Warmian-Masurian Voivodeships

Joanna Nowicka[1], Karolina Baranowicz[1], Paweł Gładysz[1], Agnieszka Świątalska[2], Anna Lass[1]

1 Medical University of Gdańsk, Poland
2 Department of Veterinary Hygiene, Kaprów 10, 80–316 Gdańsk, Poland

Abstract

Echinococcus multilocularis is the most dangerous parasite existing in Northern Hemisphere that causes alveococcosis. Humans become infected by ingestion of its eggs excreted by definitive hosts, mainly red foxes (*Vulpes vulpes*). In some areas also dogs can play this role. However, in Poland data on *E. multilocilaris* infection in dogs is fragmentary. Thus, the aim of the study was to compare the prevalence of *E. multilocularis* infection in dogs living in shelters located in two neighboring voivodeships in northern Poland: Pomerania and Warmia-Masuria. In total, 464 dog faecal samples were collected with the help of shelter workers along with a questionnaire describing the dog condition and investigated with the use of nested-PCR and Sanger sequencing. We detected *E. multilocularis* DNA in 16 samples, with an overall prevalence of 3,5%. Presence of the tapeworm was confirmed in 7,6% of samples collected in Pomerania Voivodeship compared to 0,7% positive of samples collected in Warmia-Masuria Voivodeship. Positive samples will be subsequently genotyped. Results of this preliminary study show that *E. multilocularis* occurs in shelter dogs in both studied voivodeships. They contribute to our understanding of the abundance of *E. multilocularis* in dogs living in shelters in Poland as well as potential role of these dogs in parasite transmission. In consequence, investigations proposed in this study will complement knowledge about epidemiology of *E. multilocularis* infection, which is of high both local and world interest. Moreover, biomonitoring of prevalence of *E. multilocularis* infection in dogs from shelters will help to understand if, and where dogs may pose a threat for humans, especially shelter workers and future owners.

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Keywords: *Echinococcus multilocularis*, biomonitoring, alveococcosis, dogs

PS – poster session

Session: [SE3] Emerging parasitoses and zoonoses in Europe – questions about the future

Cysticercosis of the muscles and central nervous system

Dimitar Vuchev, Galya Popova

Bulgaria

Abstract

Intestinal infections caused by *Taenia solium* have not been registered in our country in the last 30 years, and cysticercosis in pigs is very rare. Nevertheless, cysticercosis in humans is still sporadically diagnosed using imaging (X-ray, computed tomography, MRI). We observed seven cases of cysticercosis in the central nervous system and another three - in the lumbo-sacral region and muscles of the lower limbs. In case of cerebral localization, the patients mainly complained of headache, which occured as cluster headache and it was not significantly relieved by analgesics. Patients had also imbalance, nausea, vomiting, seizures with loss of consciousness, urinary incontinence, discordance, etc. In case of muscle invasion, they complained of permanent myalgia. Hematological and biochemical tests were within normal values. Serological tests (ELISA) were positive only in two cases. The differential diagnosis was confirmed by imaging after excluding other diseases of the CNS such as meningoencephalitis, multiple sclerosis, tumors, syphilis, etc. Cysticerci were from one to numerous, disseminated in the CNS. The presence of calcifications eliminated the need for etiological treatment with albendazole or praziquantel, as only symptomatical treatment was required. Neurocysticercosis proceeds dramatically, sometimes with exitus. The newly diagnosed cases are a signal of hidden incidence of taeniasis.

Keywords: cysticercosis, taeniasis, headache, myalgia

PS – poster session

Session: [SE3] Emerging parasitoses and zoonoses in Europe – questions about the future

High prevalence of *Eucoleus* detected in shelter cats while the endoparasite prevalence of dogs and pet cats is low in Finland

Pia Rapp[1], Antti Sukura[1], Annika Pynnönen[1], Veera Gindonis[2], Anu Näreaho[1]

1 University of Helsinki, Department of Veterinary Biosciences, Finland
2 Environmental Health Services, City of Porvoo, Finland

Abstract

In this study the current prevalence of endoparasites in Finnish dogs and cats was examined. Background data from studied animals was collected for risk factors linked to Toxocara infections. Fecal samples from 664 dogs and 379 cats (including 46 shelter cats), over six months old, were examined with quantitative flotation method (Mini-Flotac®) using mainly zinc sulphate flota-

tion solution, and magnesium sulphate for some samples. Fecal samples from 396 dogs and 89 cats were also examined with Baermann method in order to detect nematode larvae.

The overall prevalence of endoparasites was 3.5% in dogs, 3.6% in pet cats and 41.3% in shelter cats. Strongylids (1.7%; probably pseudoparasites due to coprophagy) were most common findings in dogs followed by *Toxocara* (0.9%), trematodes (0.4%), *Eucoleus* spp. (0.3%), and *Taenia* spp., *Trichuris vulpis*, *Isospora* spp. and *Crenosoma vulpis* (0.2% each). Toxocara was the most prevalent finding in pet cats (3.3%) and shelter cats (34.8%). *Taenia* (0.6%) and trematode (0.3%) eggs were also found in pet cats. Shelter cats were also found to be infected with *Eucoleus* spp. (13.0%), *Isospora* spp. (10.9%), *Taenia* spp. (8.7%) and *Toxoplasma gondii*/*Hammondia hammondii* (2.2%).

Dogs that were most likely infected with *Toxocara* were under twelve months old. In cats, shelter background, male gender, being mixed-breed, being dewormed two to four times a year and having history of previous parasitic infections was associated with higher *Toxocara* infection prevalence. The background data collected from the questionnaire revealed that 31.2% of studied dogs were dewormed or their fecal sample was analyzed less than once a year or never, which are both unacceptable, even though the prevalence is low.

Endoparasitic prevalence in Finnish dogs and cats has reduced since past studies and is currently very low. The *Eucoleus* prevalence in shelter cats was unexpectedly high.

Keywords: *Eucoleus*, feline, canine, endoparasite, prevalence

PS – poster session

Session: [SE3] Emerging parasitoses and zoonoses in Europe – questions about the future

Survey on *Neospora caninum* prevalence in rural regions' dogs of Shabestar county, northwestern Iran

Aria Tafarroji Kasmaei[1], Mohammad Sadaghian[2], Seyed Masih Razavi[1]

1 Veterinary Medicine student, School of Veterinary Medicine, Shabestar branch, Islamic Azad University, Shabestar, Iran
2 Department of Pathobiology, School of Veterinary Medicine, Shabestar branch, Islamic Azad University, Shabestar, Iran

Abstract

Neospora caninum is an apicomplexan parasite of animals that is a major pathogen for cattle and dogs which occasionally causes clinical diseases in horses, goats, sheep, rodents and deer. Domestic dogs are the only known definitive hosts for *N. caninum* and have essential role in the initial transmission of this parasite to cattle as the other intermediate hosts, so due to increase in stray dogs' population in the area, major production problem in livestock husbandry and rural economy will be predictable. The aim of present survey is to evaluate the rate of area's rural dogs' infection, using PCR, to preparing basic information for delegates, in order to control of infection in Shabestar, Northwestern Iran.

Blood samples of 102 rural dogs were randomly collected from the area and the extracted DNA of the samples, were subjected to the real-time PCR technique, using the designed primer (targeting NC5 marker genomic sequence).

The results indicated that 10 out of the 102 (9.8%) examined samples, were PCR-positive for *N. caninum*, where 2 out of 7 (28.6%) "less than 6-month", 1 out of 14 (7.1%) "6–18 month" and 7 out of 81 (8.6%) "more than 18-month" age groups' dogs were infected. Also, the results showed that 3 out of 49 (6.1%) male dogs and 7 out of 53 (13.2%) of female dogs were PCR-positive. The rate of infection was 11.1%, 16.7% and 9.2% for herd, guard and stray dogs, respectively. Furthermore, 1 out of 3 (33.3%) and 9 out of 99 (9.1%) of the examined dogs, with and without infection related clinical signs, were positive, respectively. Statistical analysis revealed no significant differences about the rates based on age, gender, the type of dogs and related clinical signs as well as the recorded rates of different sampling regions ($P > 0.05$).

The outcome of the survey, confirms the presence and mild rates of *Neospora caninum* infection in rural dogs of Shabestar territory and emphasize on the importance of dogs' population controlling policies.

Keywords: *Neospora caninum*, Dog, PCR, Shabestar, Iran

PS – poster session

Session: [SE3] Emerging parasitoses and zoonoses in Europe – questions about the future

Epidemiological aspects and genetic diversity of *Echinococcus multilocularis* in Slovak patients, 2000-2023

Daniela Antolová[1], Viliam Šnábel[1], Júlia Jarošová[1], Serena Cavallero[2], Stefano D´Amelio[2], Yaroslav Syrota[3], Martina Miterpáková[1]

1 Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovak Republic
2 Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy
3 Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovak Republic; I. I. Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine, Kyiv, Ukraine

Abstract

In the study we evaluated the epidemiology of human alveolar echinococcosis (AE) and the haplotype diversity of *E. multilocularis* in clinical isolates from the country and investigated their genetic relatedness with haplotypes from other countries in Europe.

In the years 2000 - 2023, 137 AE cases of patients infected with *E. multilocularis* were recorded in Slovakia. The average incidence increased from 0.031 per 100,000 inhabitants between 2000 and 2011 to an average of 0.187 since 2012, i.e. about six times. The mean age at the time of diagnosis was 52.8 years. Most cases were diagnosed in the age groups 51–60 years and 61–70 years (33 cases each). Most of the patients came from the northern regions of Slovakia. A positive spatial correlation was found between incidence of human AE and prevalence of the parasite in foxes documented since 2000.

For genetic analyses, metacestodes from 21 patients collected in 2013 - 2021 were subjected to sequencing of *cob* (603 bp), *nad2* (882 bp) and *cox1* (789 bp) mt genes. As a result, 14 isolates (66.7%) were assigned to the European E5 profile of *E. multilocularis*, two isolates (9.5%) to the E5a subtype, four isolates (19%) to the E4 profile and one isolate (4.8%) to haplogroup E1/E2. The E5-type and E4 profiles were distributed throughout the country, whereas the E1/E2 profile was found in western Slovakia. According to our data and GenBank evidence, E5 dispersal was so far limited to central-eastern Europe and the variant thus seems to be indigenous to this region. The admixture with the haplotypes E4 and E1/E2 in Slovakia could have taken place from a historical endemic focus during the expansion of fox populations in the last decades. The acceleration of the AE incidence together with the relatively high haplotype diversity suggest the emergence of the disease in Slovakia.

Acknowledgements: The research was supported by the grants APVV-21-0166, VEGA 2/0107/20, and the NextGenerationEU funding, project No. 09I03-03-V01-00046.

Keywords: Slovakia, *Echinococcus multilocularis*, incidence, haplotype, patients

PS – poster session

Session: [SE3] Emerging parasitoses and zoonoses in Europe – questions about the future

Genotypes G1 and G3 of *Echinococcus granulosus* sensu stricto – host distribution, cyst fertility and organ tropism.

Viliam Šnábel

Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovak Republic

Abstract

From the species complex of *Echinococcus granulosus sensu lato* causing cystic echinococcosis (CE), the *E. granulosus sensu stricto* (*s.s.*) sp. is the most common cause of human infections, implicated in 88.4% of cases worldwide (Alvarez Rojas et al., 2014). In the present study, data were collected from published resources regarding the host distribution of the valid G1 and G3 genotypes of *E. granulosus s.s.*, with evaluation of differences in viability and organ tropism in cysts induced by G1 and G3.

Global data on the distribution of *E. granulosus s.s.* variants in intermediate hosts showed a predominance of G1 genotype, which was particularly pronounced in humans (recorded in 92.2% patients infected with *E. granulosus s.s.*), goats (91.7%), cattle (90%), sheep (89.4%) and camelids (camel, alpaca; 86.3%). The highest occurrence of G3 was documented in water buffalo, in which this type (´buffalo strain´) was originally described. G3 was found in 33.1% of buffalo individuals carrying *E. granulosus s.s.*

To assess the susceptibility of hosts to infection with G1 and G3, data involving the genotyping of *E. granulosus* s.s. and cyst viabilities were gathered. Cysts with viable protoscolices were considered fertile, while sterile cysts and cysts with degenerative modifications (cysts with caseous necrosis and/or calcified cysts) were scored as infertile. No statistically significant difference ($p = 0.359$, Fisher's exact test) was found between the viability of hydatid cysts due to G1/G3 across different hosts.

The liver is the most commonly affected organ in intermediate hosts suffered from CE, followed by the lungs. No significant difference ($p = 0.877$) was found in the number of isolates identified as G1 and G3 with respect to their allocation to liver and lungs. Overall, the distribution of G1 and G3 in liver and lung was similar (the liver prevailed over the lungs 7.18 and 7.04 times, respectively).

Acknowledgements: The research was supported by the grant VEGA 2/0157/22.

Keywords: *Echinococcus granulosus*, genotypes, host distribution, cyst fertility, tropism

PS – poster session

[SE4]
Fish parasitology

Session: [SE4] Fish parasitology

Biodiversity and infestations by *Cryptocotyle* (Digenea) of fish in the ecosystems of the English Channel and the North Sea

Maureen Duflot[1], Pierre Cresson[2], Odile Bourgau[1], Mélanie Gay[1]

1 Anses, Laboratory for Food Safety, Boulogne-sur-Mer, France
2 Ifremer, RBE/HMMN, Laboratoire Ressources Halieutiques Manche Mer du Nord, F-62200 Boulogne sur Mer, France

Abstract

Marine fish are often speckled with black spots caused by larval trematode infection, inducing a host response. These unesthetic spots and the presence of parasites may lead to important economic loss in fishery and may have impacts on public health. As many other zoonotic trematodes, *Cryptocotyle* (Lühe, 1899) are present in marine fish species although they belong to under-studied parasites of the biodiversity. So far, its impact on human health is still unknown and few publications exist dealing with its distribution among commercially important fish. This study investigated the *Cryptocotyle* distribution in the ecosystems of English Channel and the North Sea.

An epidemiological study was performed on seven commercial fish species: herring (*Clupea harengus*), sprat (*Sprattus sprattus*), whiting (*Merlangius merlangus*), pout (*Trisopterus luscus*), dab (*Limanda limanda*), plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*). A total of 1,586 fish were sampled during sea campaigns in January 2019 and 2020. Infestations were estimated for each geographical areas and fish species by counting visible black spots or lesions due to parasite infection on the fish skin. Metacercariae were isolated and identified from morphological and molecular perspectives.

Variations of the prevalence, intensity and abundancy were observed according to fish species and fishing areas. Encysted metacercariae were found with a total prevalence of 20.5%. Whiting and pout were the most infected species for all sampling areas. *C. lingua* was mainly identified and some *C. concava* and Bucephalidae were also observed.

This survey constitutes the first description of *Cryptocotyle* metacercariae in these marine ecosystems. It will contribute to better understanding of the ecology of these trematodes.

Acknowledgements: The authors thank the Hauts-de-France region and Anses for the financial support, and all the people involved in sampling and data collection.

Keywords: *Cryptocotyle*, trematode, distribution in ecosystems

OS – oral session

Session: [SE4] Fish parasitology

Anisakidae in the English Channel and the North Sea: review of the literature, gap identification and preliminary data

Alice Cappelletti[1], Maureen Duflot[2], Odile Bourgau[2], Clémence Couvreur[1], Rémy Cordier[1], Gay Mélanie[2], Pierre Cresson[1]

1 Ifremer, HMMN, Channel and North Sea Fisheries Research Unit, F-62200 Boulogne sur Mer, France
2 Anses, Laboratory for Food Safety, Boulogne-sur-Mer, France

Abstract

Parasitic nematodes from the Anisakidae family are very common in commercially and ecologically important fish species. Their life cycle involves zooplanctonic crustaceans, fish and/or cephalopods and marine mammals or piscivorous birds. Many distribution studies have been published but are often scattered and specific for one or a few species. Comparison is thus tricky, considering differences in methods, aims and species. On another hand, the fishing grounds covered by the English Channel and the North Sea are a crucial area for the fishing economy and industry in Europe. Valuable data are thus requested by stakeholders to assess and manage sanitary hazards. Moreover, from an ecological perspective, considering their wide distribution and potentially high infestation levels, the influence of Anisakidae on the marine ecosystem is to be considered for food web studies.

Within AniMode project, aimed at describing the ecology of Anisakidae in the English Channel and North Sea, the the present study reviewed the literature available on the distribution and diversity of Anisakidae in this area. Specific research strings were designed for PubMed, Google Scholar and Scopus including the type of studies, the geographical area, the parasites and the hosts (fishes, crustaceans and marine mamals). Major conclusions confirmed the scattered results and the difficulty to compare outcomes of different protocols. Surprisingly, infestation levels in species of major economic and/or ecological interest (*i.e.* cephalopods, zooplankton-feeding fish, or flatfish) were missing or old. Preliminary data on plaice obtained during this project will begin filling this gap.

AniMode project was funded by the Hauts-de-France Regional Council. AC is a PhD candidate, within the IHOP project, funded by the ANSES and IFSEA.

Keywords: Anisakidae, infestation levels, English Channel, North Sea, ecology

OS – oral session

Session: [SE4] Fish parasitology

Genetic variability in the anisakid *Contracaecum osculatum* B over a temporal scale: a tool for monitoring the food webs in sub-Arctic Sea?

Beatrice Belli[1], Marialetizia Palomba[2], Paolo Cipriani[3], Miguel Bao[3], Lucilla Giulietti[3], Arne Levsen[3], Giuseppe Nascetti[2], Daniele Canestrelli[2], Simonetta Mattiucci[1]

1 Department of Public Health and Infectious Diseases, Section of Parasitology, Sapienza University of Rome, Italy
2 Department of Ecological and Biological Sciences, Tuscia University, Viterbo, Italy
3 Institute of Marine Research (IMR), Nordnes, Bergen, Norway

Abstract

Anthropogenic changes in marine Arctic ecosystem altering the demography of fish and seal populations can even affect their endoparasites. Reduced hosts population size can alter anisakid population size as well, leading to possible genetic erosion in their gene pools (Mattiucci & Nascetti 2008). The parasite species *C. osculatum* B is a sibling species of the *C. osculatum* (s.l.) complex (Nascetti et al., 1993), parasitizing Arctic and sub-Arctic seals and fish species such as the Arctic cod (Levsen et al., 2016; 2023). This study aims to investigate the genetic variability at both nuclear and mitochondrial level of *C. osculatum* B from these areas over a 35-year time scale. Historical specimens of *C. osculatum* B collected from Barents and Norwegian Seas in 1985-1986 were genetically compared with contemporary ones sampled from cod and capelin in the same areas in 2021-22. Specimens were first sequenced at the diagnostic mtDNA *cox2* gene, and then genotyped at 7 DNA SSRs loci, here developed for the first time. High polymorphism was observed at both nuclear and mitochondrial level in the two samples. However, in the current population, rare alleles at some polymorphic SSRs loci and rare mtDNA haplotypes seem to be lost. In contrast, the current population showed novel unique alleles and haplotypes, not observed in the historic samples. Demographic inference analysis performed on the genetic data set, over the considered time scale, showed an initial parasite population decrease followed by a period of demographic stability, and, more recently, a population increase. The results suggest demographic fluctuations of the parasite populations in the actual area over the study period, likely reflecting fluctuations recorded in their hosts (Gjøsæter et al., 2009). Monitoring genetic variability and population size of anisakids in sub-Arctic waters across spatio-temporal scales may help assess the stability of marine trophic webs.

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Keywords: *Contracaecum osculatum* B, sub-Arctic Sea, genetic variability, stability of marine trophic webs

OS – oral session

Session: [SE4] Fish parasitology

Emerging parasites of salmon *Salmo salar* sampled in the Polish marine waters, southern Baltic Sea

Katarzyna Nadolna-Ałtyn, Joanna Pawlak, Magdalena Podolska, Adam Lejk

National Marine Fisheries Research Institute

Abstract

Salmon *Salmo salar* belongs to the most important commercially fished species. Despide of that, little is known about parasite fauna of that fish in the southern Baltic Sea. ICES grouped salmon Baltic rivers into 6 Assessment Units based on management objectives, biological and genetic characteristics of the river stocks contained in a unit. Southern Baltic is a feeding area where mixture of the stocks was reported. Poor status of some stocks led to a fishing ban of salmon in the southern Baltic since 2022. The aim of our studies was to analyse the condition of salmon sampled in Polish marine waters between 2006-2023. Additionally the diet composition and presence of liver and intestines parasiets in samples collected between 2020-2023 were analysed. Generalized Linear Models show statistically significant decline in salmon condition. All analysed fish have been found infected with intestinal parasite *Eubothrium* sp., that may significantly affect the intake of nutrients. Presence of Anisakidae nematodes *Contracaecum* sp. and *Pseudoterranova* sp. were detected in livers of salmon. The main components of the diet were sprat (*Sprattus sprattus*), three-spined stickleback (*Gasterosteus aculeatus*) and invertebrate *Mysis mixta*. Diet and presence of the parasites may affect the condition of the salmon. Food components are not only source of nutrients, but also way how fish become infected. (Funding: EU Multiannual Programme for Fisheries Data Collection; own research fund of National Marine Fisheries Research Institute: DOT ParaSalmon).

Keywords: salmon, *Eubothrium*, Anisakidae, Baltic

OS – oral session

Session: [SE4] Fish parasitology

Small pelagic fish as transmitters of the Anisakidae nematodes in the southern Baltic Sea

Joanna Pawlak[1], Katarzyna Nadolna-Ałtyn[1], Magdalena Podolska[1], Beata Szostakowska[2], Marzenna Pachur[1]

1 National Marine Fisheries Research Institute, Gdynia, Poland
2 Institute of Maritime and Tropical Medicine Medical University of Gdansk, Poland

Abstract

A dispersion of Anisakidae nematodes among marine organisms in the southern Baltic Sea has been reported over the last decade. Most attention was paid to zoonotic nematode species (*Pseudoterranova* sp., *Anisakis* sp., *Contracaecum* sp.), which were noted in commercially important fish, like cod *Gadus morhua* or salmon *Salmo salar*. Small pelagic fish, especially sprat *Sprattus sprattus*, are the main components of the diet of piscivorous fish predators and were considered as the source of infection. The aim of our studies was to determine the Anisakidae nematodes composition in sprat from the Polish marine waters. Sprats were collected and analysed between 2015-2016 as well as 2019 and 2021. Visual inspection of the body cavity was performed during ichthyological analysis. Molecular analysis of all collected Anisakidae larvae were conducted. Representatives of *Pseudoterranova*, *Anisakis* and *Contracaecum* genera were detected. In 2016 the prevalence of sprat infection with *C. osculatum* in the central off-shore area of the Polish marine waters was 6.67%. *Pseudoterranova* sp. has been detected in sprat from the Baltic Sea for the first time in August 2019 with the prevalence of infection up to 15.9%. A single larva of *Anisakis* sp. was detected in sprat sampled in February 2021 in the Gulf of Gdańsk. Presented level of sprat infection with Anisakidae nematodes are elevated compared with results from the 1980s-1990s. That is in line with an increase in the number of marine mammals, especially grey seals, that act as final hosts of these parasites, and are thus indispensable for the completion of their life cycles. Sprat as a main component of the diet of cod or salmon, may play an important role as a transmitter of Anisakidae nematodes. Dispersion of these parasites in these commercially important fish poses also a risk to human health.

Keywords: Sprat, Anisakidae, Baltic Sea

OS – oral session

Session: [SE4] Fish parasitology

Chub enteric tapeworm: cestode glandular products - a sign of parasite defence mechanism against fish immune response?

Bahram Sayyaf Dezfuli[1], Massimo Lorenzoni[2], Antonella Carosi[2], Giampaolo Bosi[3], Larisa Poddubnaya[4]

1 University of Ferrara, Italy
2 University of Perugia, Italy
3 University of Milan, Italy
4 Institute for Biology of Inland Waters, Borok, Russia

Abstract

Most fish in wild and farmed populations are infected with parasites. The endemic chub *Squalius tenellus* (Heckel, 1843) was introduced more than one hundred years ago to Lake Blidinje in the west-central part of what is today Bosnia-Herzegovina. In a sample of 35 chubs the sole species of enteric helminth present was the tapeworm *Caryophyllaeus brachycollis* (Janiszewska, 1953). Our histopathological investigation identified innate immune cells involved in the fish-cestode system and evaluated glandular products within the scolex tegument of the cestode. The results showed a very close contact between the parasite's anterior body and the fish intestinal wall with an increase in the number of mucous cells and rodlet cells (RCs) within the fish intestinal epithelium. Also, within the tunica propria-submucosa, beneath the site of scolex attachment, numerous neutrophils and mast cells (MCs) were detected. Transmission electron microscopy analysis showed the occurrence of a multicellular, syncytial glandular complex within *C. brachycollis* scolex, within which membrane-bound secretory granules of moderately dense, fibrillar contents are formed. The glandular cytoplasmic processes containing granules extend into the distal tegumental cytoplasm, with granules distributed all around the tapeworm body scolex. Large rod-shaped granules up to 2 µm in length are located perpendicular to the surface membrane and in close contact with it, and appear to utilize a microapocrine secretion mechanism for discharge of granule contents from the scolex. Helminths establish long-term infections by modulating the host immune response for survival, releasing excretory-secretory products. Different sizes lipid droplets likely originating from both the parasite excretory system and the fish intestine were observed at the fish-parasite interface area. Our results are among the first to provide evidence on the sophisticated relationship between fish immune cells and cestode products.

Keywords: tapeworm, glandular products, fish innate immune cells, histopathology, transmission electron microscopy

OS – oral session

Session: [SE4] Fish parasitology

Polyparasitism in sea bass: magnificence of parasites adaptation and host tolerance

Bahram Sayyaf Dezfuli[1], Emanuela Franchella[1], Joseph DePasquale[2], Mattia Lanzoni[1], Luisa Giari[1]

1 University of Ferrara, Italy
2 Morphogenyx, New York, USA

Abstract

We had the opportunity to study the co-occurrence of 4 different helminths species in organs of sea bass, *Dicentrarchus labrax* (L.) obtained from a lagoon in the River Po delta. *D. labrax* harbored both ectoparasites, the gill monogenean *Diplectanum aequans*, and endoparasites, the digenean *Timoniella imbutiformis* in the gut, and, in extra-intestinal sites, encysted larvae of nematode *Contracaecum rudolphii* and acanthocephalan *Southwellina hispida*. Histopathological examination of host tissues was conducted using light and transmission electron microscopy. A marked erosion and inflammation of the epithelium of the primary and secondary lamellae was observed in monogenean infected bass, which was associated with elevated numbers of mucous and rodlet cells. Intestinal pathology of digenean *T. imbutiformis* infection revealed the main damage to be necrotic destruction of the mucosal epithelium of the villi. Near the site of digenean attachment, several rodlet cells and mucous cells were observed in the epithelium. Co-occurrence of *C. rudolphii* and *S. hispida* larvae was common and often both parasites were loosely embedded in the visceral peritoneal serosa, where sparse dead dark-brownish melanised larvae of nematode were also noticed. Encysted larvae of nematode and acanthocephalan were surrounded by loose connective tissue with very few scattered mast cells and macrophage aggregates present. *Phalacrocorax carbo sinensis*, a cormorant native to the study area, acquires helminth larvae from feeding on *D. labrax,* which act as their paratenic host. Encapsulation of larvae in an extra-intestinal location enables the survival of the parasites while preventing further migration of the nematode and acanthocephalan to other organs. Thus isolated, live larvae do not excessively compromise the function of the alimentary canal and the overall health of fish. While the full killing mechanisms of the immune cells are inhibited, further development of the larva is prevented.

Keywords: seabass, monogenean, digenean, nematode, acanthocephalan

OS – oral session

Session: [SE4] Fish parasitology

Zoonotic parasites in common freshwater fish species in Serbia

Stanislav Simin[1], Srđan Subotić[2], Katarina Breka[2], Nikola Betić[3], Milica Kurućki[2], Aleksandra Uzelac[4], Ivana Klun[4]

1 University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, Novi Sad, Serbia
2 University of Belgrade, Faculty of Biology, Belgrade, Serbia
3 University of Belgrade, Institute for Meat Hygiene and Technology, Belgrade, Serbia
4 University of Belgarde, Institute for Medical Research, Belgrade, Serbia

Abstract

The Sava and Danube rivers are the source of freshwater fish species sold at the markets in Serbia. As only a few reports from Serbia on zoonotic parasites transmitted by fish have been published, the risk to consumers and the role of fish in the transmission of parasites to terrestrial hosts is not well established. For this study, fish fillets were cut for examination for plerocercoids of diphyllobothriids and *Spirometra* spp. The coelom, internal organs and musculature were inspected for *Eustrongylides* spp. larvae. Fillets were pooled by species and location and artificial digestion using trypsin was performed. The digested sediment was examined by microscopy prior to DNA extraction and detection of *Metagonimus* spp., *Apophallus* spp., *Methorchis* spp. and *Opistorchis* spp. by PCR. A total of 85 (48 from the Danube, 37 from the Sava River) specimens of European perch (*Perca fluviatilis,* n=14), common roach (*Rutilus rutilus*, n=30), common *bream* (*Abramis brama*, n=27), vimba bream (*Vimba vimba*, n=9), and white-eyed bream (*Ballerus sapa*, n=5) were examined. *Eustrongylides* spp. larvae were detected in 10/14 European perch (8/12 from the Danube, 2/2 from the Sava), and in 1/10 common bream from the Sava. Metacercariae were visually detected in pools of the common roach and vimba bream from the Sava. PCR confirmed the presence of *Metagonimus* spp. in the common bream from the Sava and Danube as well as the common roach from the Danube. The results suggest that European perch are heavily parasitized by *Eustrogylides* spp., as evident in some fish from the Danube. DNA of *Metagonimus* spp. with confirmed zoonotic potential was detected in common bream and roach. These findings indicate that fish caught for human consumption in the two largest rivers in Serbia may be a source of zoonotic parasites.

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Keywords: Zoonotic parasites, freshwater fish, Danube, *Eustrongylides* spp., *Metagonimus* spp.

OS – oral session

Session: [SE4] Fish parasitology

Trematode *Diplostomum pseudospathaceum* inducing differential immune gene expression in sexual and gynogenetic gibel carp (*Carassius gibelio*), an invasive freshwater fish

Andrea Vetešníková Šimková[1], Md Mehedi Hasan Fuad[1], Tomáš Tichopád[2], Markéta Ondračková[3], Kristína Křížová[1], Mária Seifertová[1], Kristýna Voříšková[1], Martin Demko[4], Lukáš Vetešník[1]

1 Faculty of Science, Masaryk University, Brno, Czech Republic
2 University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Vodňany, Czech Republic
3 Institute of Vertebrate Biology of the Czech Academy of Sciences, Brno, Czech Republic
4 Central European Institute of Technology, Masaryk University, Brno, Czech Republic

Abstract

Diplostomum pseudospathaceum is a widely-distributed trematode parasite infecting the eyes of a wide range of the fish species. Gibel carp (*Carassius gibelio*) may act as one of the potential second intermediate host. This invasive fish species often forms populations composed of sexual and asexual (reproducing by gynogenesis) specimens. We hypothesized that parasite-mediated selection is one of the potential mechanisms contributing to the coexistence of asexual-sexual complexes. The experimental infection of gynogenetic and sexual gibel carp using *D. pseudospathaceum* was performed, and the transcriptome profile of the fish spleen was applied to reveal the differentially expressed immunity-associated genes related to *D. pseudospathaceum* infection. Gynogenetic fish were more parasitized when compared to sexuals. Our analyses showed that eye trematode situated in an immune-privileged organ may induce an immune response in gibel carp. Differential gene expression induced by *D. pseudospathaceum* infection was revealed, with many genes documenting different impacts on gynogenetic and sexual gibel carp, specifically mostly DEGs upregulation in sexuals, and downregulation in asexuals. GO analyses revealed the importance of immune genes assigned to the following GO terms: immune function, the Notch signaling pathway, MAP kinase tyrosine/threonine/phosphatase activity, and chemokine receptor activity. The 12 immunity-associated pathways revealed by KEGG pathway enrichment analysis were affected by *D. pseudospathaceum*. Our study implies that parasite-mediated selection and the modulation of organismal responses to parasite infection may represent the key mechanism contributing to the coexistence of asexual and sexual forms in gibel carp. We revealed the limited potential of asexual gibel carp to cope with parasite infection likely indicating a loss of capacity to induce an effective immune response.

The study was funded by the Czech Science Foundation, Project No. 22-27023S.

Keywords: trematodes, gibel carp, RNAseq, differential gene expression, immune genes

OS – oral session

Session: [SE4] Fish parasitology

Distribution of *Anisakis* sp. larvae in the muscle of European hake (*Merluccius merluccius* L., 1758) of the south-east of Ireland and methods to reduce its infection risk

Fernando Atroch[1], Luis Filipe Rangel[2], Paula Ramos[3], Camilo Ayra-Pardo[4], Maria João Santos[5]

1 Porto University/ CIIMAR /Portugal
2 CIIMAR/ Portugal
3 IPMA/ Portugal
4 CIIMAR/Portugal
5 Porto University/ CIIMAR/ Portugal

Abstract

European hake is a fish that is very highly appreciated in the fish market; however, it is recognized as being usually heavily infected by *Anisakis* sp. worms. Moreover, it has a high zoonotic risk and problems with food security.

This work aimed to evaluate the distribution of worms in their edible portion, the muscle, and to recommend safety measures to decrease their potential risk to human health.

Fifteen hakes, fully eviscerated, caught in South East Ireland at FAO zone 27, were analyzed during the spring of 2023. The muscle was divided into 8 portions: left and right sides, dorsal and ventral (above or under the middle line of the fish), and anterior and posterior (forward and behind the anus aperture). The symmetry of the infection, right versus left side, was analyzed. The fish analyzed had a range for their total weight of 646-154g and a total length of 47-66cm.

All the hakes were infected with *Anisakis* larvae, and we reported a total intensity in the muscle of 11,148 worms in the fish analyzed. The sections most infected were the ventral anterior sections. Possible methods to reduce the presence of *Anisakis* in the muscle, including mechanical cutting, will be discussed to reduce the risk of infection by human consumption.

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Keywords: *Anisakis*; European hake; *Merluccius merluccius*; infection risk

PS – poster session

Session: [SE4] Fish parasitology

Grillotia sp. (Cestoda: Trypanorhyncha) of pouting (*Trisopterus luscus* (L., 1758)) collected in Portuguese waters and their risk to food safety

Fernando Atroch[1], Paula Ramos[2], Yangrong Zhu[3], Luis Filipe Rangel[4], Camilo Ayra-Pardo[4], Maria João Santos[3]

1 Porto University/ CIIMAR/ Portugal
2 IPMA / Portugal
3 Porto University/ CIIMAR /Portugal
4 CIIMAR/ Portugal

Abstract

Infection of humans by Trypanorhyncha larvae is rare, but recent studies have pointed to the allergenic potential of some species of the group. This work aimed to study the parasitic infection of pouting by *Grillotia* sp. (plerocercoids) and the risk to food safety. Pouting samples were collected in Portuguese waters (FAO zone 27) in the Porto-North and Lisbon-South, seasonally during 2023. The cestodes found were reported and identified, and the infection levels were determined. The food risk generated by the *Grillotia* sp. took into account the infection levels. 339 fish were surveyed for worms detection. Forty-five fish were infected. A total of 106 worms of the *Grillotia* sp. were collected. They recorded no evident seasonal pattern. The worms were found on the viscera of the fish. Species of the order Trypanorhyncha have been reported to have zoonotic and allergenic potential. By its infection site, *Grillotia* sp. is evidence of low food risk at first analysis. However, further studies should be carried out to understand the zoonotic and allergenic risks the species poses.

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Keywords: *Grillotia*, Trypanorhyncha, pouting, food safety

PS – poster session

Session: [SE4] Fish parasitology

Microsporidia parasite of pouting (*Trisopterus luscus* (L., 1758)) collected in Portuguese waters and their risk to food safety

Fernando Atroch[1], Yangrong Zhu[2], Camilo Ayra-Pardo[3], Paula Ramos[4], Maria João Santos[5], Luis Filipe Ragel[3]

1 Portugal
2 Porto University/ CIIMAR /Portugal
3 CIIMAR/ Portugal
4 IPMA/ Portugal
5 Porto University/ CIIMAR/ Portugal

Abstract

Microsporidia infects an extensive host range, including vertebrates (as humans) and invertebrate hosts. 220 microsporidia genera described are known to infect aquatic organisms, with about 20 genera infecting fish. This work aimed to study the parasitic infection of pouting by microsporidia (cysts) and evaluate its risk to food safety. Four seasonal pouting samples were carried out in Portuguese waters (FAO zone 27) in Porto during 2023. The results presented in this study are partial. The cysts found were counted and identified, and the infection levels were obtained. The food risk generated by the cysts took into account the infection levels and their infection site. A total of 119 fish were

analyzed. The cysts were recorded in the musculature of 79 fishes, with a mean abundance of 15.4±28.3 cysts. A total of 1,836 cysts of the Microsporidia were collected. The site of infection of this parasite can generate food risk to consumers. However, further studies should be carried out to understand the zoonotic risk this parasite poses.

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Keywords: Cysts, Microsporidia, pouting, *Trisopterus luscus*

PS – poster session

Session: [SE4] Fish parasitology

Parasite diversity in Antarctic fishes (Teleostei: Nototheniinae) off the Livingston Island in the Southern Ocean, West Antarctica

Simona Georgieva[1], Petya Orozova[2], Zornitza Zaharieva[3], Eliza Uzunova[4]

1 Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences
2 National Reference Laboratory for Fish, Mollusks and Crustacean Diseases, National Diagnostics Research Veterinary Institut
3 Department of General and Applied Hydrobiology, Faculty of Biology, Sofia University "St. Kliment Ohridski"
4 Department of General and Applied Hydrobiology, Faculty of Biology, Sofia University "St. Kliment Ohridski"

Abstract

West Antarctica is known to experience increased worming by 3°C since the mid-20th century. Such shifts in temperature, increased ice melt, and desalinated water are expected to have profound effects on host-parasite interactions and the dynamics of disease in the Southern Ocean. Notothenioid fishes from the West Antarctic have been already reported as severely affected with parasite disease outbreaks because of the rapidly changing environment. As part of a broader survey on the Antarctic fish pathogens off the Livingston Island (South Shetlands Islands, West Antarctica) we examined five notothenioid fishes, *Parachaenichthys charcoti*, *Notothenia coriiceps*, *N. rossii*, *Trematomus bernacchii*, and *Chaenocephalus aceratus*, for metazoan parasite infections. Fishes were caught during the 31st and 32nd Bulgarian Antarctic expeditions, during the Austral summers of 2023 and 2024, respectively, at depths of 15–35 m. Parasites recovered were identified morphologically and/or molecularly. Presence of multiple species from six major taxa, i.e., Digenea, Cestoda, Nematoda, Acanthocephala, Copepoda, and Myxosporea were recovered. Of these, three myxosporeans recovered represent putative new species. The cestodes and nematodes were represented only by larval stages. Our results: (i) suggest a diverse and yet unexplored fish parasite fauna in the West Antarctic, (ii) are important contribution to the better understanding of the biology behind Antarctic fish infections that would help make projections about the future impact of the worming in the Southern Ocean on the local biota, host-parasite interactions, and disease outbreaks.

The study was funded through the National programme for polar research, project "Potential ichthyopathogens in Antarctic fish under a changing climate" contract #70-25-28/2023, and managed by Sofia University "St. Kliment Ohridski".

Keywords: Antarctica, Southern Ocean, Nototheniinae, parasite diversity

PS – poster session

Session: [SE4] Fish parasitology

In vivo study on the zoonotic potential of *Cryptocotyle lingua* (Digenea)

Maureen Duflot[1], Odile Bourgau[1], Mélanie Gay[1], Grégory Karadjian[2]

1 Anses, Laboratory for Food Safety, Boulogne-sur-Mer, France
2 JRU Parasite Immunology and Molecular Biology (BIPAR), ENVA-INRAE-ANSES, Animal Health Laboratory, Maisons-Alfort, France

Abstract

Over the last two decades, the diffusion of new eating habits and the increase of fish products demand lead to a raising risk for consumers due to fish-borne parasitic zoonosis. *Cryptocotyle* (Lühe, 1899) are present in marine fish species. Human may be infected by consumption of raw or undercooked fish products. Characterized as zoonotic by Chai and Jung (2017) based on Babbott et al. (1961) human case description in 1999, its impact on human health is still unclear. For the first time, this study intends to characterize *Cryptocotyle* zoonotic potential.

Beforehand conditions of conservation of *Cryptocotyle* metacercariae were set to organize scheduling of isolation of metacercariae from fish. Then, *in vivo* trials were performed on mice. After ingestion of 500 *Cryptocotyle ligua* metacercariae per mouse, daily monitoring of mice was undertaken by observation of their behavior, weight record and fecal time report. Mice were sacrificed twenty-one days post infection and organs were collected for observations of parasites or lesions.

This survey constitutes the first analysis of *Cryptocotyle lingua* zoonotic potential by *in vivo* experiments.

Acknowledgements: The authors want to thank the Hauts-de-France region of France for financial support.

Keywords: zoonotic parasite, *Cryptocotyle*, trematode, in vivo experiment

PS – poster session

Session: [SE4] Fish parasitology

Multi-year assessments on the *Notothenia coriiceps* helminth community dynamics: a summary of 2002–2021 non-annual observations near the Ukrainian Antarctic Station "Akademik Vernadsky"

Tetiana A. Kuzmina[1,2], Yaroslav Syrota[1,2], Olga I. Lisitsyna[1,2], Oleksander Salganskiy[3], Ihor V. Dykyy[4], Anton Y. Puhovkin[5,6,7], Yuriy Kuzmin[1]

1 I. I. Schmalhausen Institute of Zoology NAS of Ukraine, Kyiv, Ukraine
2 Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovak Republic
3 Kyiv, Ukraine
4 Ivan Franko National University of Lviv, Lviv, Ukraine;
5 State Institution National Antarctic Sc Parasites in domestic horses ientific Center of the Ministry of Education and Science, Kyiv, Ukraine
6 Masaryk University, Department of Experimental Biology, Brno, Czech Republic
7 Institute for Problems of Cryobiology and Cryomedicine of the NAS of Ukraine, Kharkiv, Ukraine

Abstract

This study aimed to assess the species richness and analyze the fluctuations in the helminth infracommunities of Antarctic rockcod *Notothenia coriiceps* in coastal waters of the Ukrainian Antarctic station "Akademik Vernadsky", Argentine Islands, West Antarctica.

Between 2002 and 2021, 323 *N. coriiceps* specimens were examined by parasitological techniques. In total, 60,137 helminth specimens were collected and identified. A negative binomial regression model was elaborated to estimate variations in the helminth infracommunities during two decades.

All *N. coriiceps* individuals were infected with helminths: 94% of fishes were infected with Nematoda, 87% with Cestoda, 92% with Trematoda, 99% with Acanthocephala, 63% with Monogenea. Thirty helminth species were found: 5 species of Nematoda, 4 Cestoda, 9 Trematoda, 11 Acanthocephala, and 1 Monogenea. The mean number of helminth species per host ranged from 3–14. The regression model revealed that the overall mean abundance of *N. coriiceps* helminth infection was lower in 2014 and higher in 2021 compared to the base 2002 year. PERMANOVA demonstrated the presence of significant differences in the helminth component community composition in separate years. Eight species (*Macvicaria pennelli*, *Diphyllobothrium* sp., *Pseudoterranova* sp., Bilocular metacestode, *Metacanthocephalus* spp., *Genolinea bowersi*, *Elytrophalloides oatesi*, *Neolebouria antarctica*) added the highest contribution to helminth community dissimilarities. A rarefaction curve constructed to estimate the sufficiency of the fish sample size for the detection of the species richness indicated that the survey of 90–108 *N. coriiceps* individuals resulted in the detection of 96% of the total helminth species number; increasing the sample to 323 individuals led to the detection of one more species (4%) in the studied area.

The study was supported by National Antarctic Scientific Center of the Ministry of Education and Science of Ukraine (project H/05-2023).

Keywords: Antarctic rockcod, helminth community dynamics, component community composition, species richness, Ukrainian Antarctic station

PS – poster session

Session: [SE4] Fish parasitology

Acanthocephalans from freshwater fishes in Northeast Thailand

Olga Lisitsyna[1], Daniel Barčák[1], Alexis Ribas Salvador[2], Srisupaph Poonlaphdecha[2], Mikuláš Oros[1]

1 Institute of Parasitology SAS, Slovak Republic
2 University of Barcelona, Spain

Abstract

During the ichtyoparasitological survey conducted in Udon Thani and Nong Khai provinces of Thailand in 2015, four species of acanthocephalans were found in four species of freshwater fishes. Adult stages of *Pallisentis rexus* and *Raosentis* sp. (Eoacanthocephala, Quadrigyridae) were isolated from *Channa striata* (Anabantiformes, Channidae) and *Mystus mysticetus* (Siluriformes, Bagridae), respectively. Cystacanths of *Arhythmorhynchus* sp. (Palaeacanthocephala, Polymorphidae) were found on the viscera of *M. albolineatus* (Siluriformes, Bagridae). Adult acanthocephalans of the subgenus *Acanthosentis* isolated from *Barbonymus schwanenfeldii* (Cypriniformes, Cyprinidae) differed morphologically from all described species of the subgenus. These differences included the arrangement of circular rows of tegumental spines in two fields with a more or less pronounced distance between them and by the presence of a dome-shaped process with a circular row of small spines at the base at the posterior end in females. Molecular data were obtained for three species, and phylogenetic analysis based on 18S rDNA clearly supported the (sub)generic identification of *Acanthogyrus* (*Acanthosentis*) sp. n. and *P. rexus*. While the phylogenetic position of the former species within the subgenus could not be clarified, the latter species formed a well-supported sister lineage in the clade with isolates of *P. roparensis*, *P. nagpurensis*, *P. paranandai* and *P. nandai*. In summary, *Acanthogyrus* (*Acanthosentis*) sp. n. will be formally described as a new species, the first genetic data for *P. rexus* were generated, and the species of the genus *Raosentis* was found for the first time outside the Indian subcontinent.

The study was funded by the Next Generation EU through the Recovery and Resilience Plan for Slovakia (No. 09103-03-V0l-00016).

Keywords: Acanthocephala, fish, Thailand, taxonomy, DNA

PS – poster session

Session: [SE4] Fish parasitology

Nybelinia sp. (Cestoda: Trypanorhyncha) of pouting (*Trisopterus luscus* (L., 1758)) collected in Portuguese waters and its risk to food safety

Fernando Atroch[1], Paula Ramos[2], Ana Catarina Cunha[1], Yangrong Zhu[1], Sandra Regalado[2], Luis Filipe Rangel[3], Camilo Ayra-Pardo[3], Maria João Santos[1]

1 CIIMAR, Department of Biology, Faculty of Sciences, Porto University, Porto, Portugal1; CIIMAR, University of Porto, Matosinhos, Portugal;
2 CIIMAR, University of Porto, Matosinhos, Portugal; IPMA, I.P., Portuguese Institute for the Ocean and Atmosphere, Lisboa, Portugal.
3 CIIMAR, University of Porto, Matosinhos, Portugal

Abstract

Parasitic human diseases transmitted by fishery products include those caused by cestoda, trematoda, and nematode. This work aimed to study the parasitic infection of pouting by *Nybelinia* sp. (plerocercoids) and evaluate their possible risk to food safety. Four seasonal pouting samples were carried out in Portuguese waters (FAO zone 27) during 2023. A total of 339 fish were analyzed. The cestodes found were counted and identified, and the infection levels were obtained. The food risk generated by *Nybelinia* sp. took into account its infection levels and site. A total of 6,643 specimens of the *Nybelinia* sp. were collected. The worms were found in the musculature and viscera of the host. In winter, a higher Total Intensity was detected. Other species of Trypanorhyncha, belonging to the genus *Nybelinia*, have been reported with zoonotic potential; since our *Nybelinia* sp. worms show high parasitic infection levels and infect the muscle, we assume that they may represent a risk to food safety. However, further studies should be conducted to understand its zoonotic risk better.

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Keywords: *Nybelinia*, Trypanorhyncha, pouting, food safety

PS – poster session

Session: [SE4] Fish parasitology

The potential of methods to visualize and isolate larval *Anisakis* applied to the assessment of the number of larvae in the viscera and the prediction of parasite load in scabbardfish muscle

Paula Ramos[1], Sandra Regalado[1], Fernando Atroch[2], Luis Filipe Rangel[3], Camilo Ayra-Pardo[3], Maria João Santos[2]

1 IPMA, I.P., Portuguese Institute for the Ocean and Atmosphere, Lisboa, Portugal1; CIIMAR, University of Porto, Matosinhos, Portugal

2 CIIMAR, University of Porto, Matosinhos, Portugal; CIIMAR, Department of Biology, Faculty of Sciences, Porto University, Porto, Portugal.
3 CIIMAR, University of Porto, Matosinhos, Portugal;

Abstract

The black scabbardfish, *Aphanopus carbo* is an Atlantic Ocean fish with important commercial value. 120 adult black scabbardfish specimens were obtained during 2023 along the South Portuguese coast (FAO zone 27). A parasitological survey was carried out to evaluate the intensity of the zoonotic parasite *Anisakis*. The parasite was analyzed in different organs and tissues for each fish through visual inspection, artificial digestion, and UV-press methods. Parasites were identified and preserved for subsequent molecular analysis. Four degrees of parasite intensity were defined based on visual inspection of the visceral cavity. Attempts had been made to associate the degree of infection with the presence of the L3 larvae stages in the belly flap. Results are discussed in relation to the potential methods used to recover the *Anisakis* larvae in the belly flaps to provide information to prevent human infection.

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Keywords: black scabbardfish, Anisakis, zoonotic parasite, food control

PS – poster session

Session: [SE4] Fish parasitology

Nematoda parasites from European hake (*Merluccius merluccius*)

Iara Santos Mourão[1], Fernando Atroch[1], Luis Filipe Rangel[2], Camilo Ayra-Pardo[2], Paula Ramos[3], Maria João Santos[1]

1 CIIMAR, Department of Biology, Faculty of Sciences, Porto University, Porto, Portugal and CIIMAR, University of Porto, Portugal
2 CIIMAR, University of Porto, Portugal
3 CIIMAR, University of Porto, Portugal and IPMA, I.P., Portuguese Institute for the Ocean and Atmosphere, Lisboa, Portugal

Abstract

Among the products obtained by fishing activity, European hake (*Merluccius merluccius*) stands out, leading to the need to monitor its quality in terms of the occurrence of parasites of zoonotic interest, such as nematodes of the genus *Anisakis* – taking into account the frequency of its parasitism and the risk to humans. 30 samples collected during the autumn of 2023 on the Biscayan golf were analyzed to quantify the nematodes found and examine their consumption's food safety. For parasitological analysis, one belly flap and viscera were examined using the UV-press method to find Nematoda, which was identified by morphology. Finally, nematode distribution per site and prevalence was determined. A 100% prevalence of nematode infection was observed in the fish analyzed, with a total intensity of 9,492 worms in the viscera and 11,134 worms in the muscle. 98.4% of the worms belonged to the genus *Anisakis* and 1.6% to *Hysterothylacium sp.* In the viscera, the worms were distributed: 36.8% (3,491) in the liver, 28.9% (2,752) in the gonads, 18.1% (1,714) in the stomach, and 16.2% (1,535) in the intestine. The results show a high number of nematodes found, particularly in the muscle of the belly flap analyzed, that overpass the value recorded in the viscera. The data from this study contribute to assessing the risks presented by the consumption of parasitized fish and updating the epidemiological picture in this area.

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Keywords: *Merluccius merluccius*, Nematoda, Anisakidae, zoonotic parasites, food safety

PS – poster session

Session: [SE4] Fish parasitology

Preliminary results of the analysis of the community of gill macroparasites of pouting, *Trisopterus luscus* (L., 1758) off the Portuguese waters

Maria João Santos[1], Fernando Atroch[1], Ana Catarina Cunha[1], Sandra Regalado[2], Luis Filipe Rangel[3], Camilo Ayra-Pardo[3], Paula Ramos[2]

1 CIIMAR, Department of Biology, Faculty of Sciences, Porto University, Porto, Portugal1; CIIMAR, University of Porto, Matosinhos, Portugal
2 CIIMAR, University of Porto, Matosinhos, Portugal; IPMA, I.P., Portuguese Institute for the Ocean and Atmosphere, Lisboa, Portugal
3 CIIMAR, University of Porto, Matosinhos, Portugal

Abstract

The biodiversity of macroparasites of pouting, *Trisopterus luscus* (Linnaeus, 1758) was analyzed in two sets of fishes, one from the North (Porto) n=119, and the other from the South (Lisbon), n= 120, both off the Portuguese coast (FAO zone 27). The fish were caught in sets of around 30 per season during the years 2023-2024. Morphometric features of the hosts were measured. The fish length and weight values in the North (N) ranged between 18.5–32.5 cm and 65.0-362.0g, and 19.5-30.6cm and 65.0-377.6g, in the South (S),respectively.

When the fishes arrived at the laboratory, they were dissected, and the gills extracted and promptly frozen. Later on, they were defrosted and analyzed under a dissection microscope for parasite detection. The epidemiological data for each parasite species found was recorded.

The gills community of macroparasites recorded in the N and in the S was different. In the N, we found 3 parasite species less, belonging to the Crustacean (Isopoda) and the Trematoda taxa, than in the S., where we retrieved 6 species. The common species of parasites found were 1 Monogenea - Polyopisthocotylea, 1 Cestoda - Trypanorhyncha, and 1 Crustacea - Copepoda.

For the seasonal variation analysis, the Cestoda andthe Copepoda show very high levels of infection during the Winter season, probably profiting from the low immunological system defences of the fish during low water temperatures.

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Keywords: Pouting, gill macroparasites, community

PS – poster session

Session: [SE4] Fish parasitology

MHC diversity and parasite load driving invasive success of gibel carp (*Carassius gibelio*): temporal changes in host-parasite interactions

Lukáš Vetešník, Tomáš Pakosta, Andrea Vetešníková Šimková

Faculty of Science, Masaryk University, Brno, Czech Republic

Abstract
Gibel carp (*Carassius gibelio*) is an invasive species widely distributed in Europe. The populations of gibel carp are often composed of asexual and sexual specimens. Asexual females reproduced by gynogenesis when the eggs are activated by the sperm of conspecific or phylogenetically related males. In the line with the Red Queen hypothesis, the asexual form or the most common genetic clone of asexuals is the target of parasite adaptation, whilst sexual form may escape from parasites. The genes of major histocompatibility complex (MHC) genes play the key role in vertebrate adaptive immunity and resistance to parasites. We investigated the variability of major histocompatibility (MHC) genes of the class IIB and the associations between MHC genes and parasitism in the selected population of gibel carp during three consecutive years. We showed the temporal differences in parasite load as well as in MHC allelic profile of sexual and asexual forms, and revealed several associations between parasite species and MHC alleles.

The study was funded by the Czech Science Foundation, Project No. 22-27023S.

Keywords: MHC genes, parasitism, gibel carp, host-parasite interactions

PS – poster session

Session: [SE4] Fish parasitology

Decoding the rich diversity of monogeneans on catostomid fishes (Catostomoidei, Cypriniformes): A first insight into the internal phylogeny of the Pseudomurraytrematidae

Eva Řehulková, Mária Seifertová, Andrea Vetešníková Šimková

Faculty of Science, Masaryk University, Brno, Czech Republic

Abstract
A survey was initiated to investigate the diversity of monogeneans infecting North American catostomid fishes. Fifteen catostomid species from seven US states (Arkansas, Illinois, Michigan, Mississippi, New York, Texas, and Wisconsin), the Canadian province of Québec, and northern Mexico have been surveyed to date. Ten of the fifteen species of catostomids examined were positive for pseudomurraytrematid parasites. A total of 23 monogenean species across three genera (*Anonchohaptor*, *Icelanonchohaptor*, and *Pseudomurraytrema*) were collected from the gills and/or fins of the examined catostomid hosts. *Pseudomurraytrema* was the most diverse genus with twelve species, followed by *Anonchohaptor* with seven species and *Icelanonchohaptor* with four species. The greatest species richness of monogeneans was recorded on *Hypentelium nigricans* (Arkansas), which hosted four species of *Pseudomurraytrema*, and *Ictiobus bubalus* (Illinois, Mississippi, Texas) hosted three species of *Anonchohaptor* and one species of *Icelanonchohaptor*. Most species of the Pseudomurraytrematidae were recorded on gills, only three species of *Icelanonchohaptor* were found on fins of the examined hosts (*Carpiodes carpio*, *I. bubalus*, *Ictiobus niger*). A phylogenetic analysis including all 23 species of the Pseudomurraytrematidae and five species of the Diplectanidae used as an outgroup revealed two well-supported clades: One represented by species of *Pseudomurraytrema* from species of Catostominae (*Catostomus*, *Erimyzon*, *Hypentelium*, *Minytrema*, and *Moxostoma*), another included species of *Anonchohaptor* and *Icelanonchohaptor* mostly from the Ictiobinae (*Carpiodes*, *Catostomus*, *Ictiobus*, *Minytrema*, *Moxostoma*). *Pseudomurraytrema asiaticus* parasitizing Chinese sucker, *Myxocyprinus asiaticus*, was in basal position to the Nearctic Pseudomurraytrematidae, suggesting that this species represents an earlier evolutionary branch within this family.

The study was funded by the MEYS of the Czech Republic, project no. LUAUS23080.

Keywords: Monogenea, Pseudomurraytrematidae, North America, catastomid fish

PS – poster session

Session: [SE4] Fish parasitology

New discovery in the USA and phylogenetic position of *Salsuginus* parasite (Monogenea: Dactylogyridae) associated with the western mosquitofish, *Gambusia affinis*

Chahrazed Rahmouni[1], Mária Seifertová[1], Megan Bean[2], Andrea Vetešníková Šimková[1]

1 Faculty of Science, Masaryk University, Brno, Czech Republic
2 Texas Parks and Wildlife Department, TX, USA

Abstract
The Western mosquitofish, *Gambusia affinis* (Baird and Girard, 1853), belonging to Poeciliidae, is naturally found across the Mississippi and Gulf Coast drainages in North America. However, it has been widely introduced globally to control mosquito-borne diseases. Herein, we document the presence of a commonly distributed dactylogyrid monogenean parasite associated with *G. affinis*, *Salsuginus seculus* (Mizelle and Arcadi, 1945) Murith and Beverley-Burton, 1985, found in the North Fork Guadalupe and Brazos River Basins in Texas, USA. Our discovery marks the fourth recorded instance of *S. seculus* on *G. affinis* in Texas. The meristic characteristics of *S. seculus* largely coincide with those observed in previous studies conducted in Texas. However, we observed intraspecific variability when comparing our findings with type-material collected from the Western USA, as well as with specimens of *S. seculus* found to parasitize non-indigenous *G. affinis* in Japan. Analysis of 28S rDNA sequences places *Salsuginus* as a sister taxon to a monophyletic group of dactylogyrids (*Gussevia*, *Sciadicleithrum*, and *Parasciadicleithrum*) associated with Neotropical Cichlidae. Our study emphasizes the importance of investigating the potential co-introduction of monogeneans along with *G. affinis* into new freshwater systems and its impact on native fish populations.

This study was funded by the Ministry of Education, Youth and Sports of the Czech Republic, project no. LUAUS23080. Fish sampling and processing was funded by US Fish and Wildlife Service's Sport Fish Restoration Grant to Texas Parks and Wildlife Department.

Keywords: *Gambusia affinis*, Poeciliidae, Monogenea, *Gyrodactylus*, parasite co-introduction

PS – poster session

[SE5]
Vector-borne pathogens and diseases

Session: [SE5] Vector-borne pathogens and diseases

The role of fleas as a vectors of *Bartonella* sp. and the genetic diversity of *Bartonella* sp. in rodents and fleas

Mohammed Alsarraf[1], Lukasz Banasiak[2], Katarzyna Tolkacz[1], Dorota Dwużnik-Szarek[1], Maciej Grzybek[3], Jolanta Behnke-Borowczyk[4], Mustafa Alsarraf[1], Jerzy M. Behnke[5], Anna Bajer[1]

1 Department Of Eco-Epidemiology Of Parasitic Diseases, Institute Of Developmental Biology And Biomedical Sciences, Faculty Of Biology, University Of Warsaw, Warsaw, Poland, Miecznikowa 1, 02-096 Warsaw, Poland
2 Institute of Evolutionary Biology, Faculty of Biology, Biological and Chemical Research Centre, University of Warsaw, Żwirki i Wigury 101, 02 089 Warsaw, Poland
3 Department of Tropical Parasitology, Medical University of Gdańsk, Powstania Styczniowego 9B, 81-519 Gdynia, Poland
4 Department of Forest Phytopathology, Faculty of Forestry, Poznań University of Life Sciences, Poznań, Poland
5 School of Life Sciences, University of Nottingham, University Park, Nottingham, NG7 2RD, UK

Abstract

Bartonella sp. is a group of parasites of mammalian erythrocytes and endothelial cells transmitted by blood-feeding arthropod ectoparasites. Different species of rodents constitute the main hosts of *Bartonella*, including several zoonotic species. *Bartonella* sp. can be transmitted by fleas. Fleas change hosts and come into contact with domestic animals as well as humans and can potentially infect them. For that reason, it is important to know which species of fleas can transmit *Bartonella* sp.

The aim of this study was to identify the species/genotypes and the diversity of *Bartonella* sp. in rodents and their fleas. Seven species of rodents were trapped between 2010- 2018 in five sites in Poland. From blood samples genomic DNA was extracted. Additionally, we have extracted DNA from the heart of two moles *Talpa europaea*. The DNA isolates were genotyped by the amplification and sequencing of *Bartonella*-specific 860-bp gene fragment of *rpoB*. Fleas collected from these rodents were identified to the species and sex then undergone DNA extraction. The phylogenetic analysis was conducted using the Mr. Bayes method and RAxML method.

Five species of fleas were identified, *Ctenophthalmus agyrtes, C. assimilis, Megabothris turbidus, M. walkeri* and *Histrychopsylla talpae*. The phylogenetic analysis revealed that two major species of *Bartonella* were detected in rodents and fleas, *B. grahamii* (n=89), *B. taylorii* (n=49), one species less frequent in Poland, *B. rochalimae -like* (n=3) and unidentified *Bartonella sp.* (n=12). Moreover, the Minimum Spanning Network for the *rpoB* gene revealed 17 haplotypes of *B. taylorii*, whereas *B. grahamii* has 9 haplotypes. The isolates from *T. europaea* clustered in a unique separate group which could indicate the occurrence of a novel species. The role of fleas for *Bartonella* sp. transmission to rodents has been confirmed. Our study confirmed great diversity of *Bartonella* spp. in rodents and in flea vectors.

Keywords: *Bartonella* sp., flea, rodents, moles, rpoB gene

OS – oral session

Session: [SE5] Vector-borne pathogens and diseases

Dirofilaria spp. spread and genetic diversity in north-eastern Europe

Anna Bajer, Mustafa Alsarraf

Department of Eco-Epidemiology of Parasitic Diseases, Institute of Developmental Biology and Biomedical Sciences, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096 Warsaw, Poland

Abstract

Dirofilariasis is a fast-spreading disease of dogs and other carnivores and humans. In present study we monitored the spread of filariae northward, by screening of dogs from the Baltic countries and confirmed endemic status of *Dirofiaria* spp. in Poland by examination of wild carnivores. We investigated genetic diversity of *Dirofilaria* spp. and distribution of haplotypes.

Blood samples were collected from dogs (n=424) across Poland, Baltic states and other countries. Samples from seven species of free-living carnivores from different regions of Poland were examined. Number of *D. repens*- and *D. immitis*-positive samples were obtained from different regions of Europe. PCR amplification and sequencing of mitochondrial markers and ITS1 were used for screening and genotyping.

The DNA of *D. repens* was detected in dogs from Poland, Ukraine, Lithuania, Latvia and Belarus, with the highest prevalence in Lithuania (38%). Among wild carnivores, *D. repens* was detected in three species (prevalence 3.1%).

Genetic diversity: 18 *D. repens* haplotypes were identified in combined mtDNA markers (COI+NADH) in 95 analysed samples. Haplotype DR1 was most common (66 isolates). Other haplotypes grouped around DR1 separated by 1–5 SNPs, forming a star-like shape. Among 122 *D. immitis* samples, 9 haplotypes were delineated. Haplotype Di1 was the dominant haplotype (91) occurring in 9 countries and 4 host species. There was evidence for geographical segregation of haplotypes, with 3 unique haplotypes in Italy.

D. repens became endemic in Poland, infecting dogs and free-living carnivores. It has spread to the Baltic states and occurs in Belarus and Ukraine. Further spread to the Nordic countries may be expected. *D. repens* is more genetically diverse than *D. immitis*, it may facilitate its spread. In Italy unique haplotypes of both *Dirofilaria* spp. have been recorded.

Acknowledgements: The study was funded by the National Science Centre (NCN) OPUS grant no. 2017/27/B/NZ6/01691.

Keywords: *Dirofilaria repens*, *Dirofilaria immitis*, epidemiology

OS – oral session

Session: [SE5] Vector-borne pathogens and diseases

Genotyping of *Babesia* spp. in deer and cats in Poland

Anna Bajer[1], Natalia Kartawik[2], Jolanta Behnke-Borowczyk[2], Mustafa Alsarraf[1], Dagmara Wężyk[1], Dorota Dwużnik-Szarek[1]

1 Department of Eco-epidemiology of Parasitic Diseases, Faculty of Biology, University of Warsaw, Warsaw, Poland
2 Department of Forest Pathology, Faculty of Forestry, Poznań University of Life Sciences, Poznań, Poland

Abstract

Babesiosis is an emerging infectious disease in Europe which affects domestic animals, livestock, wildlife and humans. Different deer species are known reservoir of several *Babesia* species, including zoonotic *Babesia divergens*. Studies on *Babesia* and other piroplasm in cats in Europe are rare and incomplete.

The aim of the current study was to determine molecular identity of babesiae infecting free-living deer species and free-roaming cats in Poland. Piroplasm species was determined by PCR amplification and sequencing of 18S rRNA gene fragment, followed by phylogenetic analysis.

Three *Babesia* spp. were identified among deer, including *B. divergens* and newly identified *B. odocoilei*-like 'deer genotype' of *Babesia*. Surprisingly, only *Babesia canis* vectored by *Dermacentor reticulatus* ticks was identified among cats, including asymptomatic individuals and individuals treated because of babesiosis.

Deer in Poland can be infected with several species of deer-specific babesiae, however infections in cats are mostly due to canine piroplasm, *B. canis*.

The study was financially supported by the National Science Centre (NCN) grant OPUS no. 2022/45/B/NZ7/01017 (AB).

Keywords: *Babesia canis*, *Babesia divergens*, *Babesia odocoilei*-like

OS – oral session

Session: [SE5] Vector-borne pathogens and diseases

Brown dog ticks collected in Romania and Hungary: molecular identification and pathogens

Sajjad Ghodrati[1], Ludek Zurek[2], David Modry[1], Andrei Daniel Mihalca[3]

1 *Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic*
2 *Department of Microbiology, Nutrition and Dietetics, Czech University of Life Sciences, Prague, Czech Republic*
3 *USAMV Cluj-Napoca, Romania*

Abstract

Rhipicephalus sanguineus sensu lato is a group of tick species commonly called brown dog ticks. *R. sanguineus* s.l. are well-known vectors of a range of pathogens of medical and veterinary importance. These include *Anaplasma*, *Ehrlichia*, *Rickettsia*, and piroplasmids such as *Babesia*, *Theileria*, and *Hepatozoon*. The taxonomy of *R. sanguineus* s.l. ticks has been questioned and debated for many years. Accurate identification of *R. sanguineus* s.l. species is crucial for understanding their role as disease vectors. Only in the last three years, three new species have been distinguished, raising questions about their roles in disease transmission.

Descriptions and re-descriptions of new species over the last decade suggest that many ticks "superficially" identified as *R. sanguineus* may actually be other species. The *Rhipicephalus* ticks, and their pathogens situation in Hungary, and Romania is unclear and likely underestimated. The genetic complexity of the brown dog tick led us to conduct genetic identification of brown dog ticks, and the presence of aforementioned pathogens.

A total of 169 *Rhipicephalus* ticks were collected from Hungary and 119 from Romania. All the ticks were analyzed by amplification and sequencing of *COI*, 16S rDNA, and 12S rDNA. Maximum-likelihood (ML) phylogenetic trees were constructed for each gene. After genetic characterization, all samples were screened for pathogens, including *Ehrlichia*, *Anaplasma*, *Rickettsia*, *Babesia*, *Theileria*, and *Hepatozoon*.

Through maximum likelihood analysis, all 169 Hungarian ticks identified as *R. sanguineus s.s.* for all three genes. For the Romanian ticks (73/119), phylogenetic analysis revealed the majority (n=62) to be *R. rossicus*, followed by *R. sanguineus s.s.* (n=10), and *R. rutilus* (n=1) in all the genes. Of 169 Hungarian ticks,168 tested negative for all pathogens assessed, with only one tick positive for *A. phagocytophilum*. None of the 119 Romanian ticks tested positive for *Anaplasma*, with the study still in progress.

Keywords: *Rhipicephalus sanguineus* s.l., tick, tick-borne disease, Hungary, Romania

OS – oral session

Session: [SE5] Vector-borne pathogens and diseases

Coexistence of *Borrelia* with different tick-borne pathogens in *Ixodes ricinus* ticks from Poland - do coinfections influence occurrence and loads of pathogens in ticks?

Julia Koczwarska[1], Agnieszka Pawełczyk[2], Renata Welc-Falęciak[1]

1 *Department of Parasitology, Faculty of Biology, University of Warsaw, Warsaw, Poland*
2 *Department of Immunopathology of Infectious and Parasitic Diseases, Medical University of Warsaw, Warsaw, Poland*

Abstract

Ixodes ricinus tick is the main vector of *Borrelia* spp. and a broad spectrum of other tick-borne pathogens in Europe. Multi-species infections are common among ticks, which creates a possibility for co-transmission of pathogens. Co-infections in humans and animals might enhance disease severity and can lead to significant consequences in terms of tick-borne disease treatment and diagnosis. The mechanism by which *Borrelia* co-exist with other pathogens within the tick remains unexplored. Furthermore, it is unclear to what extent *Borrelia* engage in interactions or how multi-species infections might influence on pathogen loads in ticks and, consequently, on transmission to humans and pathogenicity. The aim of this study was to evaluate the impact of coinfections on occurrence and loads of *Borrelia* and other pathogens in *I. ricinus*. In the years 2021-2022, we collected 2093 *I. ricinus* from tick-bitten individuals from around Poland and analyzed individually for the presence of *Borrelia* spp., *Babesia* spp. *Rickettsia* spp. *Anaplasma phagocytophilum* and *Neoehrlichia mikurensis* using molecular methods. Loads of pathogens were determined with droplet digital PCR technique.

The presence of *Borrelia* DNA was found in 328/2093 ticks. 36,1% of *Borrelia*-infected ticks were co-infected with at least one different pathogen. *Babesia* and *N. mikurensis* demonstrated higher prevalence in *Borrelia*-positive ticks (1,3 vs 3,4% and 4,2 vs 10,2% respectively). Additionally loads of *N. mikurensis* were significantly higher in *Borrelia*-positive compared to *Borrelia*-negative ticks (mean 240,0 vs 139,2 copies/µl of reaction).

Our results suggest the possibility of interactions between *Borrelia* and two other pathogens in *I. ricinus*. Understanding the relationships between pathogens coexisting in ticks may broaden our insight into epidemiology of tick-borne diseases and improve assessing the tick-borne disease risk.

The study was supported by the National Science Center (NCN) grant no. 2020/37/B/NZ6/01587

Keywords: Ixodes ricinus, Borrelia, coinfections, tick-borne pathogens

OS – oral session

Session: [SE5] Vector-borne pathogens and diseases

Incidence of Lyme borreliosis following *Ixodes ricinus* tick bite in Poland

Agnieszka Pawełczyk[1], Julia Koczwarska[2], Renata Welc-Falęciak[2]

1 *Department of Immunopathology, Infectious and Parasitic Diseases, Faculty of Medicine, Medical University of Warsaw, Poland*
2 *Department of Parasitology, Faculty of Biology, University of Warsaw, Poland*

Abstract

In Poland, the incidence of Lyme borreliosis (LB) has increased in the two last decades since 4.79 to 66.96/100 000 cases, but the risk of developing LB after the tick bite of *Borrelia*-infected tick is still unclear and depends on many factors i.e. tick stage and species, the rate of *Borrelia* prevalence in tick population and the time of tick attachment to the skin. The aim of our study was estimated the impact of *Borrelia* load in the tick and the time of tick feeding on the risk of LB developing in human in Poland.

The 2079 of *I. ricinus* parasitic on humans were investigated (collected between 2021-2022). The duration of feeding time of ticks removed from skin was estimated from scutal and coxal index. The presence and load of *Borrelia* in ticks were detected by Real Time PCR and Droplet Digital PCR, respectively. A questionnaire method, conducted among the study participants (at the beginning of the study and after 8 weeks), was used to obtain data among others on the location and time of tick parasitism, health status of the patient, diagnosis for LB by GP and clinical symptoms suggested tick-borne infection after removing of the tick.

The clinical and diagnostic data on the incidence of LB were available for 1757 patients. Among them 16.3% were bitten by

Borrelia infected *I. ricinus*. The prevalence of *Borrelia* infection in the ticks was 15.7%. The overall risk of developing LB was 3.0% and increased with detection of *B. burgdorferi* s.l. DNA in ticks from 16.0% v 0.5% when ticks tested negative. The EM was observed in 1.9% of participant who were bitten by *I. ricinus* tick, in 10.1% of participants who were bitten by *B. burgdorferi* s.l.-infected tick(s) and in 64.8% of participants in whom LB was diagnosed. The risk of LB developing was diagnosed most often in the patients who removed *Borrelia*-positive tick after 48h of attachment.

This research was funded by the National Science Centre (Poland), grant no. 2020/37/B/NZ6/01587

Keywords: *Borrelia*, *Ixodes ricinus*, Lyme borreliosis, patients, PCR

OS – oral session

Session: [SE5] Vector-borne pathogens and diseases

Optimizing mosquito-borne disease surveillance with BG-Sentinel trap equipped with FTA card

Sara Manzi[1], Michele Pazienza[2], Onofrio Zaccaria[2], Vincenzo Abbate[2], Luca Nelli[3], Claudia Fortuna[4], Francesco Gradoni[1], Federica Toniolo[1], Riccardo De Santis[5], Ferdinando Spagnolo[6], Fabrizio Montarsi[1], Florigio Lista[5], Marco Pombi[7]

1 *Istituto Zooprofilattico Sperimentale Delle Venezie, Legnaro, Italy*
2 *Stato Maggiore della Difesa, Rome, Italy*
3 *School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, Glasgow, UK*
4 *Department of Infectious Diseases, Istituto Superiore di Sanità. Rome, Italy*
5 *Istituto di Scienze Biomediche Della Difesa, Rome, Italy*
6 *Ispettorato Generale della Sanità Militare, Stato Maggiore della Difesa, Rome Italy*
7 *Dipartimento di Sanità Pubblica e Malattie Infettive, Sapienza Università di Roma, Rome, Italy*

Abstract

Early detection of vector-borne pathogens in vectors is crucial for preventing disease outbreaks. Traditional entomological surveillance is commonly based on vector trapping and pathogen molecular identification on collected specimens. This approach faces challenges such as maintaining the cold chain and the high workload of mosquito processing.

We present a trapping approach using a modified BG-Sentinel equipped with an FTA card (BG-FTA), designed to simplify field and lab work by preserving nucleic acids at environmental temperatures, thus eliminating the need for cold chain logistics and facilitating viral detection. The BG-FTA trap leverages the infected mosquito's behaviour to release pathogen through saliva during sugar-feeding. It includes a mosquito collection chamber and a feeding system with an FTA card soaked in a honey-hydrogel solution, enabling continuous mosquito collection and feeding for several days.

To define the applicability of this surveillance system in relation to standard surveillance methods and different eco-epidemiological contexts, the BG-FTA trap was tested during two field seasons (2019 and 2021) in an endemic area of West Nile virus (WNV) and Usutu virus (USUV) of North-eastern Italy. Additionally, the BG-FTA trap was evaluated in Djibouti (2020), a region characterized by a high risk of mosquito-borne diseases.

The BG-FTA cards detected both WNV and USUV in Italy (3 and 1 positive cards out of 97, respectively), while in Djibouti 2 and 1 FTA cards out of 71 detected respectively WNV and Dengue virus (DENV), confirming the trap's reliability for viral detection in different geographical contexts.

The BG-FTA approach offers significant advantages over traditional approaches for multi-target mosquito-borne disease surveillance, especially in remote areas with limited logistic support, enhancing the efficiency and effectiveness of arboviral surveillance programs.

Keywords: pathogen detection, mosquito surveillance, Arbovirus, West Nile virus, Dengue

OS – oral session

Session: [SE5] Vector-borne pathogens and diseases

From the shadows of war: molecular surveillance of dirofilariosis in stray dogs and cats as indicators of zoonotic risk in Ukraine

Mateusz Pękacz[1], Kateryna Slivinska[2,9], Alla Vyniarska[3], Katarzyna Basałaj[4], Alicja Kalinowska[4], Agnieszka Wesołowska[4], Olesia Kysterna[5], Andrii Klietsov[6], Martina Miterpáková[7], Jakub Gawor[8], Vitaliy Kharchenko[9], Anna Zawistowska-Deniziak[10]

1 *Division of Parasitology and Invasive Diseases, Department of Preclinical Sciences, Institute of Veterinary Medicine, Warsaw University of Life Sciences (SGGW), Poland*
2 *Museum and Institute of Zoology, Polish Academy of Sciences, Poland*
3 *Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies of Lviv, Ukraine*
4 *Museum and Institute of Zoology, Polish Academy of Sciences, Poland*
5 *Sumy National Agrarian University, Ukraine*
6 *NGO "Society of Veterinary Business Owners", Ukraine*
7 *Institute of Parasitology, Slovak Academy of Sciences, Slovak Republic*
8 *Polish Advisory Council for Parasitoses of Companion Animals ESCCAP Poland, Poland*
9 *I. I. Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine, Ukraine*
10 *Department of Immunology, Institute of Functional Biology and Ecology, Faculty of Biology, University of Warsaw, Poland*

Abstract

The 2022 war in Ukraine exacerbated existing public health challenges, including a rise in stray animal populations. These animals often carry parasitic diseases, posing significant biohazard risks, especially in the context of increasing vector-borne diseases amplified by climate change. Among these, dirofilariosis presents a significant threat to both animal and human health, yet comprehensive epidemiological insights, particularly for *Dirofilaria repens* and *Dirofilaria immitis*, remain scarce within Ukraine, highlighting a critical gap in current research. This study, conducted from March to December 2023 in collaboration with ESCCAP and Ukrainian NGOs, aimed to assess the biohazard risk by examining the prevalence of *Dirofilaria* species using Real-Time PCR and species-specific primers in 465 stray animals across Kharkiv, Sumy, Zvenyhorodka, and Berdychiv. Our findings revealed the presence of *D. repens*, *D. immitis*, or *Acanthocheilonema reconditum* in 65 out of 229 canine samples, yielding in an overall prevalence of 28.38 %, and in 8 out of 236 feline samples, yielding a prevalence of 3.39 %. The first report of *A. reconditum* in Ukraine adds complexity to the country's vector-borne disease landscape, highlighting the interplay of these diseases. During conflicts like wartime, embracing the One Health approach, which links human, animal, and environmental health, becomes essential. Additionally, implementing efficient vector control measures is vital to minimize the risk of disease transmission to humans.

This research was supported by The National Centre for Research and Development, Poland under grant LIDER IX 0106/L-9/2017, MSCA4Ukraine project (AvHID 1233593) and ESCCAP.

Keywords: dirofilariosis, acanthocheilonemiosis, molecular epidemiology

OS – oral session

Session: [SE5] Vector-borne pathogens and diseases

Tick-borne microsporidiosis: ticks as a neglected source of human microsporidian infections?

Bohumil Sak[1], Michaela Fibigerová[2], Kristina Mravcová[3], Nikola Holubová[1], Silvie Šikutová[3], Jana Fenclová[4], Martin Kváč[4], Ivo Rudolf[3]

1 Biology Centre of the Academy of Sciences of the Czech Republic, v.v.i., Institute of Parasitology, České Budějovice, Czech Republic
2 University of South Bohemia in České Budějovice, Faculty of Science, České Budějovice, Czech Republic
3 Institute of Vertebrate Biology, Czech Academy of Sciences, Brno, Czech Republic
4 University of South Bohemia in České Budejovice, Faculty of Agriculture and Technology, České Budějovice, Czech Republic Biology Centre of the Academy of Sciences of the Czech Republic, v.v.i., Institute of Parasitology, České Budějovice, Czech Republic

Abstract

The tick, ectoparasite of humans, domestic and wild animals, is a widespread ectoparasite that can transmit a wide range of pathogens causing a variety of diseases, mainly due to the way it parasitizes when interacting with the host's blood. Since the tick does not exclusively parasitize mammals and its life cycle requires sucking on up to 3 different hosts, it is a potential candidate for the transmission of microsporidia from various hosts. Microsporidia, unicellular obligate parasites have long been associated with serious infections in immunocompromised individuals, but also occur in immunocompetent ones, and often cause life-threatening complications of common diseases.

This study was conducted to elucidate the prevalence and molecular characterization of microsporidia in ticks in the Czech Republic. The ticks were collected either from various hosts or using flagging low vegetation. A total of 217 samples were obtained from *Ixodes ricinus* (n=203) and *Dermacentor reticulatus* (n=14). Of these, 25 microsporidia positive ticks were molecularly detected. The sequence analyses of the internal transcribed spacer (ITS) of the SSU rDNA gene identified *Enterocytozoon bieneusi* genotype D (n=8), *Encephalitozoon cuniculi* genotype I (n=7) and genotype II (n=10). Moreover, the information available to date from experimental and natural infections indicates that microsporidia can be successfully ingested by ticks with blood meal and are able to survive the tick's life-cycle transformation.

According to the results of the study, microsporidia are ranked among the most common pathogens detected in ticks (*Ixodes ricinus*) in the Czech Republic and their possible involvement in the transmission of microsporidia has been suggested. Moreover, it appears that tick feeding may facilitate transmission of infection through the concentration of microsporidia in sites of inflammation induced by ticks.

The study was supported by the grant of Grant agency of the Czech Republic (No.24-10274S).

Keywords: microsporidia, *Ixodes ricinus*, PCR, qPCR, vector borne disease

OS – oral session

Session: [SE5] Vector-borne pathogens and diseases

Pathogens transmitted by mosquitoes in Warsaw and the surrounding area - preliminary results

Aleksandra Sędzikowska[1], Joanna Werszko[1], Aleksandra Kawińska[1], Rusłan Sałamatin[1,2], Adam Kaczmarek[2], Daniel Młocicki[1]

1 Department of General Biology and Parasitology, Medical University of Warsaw, Warsaw, Poland
2 Department of Microbiology and Parasitology, Faculty of Medicine. Collegium Medicum, Cardinal Stefan Wyszyński University in Warsaw, Warsaw, Poland

Abstract

Mosquitoes, as extremely common insects, are important vector of numerous pathogens including protozoa (e.g. *Plasmodium*), nematodes (e.g. *Dirofilaria*), and viruses (e.g. Dengue, Zika). Due to changes in the distribution of these insects, there are reports of new pathogens in areas where they have not been recorded before. The aim of the research was to determine whether mosquitoes in the Masovian Voivodeship can transmit Usutu and West Nile Viruses, as well as *Spiroplasma* bacteria and nematodes.

As part of the research, multiple field trips were carried out in Warsaw (including Kabaty, Mokotów, Bielany, Targówek, Praga) and the entire Masovian Voivodeship (among others: Marki, Powsin, Otwock, Góra Kalwaria, Brwinów, Laski, Sochaczew, Podkowa Leśna, Rusinów, Nieznamierowice, Różanów). In total, material was collected from 38 different locations, including ecologically very diverse places such as parks, gardens, forests, urban and rural areas. Various collection methods were employed: traps with dry ice, traps with a UV lamp, entomological net and aspiration method. Mosquitoes collection spanned from April 2023 to February 2024, with actively flying or feeding mosquitoes captured in spring and summer, and hibernating mosquitoes found in basements during autumn and winter. Over 19,000 mosquitoes belonging to the genera *Anopheles, Aedes, Culex, Culiseta* and *Mansonia* were collected. After identification, genetic material (RNA or DNA) was isolated from the insects. Nematodes of zoonotic and veterinary importance were detected in the collected material. Vector role of mosquitoes for Usutu or West Nile Virus and *Spiroplasma* is gradually subjected to further research.

Project funded by National Science Centre (NCN), Miniatura 6, nr 2022/06/X/NZ6/00852 "Molecular studies of the occurrence of viral and bacterial pathogens dangerous for humans and animals in mosquitoes in Poland"

Keywords: mosquito, Usutu, West Nile virus, *Spiroplasma*

OS – oral session

Session: [SE5] Vector-borne pathogens and diseases

The first report on *Hepatozoon canis* in dogs and wolves in Poland: clinical and epidemiological features

Katarzyna Tołkacz[1], Milena Kretschmer[2], Sabina Nowak[3], Robert W. Mysłajek[3], Mustafa Al-Sarraf[4], Dagmara Wężyk[4], Anna Bajer[4]

1 Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
2 Vetlab sp. z o. o., Veterinary Diagnostic Laboratory, Wrocław, Poland
3 Department of Ecology, Institute of Functional Biology and Ecology, Faculty of Biology, University of Warsaw, Biological and Chemical Research Centre, Warsaw, Poland
4 Department of Eco-epidemiology of Parasitic Diseases, Institute of Developmental Biology and Biomedical Sciences, Faculty of Biology, University of Warsaw, Warsaw, Poland

Abstract

Hepatozoon canis, a parasite causing canine hepatozoonosis, is commonly found in dogs, particularly in the Mediterranean region and, more recently, in Central European countries like Austria, Hungary and Germany. Despite its high prevalence in red foxes, there have been no reported cases of infection in dogs in Poland until now. We present the first instances of autochthonous *H. canis* infection in Polish dogs, detailing their clinical presentation, alongside reporting the prevalence of *H. canis* in grey wolves across different regions of Poland.

Blood samples collected from dogs were evaluated by microscopic examination. A total of 60 wolves and 47 dogs were tested. Infections were confirmed by PCR and sequencing. Notably, gamonts of *H. canis* were found in varying proportions of neutrophils in several dogs. Molecular typing by PCR sequencing of the *18S* ribosomal RNA gene fragment confirmed infections in 11 dogs from different regions of Poland, in 2.7% of dogs attending veterinary practices in central Poland, and in 35% of wolves from various geographical regions of Poland.

Clinical manifestations were predominantly observed in older dogs, with common symptoms including anaemia and apathy, while younger dogs typically remained asymptomatic. This marks the first documented cases of *H. canis* infection in both dogs and wolves in Poland. While the exact vector of the parasite in Poland remains unknown, veterinarians should be vigilant regarding this emerging parasitic threat, emphasising the importance of appropriate diagnostic measures. Further research is warranted to elucidate the transmission dynamics of *H. canis* among domestic and wild canids in Poland.

The collection of wolf samples was performed within research projects funded by the Polish National Science Centre (Grant No. 2020/39/B/NZ9/01829 for S.N. and Grant No. 2019/35/O/NZ8/01550 for R.W.M.) and the statutory budget of the Association for Nature "Wolf", Poland.

Keywords: *Canis lupus*, *Canis lupus familiaris*, *Hepatozoon canis*, hepatozoonosis, Poland

OS – oral session

Session: [SE5] Vector-borne pathogens and diseases

Leishmania spp. and *Dirofilaria* spp. in dogs in Armenia

Seda Adamyan[1], Sargis Aghayan[2], Oleg Scherbakov[3], Hasmik Gevorgyan[3], Roman Slabodyannik[3], Varvara Solovyova[3], Julia Walochnik[1]

1 Institute of Specific Prophylaxis and Tropical Medicine, Center for Pathophysiology, Infectiology and Immunology, Medical University Vienna, Vienna, Austria.
2 Scientific Center of Zoology and Hydroecology, Academy of Sciences, Yerevan, Armenia, Laboratory of Zoology, Research Institute of Biology Yerevan State University
3 Scientific Center of Zoology and Hydroecology, Academy of Sciences, Yerevan, Armenia

Abstract

Armenia, located on a major migration route between Asia, Africa and Europe, is experiencing accelerated ecological changes due to climate change. This poses significant risks for the spread of vector-borne diseases, with human and animal health importance. The aim of this study was to evaluate the prevalence of *Leishmania* spp. and *Dirofilaria* spp. infections in domestic dogs in Armenia. Both are insect-transmitted parasites affected by climate change and globalization. Blood samples from 60 domestic dogs from surrounding villages of Artashat and Armavir towns of Armenia were screened for *Leishmania* spp. and *Dirofilaria* spp. by rapid tests and PCR. Amplicons were further identified by DNA sequencing. The sample group included 43 female and 27 male dogs aged 1-10 years. PCR assays for *Leishmania* were negative for all samples. The screening for *Dirofilaria* identified 13 positive cases out of 60 samples. Among these positive cases, 8 were females and 5 were males. The data suggests that domestic dogs, mainly used for livestock protection and living outdoors, are more exposed to vectors, making them more susceptible to these infections. As suggested by other studies, dogs living in an indoor/outdoor mixed environment are significantly more likely to be infected with vector-borne pathogens due to increased exposure to sandfly bites. A high percentage of infected dogs live in close contact to humans, highlighting their potential role as a source of human infection.

In future work, wildlife will also be screened for vector-borne parasites. This comparative approach will improve our understanding of the routes of infection, including the forthcoming analysis of wild hosts and vectors.

The findings of this study have significant implications for both dogs' health and public health in Armenia. They also enhance our understanding of the risk factors associated with the spread of zoonotic diseases.

Keywords: *Leishmania*, *Dirofilaria*, domestic dog, PCR, sequence

PS – poster session

Session: [SE5] Vector-borne pathogens and diseases

Molecular detection of haematozoan parasites in poultry of Bangladesh

Shirin Akter[1], Anita Rani Dey[1], Nusrat Nowrin Shohana[1], Mohammad Zahangir Alam[1]

1 Department of Parasitology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Abstract

Recently developed molecular based detection system would have great advantage to conduct epidemiological surveillance of avian haematozoan parasites combined with traditional morphological diagnosis by observing blood smears. Therefore, epidemiological investigation using molecular tools in previously identified (microscopically) samples were applied to explore the genetic diversity of haematozoan parasites in poultry. Parasite DNA was extracted from the 50 blood samples (those were positive to haemoprotozoan parasites with microscopy) spotted on glass slide. Extracted DNA was used for nested-PCR to detect the partial cyt*b* gene of the avian malaria mitochondrial genome. Amplification of cyt*b* gene was performed to identify the haemoprotozoan parasites at molecular level. Of 50 microscopically positive blood smears collected from different districts, 30 *Haemoproteus*, 10 *Plasmodium* and 10 *Leucocytozoon* positive samples were used for molecular detection. Out of the 50 samples tested by nested PCR, 32 resulted positive for *Leucocytozoon* or *Haemoproteus*/*Plasmodium* spp. The sequences showed high variability, and a neighbor- joining tree clustered them into 3 distinct groups with high confidence (bootstrap values from 50 to 99 for the different genera and branches). These 3 clusters corresponded to species of *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*. In conclusion, we found a variety of haematozoan parasites in indigenous poultry through molecular analysis and our present study is the first attempt to detect and characterize haemoprotozoan parasites of poultry in Bangladesh.

Keywords: poultry, malaria parasites, molecular detection, Bangladesh

PS – poster session

Session: [SE5] Vector-borne pathogens and diseases

Occurrence of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* s.l. in *Dermacentor reticulatus* ticks in the Chornobyl Exclusion Zone, Chornobyl, Ukraine. Preliminary results.

Karolina Baranowicz[1], Beata Biernat[1], Grzegorz Karbowiak[2], Kateryna Slivinska[2,3], Kinga Oparka[4]

1 Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine, Medical University of Gdansk, Poland
2 Museum and Institute of Zoology of Polish Academy of Sciences, Warsaw, Poland
3 I.I. Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, Kiev, Ukraine
4 MSc candidate; Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine, Medical University of Gdansk

Abstract

The Chornobyl Exclusion Zone (CEZ) is an area of 2,600 km² surrounding the Chornobyl Nuclear Power Plant in Ukraine. In 1986 an explosion occurred on the site, causing heavy contamination by radioactive waste and an environmental disaster. CEZ can be considered as unaffected by human activity since 1986. Previous studies have established that *Dermacentor reticulatus* is the dominant species found in the CEZ among ticks. The following study attempts to determine the prevalence of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* s.l. in *D. reticulatus* ticks present in the CEZ area. Ticks were collected by flagging method from the vegetation around of the town of Chornobyl, in the abandoned areas named Ostrov, Puso, Altanka, Cherevach. A total of 1176 adult *Dermacentor reticulatus* ticks were collected. Amplification was performed for a 75 bp fragment of the *B. burgdorferi* s.l. 23S rRNA gene using primers Bb23Sf and Bb23Sr, and the probe sequence Bb23Sp-FAM. For amplification of the 77 bp fragment of the *A. phagocytophilum msp2* gene, the primers ApMSP2f, ApMSP2r and the probe sequence ApMSP2p-HEX were used. Analyses were performed using the AriaMx Real-Time PCR System (USA) real-time thermocycler, following the appropriate temperature-time protocol. Samples of DNA isolates of female *D. reticulatus* ticks (n=300) were analysed for the prevalence of *A. phagocytophilum* and *B. burgdorferi* s.l.. One positive sample for *A. phagocytophilum* (0.33%) and three positive samples for *B. burgdorferi* s.l. (1%) were detected. The results indicate a low positivity rate of *A. phagocytophilum* and *B. burgdorferi* s.l. in the dominant tick species in the CEZ. The study confirm the previous note on the *D. reticiulatus* ticks infection with *A. phagocytophilum*. The results presented are preliminary. The research requires further investigation.

The study was funded by the MSCA4Ukraine project (AvHID 1233593; https://cordis.europa.eu/project/id/101101923).

Keywords: ticks, *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, *Dermacentor reticulatus*, Chornobyl

PS – poster session

Session: [SE5] Vector-borne pathogens and diseases

Zoonotic tick-borne pathogens in *Ixodes ricinus* complex (Acari: Ixodidae) from urban and peri-urban locations of Kosovo

Ina Hoxha[1], Betim Xhekaj[2], Genc Halimi[2], Michiel Wijnveld[3], Margarida Ruivo[3], Adelheid G. Obwaller[4], Bernhard Jäger[5], Martin Weiler[5], Julia Walochnik[1], Kurtesh Sherifi[2], Edwin Kniha[1]

1 Center for Pathophysiology, Infectiology and Immunology, Institute of Specific Prophylaxis and Tropical Medicine, Medical University Vienna, Kinderspitalgasse 15, 1090 Vienna, Austria
2 Faculty of Agriculture and Veterinary, University of Prishtina 'Hasan Prishtina', Bulevardi 'Bill Clinton', P.N., Prishtinë 10000, Kosovo
3 Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Institute for Hygiene and Applied Immunology, Kinderspitalgasse 15, 1090 Vienna, Austria
4 Division of Science, Research and Development, Federal Ministry of Defence, Roßauer Lände 1, 1090 Vienna, Austria
5 ABC-Defence, Dabsch-Kaserne, Austrian Armed Forces, Platz der Eisenbahnpioniere 1, 2100 Korneuburg, Austria

Abstract

Ixodes ricinus is Europe's most widespread tick species, inhabiting shrubs, deciduous, mixed forests and urban areas. It is a vector for many pathogens, including tick-borne encephalitis (TBE) virus, *Borrelia burgdorferi* sensu lato (Lyme borreliosis) and *Rickettsia* spp. Also in Kosovo *I. ricinus* is the predominant tick species, but data on tick-borne pathogens, especially in urban settings, are limited. This study aimed to explore the presence of tick-borne pathogens in *I. ricinus* from urban and peri-urban areas in Kosovo. Locations were sampled by flagging 150-meter transects. Altogether, 197 ticks were morphologically identified as *Ixodes ricinus* and RNA/DNA was isolated. All individuals were screened for the presence of tick-borne pathogens. Using reverse line blotting (RLB) hybridization, DNA of nine different pathogens from four genera including *Borrelia* spp., *Rickettsia* spp., *Anaplasma* spp., and *Babesia* spp. was detected. The most frequently detected pathogens were *Rickettsia* spp., followed by *Borrelia* spp. We provide the first data on genotyping of *Borrelia burgdorferi* s. l. and detecting *Anaplasma*, *Babesia*, and *Rickettsia* from *I. ricinus* in this country. The data underline that particularly recreational (peri-)urban areas may facilitate the spillover of zoonotic tick-borne pathogens to humans in Kosovo and provide baseline data for future surveys.

Keywords: Balkan, *Ixodes ricinus*, *Borrelia*, reverse line blotting, *Rickettsia*

PS – poster session

Session: [SE5] Vector-borne pathogens and diseases

Molecular investigation of *Rhipicephalus* ticks and their pathogens from Croatia

Daria Jurković Žilić[1], Ema Gagović[1], Tomislav Dumić[2], Krunoslav Pintur[2], Relja Beck[1]

1 Croatian Veterinary Institute, Croatia
2 Karlovac University of Applied Sciences, Croatia

Abstract

Ticks of genus *Rhipicephalus* are the most complex group within the family Ixodidae. The study aimed to examine *Rhipicephalus* ticks and their pathogens to fill the gaps in understanding their distribution and pathogens they harbour.Ticks were collected from various places throughout Croatia,from animals and the environment.

A total of 2,500 ticks belonging to the *Rhipicephalus* genus were morphologically identified.Out of these,291 were selected based on origin and confirmed by sequencing of 16SrRNA and COI genes.Each tick was examined for the presence of piroplasms, members of the Anaplasmataceae family, heamotropic mycoplasms, *Hepatozoon* sp., *Coxiella burnetti,* and *Francisella tularensis.* Results were visualised using QGIS software.

R. bursa ticks (69) were found in the coastal region, infesting red foxes, chamois,cattle, sheep, and goats. The presence of DNA from *B. canis*, *B. ovis*, *Babesia* sp. Badger type A, *T. ovis*, *A. ovis*, *E. canis*, and *Ehrlichia* sp. has been confirmed.

R. sanguineus s.s. ticks (72) were obtained from dogs, primarily in coastal areas.They carried *B. canis*, *H. canis*, whereas one tick from the environment contained *R. massiliae* DNA.

R. turanicus ticks (150) found on cattle, sheep, goats, donkeys, dogs, cats, and in environment originated from coastal Croatia except one tick found on a red fox in continental Croatia. The identified pathogens were *B. canis*, *Babesia* cf. *crassa*, *Babesia* cf. *tavsan*, *T. ovis*, *A. ovis*, *A. phagocytophilum*, *R. massiliae*, and *R. aeschlimannii*. Pathogens were recognised in ticks from sheep, goats, and cattle, whereas *H. felis* was found in ticks from cats. The presence of *Francisella tularensis* subsp. holartica DNA in *R. turanicus* from environment is its first molecular proof in Southern Croatia.

The results of the current study revealed a significant variety of pathogens in *Rhipicephalus* ticks in coastal region of Croatia and capabilities of *R. bursa* and *R. turanicus* to infest a wide range of hosts,in contrast to *R. sanguineus* s.s.

Keywords: ticks, Croatia, *Rhipicephalus*, pathogens

PS – poster session

Session: [SE5] Vector-borne pathogens and diseases

Update on the occurrence of *Dermacentor reticulatus* in the Wrocław Agglomeration, SW Poland

Dorota Kiewra[1], Mariusz Szymanowski Szymanowski†[2], Aleksandra Czułowska[1], Dagmara Dyczko[1], Piotr Jawień[1], Kinga Plewa-Tutaj[1]

1 Department of Microbial Ecology and Acaroentomology, University of Wrocław, Poland
2 Department of Geoinformatics and Cartography, Institute of Geography and Regional Development, University of Wrocław, Poland

Abstract

In Europe, *Dermacentor reticulatus* is recognized as one of the most important vectors of pathogens of great epidemiological significance. Therefore, an accurate knowledge of its spread and mapping of its distribution is crucial to estimate tick-borne risk. The aim of the study was to assess the current range of *D. reticulatus* in the Wroclaw Agglomeration. Ticks were collected by flagging the vegetation from March to April 2024 at 50 sites, including 30 sites monitored in the 5-year study i.e. from 2014 to 2019 by Kiewra et al. (2021) and 20 new sites located in an additional 5 km buffer zone created south-east from the previously established area. The new sites were designated using GIS analysis based on land cover maps and random selection. Compared to 2019 year, when among 30 sites 23 were recognized as positive (i.e. during one hour collection at least one specimen of *D. reticulatus* was found) in 2024 additionally, three new sites of *D. reticulatus* occurrence were found. Moreover, among 20 new sites, 14 were found to be positive. All negative sites were located in the southeastern part of the study area, however, most of them were located next to the positive sites. Overall, regular field monitoring of ticks in the Wroclaw Agglomeration carried out over the last decade indicates new sites harboured by *D. reticulatus*, and confirms the expanding range of this species. Our local-scale observation suggests the need for further long-term studies to follow the changes in the range of *D. reticulatus* to better estimate potential public and veterinary health risks.

The research was financed by the University of Wrocław (research funding).

Keywords: tick, *Dermacentor reticulatus*, expansion, SW Poland

PS – poster session

Session: [SE5] Vector-borne pathogens and diseases

The risk of human exposure to ticks and selected tick-borne diseases on nature-educational and tourist trails in Poprad Landscape Park (Małopolskie Voivodeship)

Sylwia Koczanowicz[1], Magdalena Nowak-Chmura[1], Marek Asman[2]

1 Department of Zoology, Institute of Biology and Earth Sciences, University of the National Education Commission, Kraków, Poland
2 Department of Medical and Molecular Biology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia, Katowice, Poland

Abstract

Poprad Landscape Park, with its natural features and numerous recreational areas, is often visited by residents and tourists. *Ixodes ricinus* is the most common tick species in Poland and Europe. The aim of study was to estimate the potential risk of human exposure to tick-borne infections of *Borrelia burgdorferi* sensu lato, *Babesia* spp. and *Bartonella* spp. in the selected area of the Poprad Landscape Park. Ticks were collected during the spring peak of their activity by flagging and then identified under a stereoscopic microscope accoring to their developmental stage and sex. A total of 45 *I. ricinus* ticks (15 females, 12 males, and 18 nymphs) were tested for selected tick-borne pathogens. The DNA was isolated from single individuals using the ammonia method. Its concentration was measured spectrophotometrically. The PCR and nested PCR methods were used to detect pathogens in ticks. *Borrelia burgdorferi* sensu lato was detected in ticks with the use of two pairs of primers specific to the flagelline gene (*flaB*). In turn, to detect *Babesia* spp. and *Bartonella* spp. primers specific to the 18S rRNA gene and *rpoB* gene were used, respectively. The amplification products were separated electrophoretically in 2% ethidium bromide stained agarose gels and visualized under ultraviolet light. None of the three tested pathogens were detected in the studied material. However, this does not mean that there is no risk of exposing the local population and tourists in the park to tick-borne diseases. The research in this area was conducted for the first time as preliminary studies on a small number of ticks; it will be continued and expanded. In order to protect against ticks, it is crucial to popularize research on them and, moreover, to spread preventive methods and knowledge about the threats posed by tick-borne diseases.

Keywords: ticks, *Ixodes ricinus*, tick-borne diseases, Poprad Landscape Park

PS – poster session

Session: [SE5] Vector-borne pathogens and diseases

Spotted Fever Group *Rickettsia* spp. in questing *Ixodes ricinus* ticks in north-eastern Poland

Katarzyna Kubiak[1], Hanna Szymańska[1], Małgorzata Dmitryjuk[2], Ewa Dzika[1]

1 Department of Medical Biology, School of Public Health, University of Warmia and Mazury in Olsztyn, Poland
2 Department of Biochemistry, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Poland

Abstract

Ixodes ricinus ticks are one of the reservoirs and vectors of the human pathogenic bacteria of the genus *Rickettsia*, which are classified as the spotted fever group (SFG). The aim of the study was to determine the prevalence of *Rickettsia* spp. in questing *I. ricinius* ticks in north-eastern Poland.

Rickettsia spp. were analysed in a total of 4090 ticks, including 381 females, 450 males and 3260 nymphs (652 pools of 5 specimens). The ticks were collected between April and June 2016-2017 in Warmia and Mazury region. The tick collection sites represented the western, central and eastern part of the region and two types of habitats: forest landscapes and ecotones.

The presence of *Rickettsia* spp. was confirmed by the PCR method using two primer sets (CS409/Rp1258) specific for the citrate synthase (*gltA*) gene. The *Rickettsia* species were identified by sequencing the PCR products. The nucleotide sequences obtained were compared with the data registered in the GenBank database. A chi-square test was used to compare the infection rate between the sex of the ticks, the subregions, habitats and years of the study. Rickettsial DNA was detected in 11.3% of females, 11.8% of males and in at least 6.5% (MIR, minimum infection rate) of nymphs. The proportion of *Rickettsia*-positive ticks was significantly higher in 2016 (8.4%) than in 2017 (6.6%). The highest infection rate (9.5%) was recorded in the central subregion of Warmia and Mazury. The type of habitat did not affect the *Rickettsia* prevalence, which was 7.3% in forest areas and 7.7% in ecotones.

Sequence analysis of the partial *gltA* gene showed the presence of *R. helvetica* (n=45) and *R. monacensis* (n=1). Both *Rickettsia* species are known as human pathogens.

To summarise, the prevalence of SFG rickettsiae in *I. ricinus* ticks in north-eastern Poland is not high. However, the presence of *R. helvetica* and *R. monacensis*, indicates that *I. ricinus* ticks play a significant role as vectors of these pathogens to humans.

Keywords: *Rickettsia helvetica*, *Rickettsia monacensis*, ticks, Poland

PS – poster session

Session: [SE5] Vector-borne pathogens and diseases

Occurrence of the meadow tick *Dermacentor reticulatus* in recreational areas of the city of Poznań and associated risks

Maria Lipiecka, Bożena Sikora

Department of Animal Morphology, Faculty of Biology, Adam Mickiewicz University in Poznań Uniwersytetu Poznańskiego 6, 61-614 Poznań, Poland

Abstract

Scientific reports from the last decade clearly indicate the emergence and intensive spread of the meadow tick (*Dermacentor reticulatus*) in western Poland, This phenomenon may pose a significant threat to the public health of the inhabitants of Wielkopolska (this part of Poland has so far been considered free of this tick species).

The subject of this project is this year's monitoring of the presence and range of *D. reticulatus* in recreational areas of the city of Poznań and the preparation of a map of areas where the tick is present. Such an initiative will allow an accurate assessment of the risk of contact with the parasite in green areas and will make it possible to estimate the potential epidemiological threat to the city's residents and their pets.

The designated study areas are an integral part of the city's green infrastructure, where there are wild animals that favour the expansion of the parasite (e.g. deer, wild boar, foxes, various rodent species). The monitoring covered areas on different sides of the city, which are partly elements of green wedges running continuously through Poznań (so-called urban ecological corridors): areas around lakes, the Morasko Campus and the adjacent urban forest, as well as urban parks. The commonly used flagging of the above-mentioned areas was adopted as the primary survey method. When *Dermacentor* ticks are found in designated plots, the area is further monitored at regular intervals. Collected individuals are identified to species and life stage, and will then be quantitatively analysed. This will make it possible to determine their occurrence in different parts of the city. In the next stage of the study, the collected tick specimens will be subjected to molecular analyses to detect pathogens in them. Conclusions from the number of collected *Dermacentor* ticks will be the basis for the development of preventive measures for the inhabitants of Poznan.

ID-UB UAM 119/39/UAM/005 student project.

Keywords: *Dermacentor reticulatus*, monitoring, pathogens, environmental threats, Poznań

PS – poster session

Session: [SE5] Vector-borne pathogens and diseases

The heartworm parasite (*Dirofilaria immitis*) became endemic in Slovakia

Martina Miterpáková[1], Zuzana Hurníková[1], Daniela Antolová[1], Daniela Valentová[2]

1 Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, 040 01 Košice,Slovak Republic

2 Veterinary and Food Institute, Botanická 15, 842 52 Bratislava, Slovak Republic

Abstract

Dirofilaria immitis and *Dirofilaria repens*, mosquito-transmitted parasitic filarioids, have been of great concern in Slovakia since 2005 when both species were confirmed as autochthonous among local dogs. The first epidemiological research performed in 2007 revealed the existence of high-endemic regions for *D. repens* (responsible for subcutaneous dirofilariosis) with an overall prevalence of 34.5 %. An explicit dominance of *D. repens* persisted during the next ten years when *D. immitis*, the causative agent of fatal heartworm disease (HWD), was recorded only sporadically. However, since 2017 an unquestionable change in the pattern of *Dirofilaria* species distribution in dogs living in southwestern Slovakia has been documented when the number of dogs with HWD has increased progressively. The most current analyses exposed that between 2017 and 2021 *D. immitis* was responsible for 45 % and between 2022 and 2023 even for over 75 % of all cases of canine dirofilariosis registered by veterinarians in surveyed southwestern region of the country.

Regarding health conditions, in 12.7 % of dogs with diagnosed *D. immitis* mono-infection, heart failure-related ascites were observed and in three of them also adult worms were found post-mortem in the right heart. The mixed infection caused by both dirofilarial species was clinically manifested in more than 33 % of infected dogs with clinical signs including most often chronic cough, tachypnea, and pneumonia, followed by heart murmurs, ascites, hydropericardium syndrome, dermatitis, hepatopathy, pancreatitis and effusion in the thorax.

Many dogs with diagnosed HWD came from shelters, what´s important from the epidemiology point of view, given that high numbers of dogs kept in Slovak shelters are exported abroad, most frequently to Austria, Germany, and Switzerland, which can contribute to the expansion of the infection agents into non-endemic regions.

The research was financially supported by the project VEGA 2/0014/21.

Keywords: *Dirofilaria immitis*, heartworm, dogs, epidemiology, Slovakia

PS – poster session

Session: [SE5] Vector-borne pathogens and diseases

Vector role of *Hyalomma* ticks: Comparison of prevalence of infection between ticks collected from two CCHFV-endemic countries (Mongolia and Iraq)

Wiktoria Romanek[1], Karolina Romańczuk[2], Dagmara Wężyk[1], Mustafa Al-Sarraf[1], Mohammed Al-Sarraf[1], Wiktoria Małaszewicz[1], Anna Bajer[1]

1 Zakład Eko-Epidemiologii Chorób Pasożytniczych, Instytut Biologii Rozwoju i Nauk Biomedycznych, Wydział Biologii Uniwersytetu Warszawskiego, Miecznikowa 1, 02-096 Warszawa, Polska

2 Zakład Cytologii, Instytut Biologii Rozwojowej i Nauk Biomedycznych, Wydział Biologii Uniwersytetu Warszawskiego, Miecznikowa 1, 02-096 Warszawa, Polska

Abstract

Ticks of genus *Hyalomma*, especially of the *H. marginatum* complex, are known as vectors of Crimean-Congo hemorrhagic fever virus (CCHFV). *Hyalomma* spp. are thermophilic species inhabiting mainly dry and warm habitats (deserts, semi-deserts, pastures) of southern Europe, Middle East, Africa and Asia. Occurrence of *Hyalomma* ticks is associated with a presence of CCHFV, which is one of the most genetically diverse representatives of the family *Nairoviridae*.

The main goal of this study is to compare prevalence of CCHFV infection in two CCHFV-endemic countries, Iraq and Mongolia, including both known endemic areas and new locations.

Ticks were collected during three expeditions: in Mongolia in summer 2023 and spring 2024, and in Iraq in spring 2024. We aimed to collect and examine *H. anatolicum* and *H. dromedarii* in Iraq are and *H. asiaticum* in Mongolia.

For the detection of CCHFV in ticks one-step real-time RT-PCR assay was designed. The novel technique is based on the *Taq*-man probes technology employing the endonuclease activity of *Taq* polymerase enzyme.

During the conference, results regarding a comparison of CCHFV prevalence in *Hyalomma* ticks from two CCHFV-endemic countries will be presented as well as a description of modern molecular techniques used to detect the virus in ticks.

The study was funded by National Science Centre, OPUS grant no. 2022/47/B/NZ6/01610 (AB).

Keywords: *Hyalomma* sp., Crimean-Congo hemorrhagic fever virus

PS – poster session

Session: [SE5] Vector-borne pathogens and diseases

Molecular detection of *Trypanosoma* spp. and *Bartonella* spp. in Polish primitive horses *Equus caballus* in Poland: preliminary study

Kateryna Slivinska[1,5], Joanna Werszko[2], Tomasz Szewczyk[3], Grzegorz Karbowiak[4]

1 Museum and Institute of Zoology of Polish Academy of Science, ul. Twarda 51/55 Warszawa, Poland
2 Department of General Biology and Parasitology, Medical University of Warsaw, Chałubińskiego 5, 02-004 Warsaw, Poland
3 Institute of Parasitology PAN, Warsaw, Poland (liquidated)
4 Museum and Institute of Zoology of Polish Academy of Science, ul. Twarda 51/55 Warszawa, Poland
5 I. I. Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine, vul. B. Khmelnytskogo 15, 01030 Kyiv, Ukraine

Abstract

The blood pathogens of horses encompass protozoan and bacterial species. The genus *Trypanosoma*, flagellated protozoans prevalent in Europe, causes equine trypanosomosis. *Bartonella*, a bacterium that affects humans and mammals, included horses. Our knowledge of the distributions of these vector-borne pathogens remains incomplete.

The objective of this study was to determine the prevalence of *Trypanosoma* spp. and *Bartonella* spp. infections in Polish primitive horses in Poland. A total of 65 horses from three geographically separated locations in Poland - Biebrzański National Park (BNP),17 horses, Roztoczański National Park (RNP), n=32, and Popielno Research Station (PRS), n=16 were examined. The age of the tested horses ranged from 1 to 26 years (7.3 ± 4.5). All horses were in good condition with no obvious signs of disease. Blood samples were collected from the jugular vein into sterile vacuum tubes containing EDTA and stored at −20°C. DNA was extracted using the NucleoSpin® Blood kit. To detect *Trypanosoma* spp., nested-PCR with primers TRY927F, TRY927R and SSU561F, SSU561R were used to amplify the 18S rRNA partial gene. To detect *Bartonella* spp the primers 1400F and 2300R were used to amplify an 850 bp fragment of the rpoB gene.

Infection with *Trypanosoma* spp. was detected in 64.7% of horses from BNP, 9.38% from RNP, and 6.25% from PRS. The overall prevalence observed in all horses was 23.08%. Males and the youngest horses (<1 year) were most often infected (6/14 and 5/5, respectively). The dependence between the sex of the horse and *Trypanosoma* spp. infection was confirmed by the chi-square test. *Trypanosoma* spp. sequences (GenBank accession number: OM722123-7) were registered. Sequence analysis shows similarity to isolates from Poland originating from cattle and bison.

All observed horses tested negative for *Bartonella* infection.

This is the first report of the molecular survey of *Trypanosoma* spp. and *Bartonella* spp. infections in Polish primitive horses in Poland.

The study was funded by the MSCA4Ukraine project (AvHID 1233593; https://cordis.europa.eu/project/id/101101923).

Keywords: *Trypanosoma* spp., *Bartonella* spp., horses, vector-borne diseases, Poland

PS – poster session

Session: [SE5] Vector-borne pathogens and diseases

Investigating the contributions of native and *Aedes* invasive mosquito species to the spread of filarial nematodes in a southwestern city in Hungary

Zsaklin Varga[1,2,3], Rebeka Csiba[1,2], Ágota Ábrahám[1,2], Dorina Pásztor[1,2], Gábor Kemenesi[1,2], Kornélia Kurucz[1,2,3]

1 National Laboratory of Virology, Szentágothai Research Centre, University of Pécs, Pécs Hungary
2 Institute of Biology, Faculty of Sciences, University of Pécs, Pécs, Hungary
3 National Laboratory of Health Security, University of Pécs, Pécs, Hunga

Abstract

Dirofilaria immitis and *D. repens* are filarial nematodes transmitted by mosquitoes, and the rise in human infections may be connected to the spread of invasive mosquito species including *Aedes koreicus* and *Ae. albopictus* in Europe. In Hungary, the surveillance of these mosquitoes has been ongoing since their emergence. This study aimed to monitor filaria infection in mosquitoes in an area known for human infections and to explore the connection between mosquito infestation, seasonality and species.

Mosquitoes were collected in Pécs, Hungary from May to October in 2022 and 2023. Following identification, mosquitoes were tested for filaria using real-time PCR. Positive pools were further analyzed using conventional PCR, gel electrophoresis and Sanger sequencing to determine the filaria species.

A total of 4444 specimens in 1015 pools of mosquitoes, including both *Aedes* invasive and native species, were examined. 30 pools from different species primarily *Aedes vexans* and *Culex pipiens*, tested positive for filaria spp. Most positive samples were found in June–July in both 2022 and 2023. Our findings confirmed four cases of *D. repens* (*Ae. vexans* and *Culiseta annulata*), two cases of *D. immitis* (*Cx. pipiens* and *Ae. vexans*), and the remaining 24 positive samples were infected with *Setaria tundra* and *S. labiatopapillosa*.

Our study found a seasonal pattern in filaria infection, but no clear connection between *Aedes* species and *Dirofilaria* infestation in mosquitoes. The dry season in 2022 may have influenced mosquito activity and filaria presence in 2023. The proximity of *S. tundra* to human cases warrants further investigation. The focus of our research was on the prevalence of emerging zoonotic pathogens *D. repens* and *D. immitis*. More research is needed to understand the relationship between mosquito species and filaria prevalence and we are committed to ongoing surveillance and investigating human infections.

Keywords: *Aedes koreicus*, *Aedes albopictus*, *Dirofilaria repens*/*immitis*, prevalence, qPCR, Sanger sequencing

PS – poster session

Session: [SE5] Vector-borne pathogens and diseases

Rickettsia species in *Dermacentor reticulatus* ticks feeding on human skin and clinical manifestations of tick-borne infections after tick bite

Renata Welc-Falęciak[1], Julia Koczwarska[1], Justyna Polaczyk[1], Agnieszka Pawełczyk[2]

1 University of Warsaw
2 Medical University of Warsaw

Abstract

Dermacentor reticulatus ticks are sporadically removed from human skin and therefore the medical consequences of their feeding are neglected compared to *Ixodes ricinus*. We investigated the prevalence of pathogens in *D. reticulatus* removed from human

skin and possible clinical manifestations suggestive of tick-borne diseases after a tick bite. A total of 2153 ticks were studied and of these only 34 were *D. reticulatus*. The mean prevalence of *Rickettsia* in *D. reticulatus* was 50.0 % and *R. raoultii* was identified in 82.4% of infected *D. reticulatus* ticks. We confirmed the first case of *R. aeschlimannii* infection in *D. reticulatus* ticks. Among participants bitten by *D. reticulatus*, 13.3% reported reddening around the tick bite site and flu-like symptoms, including lymphadenopathy and 3.3% reported eschar on the tick site bite. All of the participants with flu-like symptoms after tick removal were bitten by ticks infected with *R. raoultii*. The results of this study indicate that even though *D. reticulatus* ticks bite humans sporadically, pathogenic *Rickettsia* have a remarkably high prevalence in this tick species. We can expect that the incidence of tick-borne lymphadenopathy might increase with the reported expansion of the *D. reticulatus* into new areas and its growing abundance in Central Europe.

The study was funded by the National Science Centre (NCN) OPUS grant no. 2020/37/B/NZ6/01587.

Keywords: *Dermacentor reticulatus*, *Rickettsia*, tick-borne diseases, zoonosis

PS – poster session

Session: [SE5] Vector-borne pathogens and diseases

Seroprevalence of *Toxoplasma gondii* and *Borrelia burgdorferi* infections in patients with Multiple Sclerosis in Poland

Agnieszka Pawełczyk[1], Katarzyna Donskow- Łysoniewska[2], Ludmiła Szewczak[3], Magdalena Kierasińska[4], Maja Machcińska[2], Rafał Rola[5], Renata Welc-Falęciak[3]

1 Medical University of Warsaw
2 Department of Experimental Immunotherapy, Faculty of Medicine, Lazarski University
3 Department of Parasitology, Faculty of Biology, University of Warsaw
4 Laboratory of Parasitology, General Karol Kaczkowski Military Institute of Hygiene and Epidemiology
5 Department of Neurology, Military Institute of Aviation Medicine

Abstract

Multiple sclerosis (MS) is a chronic, demyelinating disease of the central nervous system that affects mainly young people. It is believed that the autoimmune process observed in the pathogenesis of MS is influenced by a complex interaction between genetic and environmental factors, including infectious agents. The results of this study suggest the protective role of *Toxoplasma gondii* infections in MS. Interestingly, high *Toxoplasma* IgM seropositivity in MS patients receiving immunomodulatory drugs (IMDs) was identified. On the other hand, *Borrelia* infections seem to be positively associated with MS. Although the interpretation of our results is limited by the retrospective nature of the studies, the results strongly indicate that further experimental and clinical studies are needed to explain the role of infectious agents in the development and pathophysiological mechanisms of MS.

This work was supported by grants of the University of Warsaw in a programme of the Ministry of Science and Higher Education "Excellence Initiative – Research University (2020-2026)" in Actions IV.3.1 and partially from the TEAM TECH/2017-4/22 project carried out within the TEAM TECH programme of the Foundation for Polish Science, co-financed by the European Union under the European Regional Development Fund.

Keywords: multiple sclerosis, toxoplasmosis, Lyme disease, Lyme borreliosis, *Toxoplasma*, *Borrelia*

PS – poster session

Session: [SE5] Vector-borne pathogens and diseases

Associations between *Babesia*, *Borrelia* and *Theileria* and tick vectors in different ecosystems of the world

Dagmara Wężyk[1], Wiktoria Romanek[1], Mustafa Al-Sarraf[1], Anna Bajer[1], Lidia Chitimia-Dobler[2], Mohammed Al-Sarraf[1]

1 Department of Eco-Epidemiology of Parasitic Diseases, Institute of Developmental Biology and Biomedical Sciences, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096 Warsaw, Poland
2 Bundeswehr Institute of Microbiology, Neuherbergstrasse 11, 80937 Munich, Germany

Abstract

Ticks that feed on a wide variety of hosts are vectors of the widest range of pathogens globally. There are at least two major gaps in tick research, including a focus on only three tick species commonly found in Europe and the US (*Ixodes ricinus, I. scapularis, Dermacentor reticultus*), and the use of ticks collected from hosts for such studies.The main aim of our project is to examine little-studied tick species from the genera *Hyalomma, Haemaphysalis, Amblyomma* and *Rhipicephalus* spp. for *Borrelia* spp. and *Babesia/Theileria* spp. patoghens. Our research focus on ticks from vegetation from not well studied regions around the World.

The conference will present the results of research conducted in various geographic regions, particularly Mongolia and Romania. To date, we have obtained about 1,000 ticks from the aforementioned regions. Subsequent analysis has revealed the presence of *Babesia caballi* in *Dermacentor nuttalli* ticks from Mongolia, as well as the identification of *B. vogeli* in *Rhipicephalus sanguineus* ticks from Romania.

The study was funded by the National Science Centre, OPUS grant no. 2022/45/B/NZ7/01017 (AB).

Keywords: tick, *Babesia*, *Borrelia*, *Theileria*

PS – poster session

[SE6]

Host-parasite interactions (with microbiome relationship)

Session: [SE6] Host-parasite interactions (with microbiome relationship)

Fishing for dominant antigens of *Anisakis* spp. in European hake through colony immunoblotting of complementary DNA expression libraries

Camilo Ayra- Pardo[1], Fernando Atroch[2], Luis Filipe Rangel[3], Paula Ramos[4], Maria João Santos[5]

1 CIIMAR / Portugal
2 Porto University/ CIIMAR /Portugal
3 CIIMAR/ Portugal
4 IPMA/ Portugal
5 Porto University/ CIIMAR/ Portugal

Abstract

Anisakis spp., parasitic nematodes commonly found in marine fish, pose a significant health risk to consumers, with European hake being a prominent host species. Here, we aimed to identify the dominant antigens of *Anisakis simplex* and *A. pegreffii* in European hake by colony immunoblotting of complementary DNA (cDNA) expression libraries. Total RNA was extracted and pooled from 10 halves of L3 larvae of each species, while the other halves were used for species identification based on the mitochondrial *COI* gene. RNA pools were used to generate cDNA expression libraries using a commercial kit. These libraries were cloned into a customised vector containing two asymmetric *Sfi*I sites flanked by the T7 promoter and the rrnB T2 terminator and introduced into competent BL21 (DE3) cells by heat shock. The recombinant bacteria were induced with IPTG and screened with immunoglobulins from highly infected hake fish sera obtained by precipitation at 50% ammonium sulphate saturation. Analysis of 8 immunoreactive clones - 5 from *A. simplex* and 3 from *A. pegreffii* libraries- by DNA sequencing and subsequent BLAST analysis revealed that all sequences, except one homologous to a truncated *A. pegreffii* HSP90-alpha, have no known domains or motifs and could not be assigned to any protein family. Thus, their functions are also unclear. Interestingly, one of the clone sequences contained up to 27 unique tandem repeats of 7 amino acids. Antigenic proteins with short repetitive sequences that trigger strong humoral responses have already been found in *Anisakis* allergens and other parasites and are thought to play a role in host-parasite interactions.

This work was funded by Innovation Pact, Project No. C644915664-00000026, known as the "Blue Bioeconomy Pact", employment contract 2022_096_IJ_BioeconomiaAzul (CAP), and research grant 2022_105_BI_BioeconomiaAzul (FA); and by FCT, employment contract CEECIND/03501/2017 (LFR), projects UIDB/04423/2020 and UIDP/04423/2020.

Keywords: fish parasites, host-parasite interaction, immunochemistry

OS – oral session

Session: [SE6] Host-parasite interactions (with microbiome relationship)

Analysis of the influence of extrinsic and intrinsic factors on host-microbiome-endoparasite relationships in wild rodents

Martyna Krupińska[1], Joanna Nowicka[1], Aleksander Goll[1], Łukasz Rąbalski[2], Anna Bajer[3], Maciej Grzybek[1]

1 Medical University of Gdańsk
2 Intercollegiate Faculty of Biotechnology UG&MUG
3 Warsaw University

Abstract

While there's growing interest in microbiome studies, most research is confined to humans and lab animals in controlled settings, leaving wildlife microbiomes poorly understood. Laboratory studies are important to learn the mechanistic basis of host-microbiota interactions, but they cannot fully capture the complexities of natural conditions. Intestinal bacteria and helminths evolved together while inhabiting the same environmental niche. Intestinal helminths are known to alter intestinal physiology, mucous secretion and the production of antimicrobial peptides – all of which may impact the host microbiome. The scientific goal of this study is to analyze the composition of the gut microbiota of wild rodents and to investigate the influence of intrinsic (host age, sex, reproductive status) and extrinsic factors (year of the study, study site) on the composition of the microbiome and possible interactions between gut microbiota and endoparasites. We sampled 457 wild rodents belonging to 7 species (*Myodes glareolus, Apodemus flavicollis, Apodemus sylvaticus, Apodemus agrarius, Microtus agrestis, Microtus arvalis, Mus musculus*) in the northeastern part of Poland between 2018 and 2022. During the rodent dissections, gastrointestinal tracts were searched for the presence of endoparasites and fecal samples were collected for DNA isolation. Amplification of full 16S rRNA amplicons and Oxford nanopore sequencing was conducted to assess the taxonomic composition of rodent gut bacteria and evaluate the influence of the presence of gut parasites, as well as internal factors like gender, species, age, reproductive status, and external factors such as the year and site of study.

Keywords: microbiome, intestines, wildlife, wild rodents, helminths

OS – oral session

Session: [SE6] Host-parasite interactions (with microbiome relationship)

Characterizing the bacterial microbiome of the invasive vector *Aedes albopictus* in Hungary: a pilot study using Oxford Nanopore sequencing

Kornélia Kurucz[1], Ágota Ábrahám[1], Zsaklin Varga[1], Rebeka Csiba[1], Gábor Kemenesi[1], Alejandro Cabezas-Cruz[2]

1 1) National Laboratory of Virology, Szentágothai Research Centre, University of Pécs, Pécs, Hungary, 2) Institute of Biology, Faculty of Sciences, University of Pécs, Pécs, Hungary
2 ANSES, INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR BIPAR, Laboratoirk e de Santé Animale, Maisons-Alfort, France

Abstract

The microbiome of mosquitoes (Culicidae) plays a critical role in their vector competence, namely influencing their capacity to transmit pathogens such as Dengue and West Nile virus or *Dirofilaria* nematodes. Understanding the bacterial communities within vector species is essential for developing strategies to control vector-borne diseases. In the current pilot study, we aimed to investigate the invasive *Aedes albopictus* mosquitoes' bacterial microbiome to reveal dominant bacterial genera with their potential impact on vector competence in the Hungarian populations. In the analysis, we used 12-12 mosquito specimens originating from two different populations in the country's southwestern part (Somogy and Baranya counties). Oxford Nanopore sequencing was performed using a standard 16S rRNA-based metagenome approach to profile the bacteria composition. Then, sequence data were classified with Kraken2 software using the Silva database. Our findings unveiled a rich bacterial landscape, with distinct dominant genera in mosquitoes from each location: notably, members of *Wolbachia* (71%) and *Acinetobacter* (18%) genera were prevalent in one area, while *Zymobacter* (42%) was also present in the other population. Additionally, we detected *Escherichia-Shigella*, *Stenotrophomonas*, *Delftia*, and *Halomonas* spp. (each less than 5% ratio).

This research marks the first comprehensive profiling of the bacterial community structure in Hungarian *Ae. albopictus* populations. Although *Wolbachia* is this species' most studied bacterial symbiont, the occurrence of further bacteria in hematophagous groups is poorly documented. Our study highlights the complexity and local variation in mosquitoes' microbiomes, which may influence their vectorial capacity and interactions with pathogens. As a follow-up, we plan to analyse the microbial interaction network to reveal co-exclusionary patterns.

Keywords: invasive species, vector-competence, meta genome analysis, tiger mosquito

OS – oral session

Session: [SE6] Host-parasite interactions (with microbiome relationship)

Effects of Giardiavirus infection in the course of *Giardia duodenalis*/human enterocyte *in vitro* interaction

Marco Lalle[1], Serena Cecchetti[2], Giovanna Nunziata[3], Francesca Pedini[4], Rosaria Coscione[2], Simona Cherchi[5], Gianluca Marucci[5]

1 Unit of Foodborne and Neglected Parasitic Disease, Department of Infectious Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy;

2 Core Facilities, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy

3 Unit of Foodborne and Neglected Parasitic Disease, Department of Infectious Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy;

4 Department of Oncology and Molecular Medicine, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy

5 Unit of Foodborne and Neglected Parasitic Disease, Department of Infectious Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy

Abstract

Giardia duodenalis is a protozoan parasite able to infect the upper-small intestine of mammals causing giardiasis, a diarrheal disease. Symptoms of human giardiasis range from asymptomatic to acute or chronic. The molecular mechanisms underlying the variability of the clinical manifestations of giardiasis are yet to be fully understood, partially due to lack of appropriate *in vitro* models that can recapitulate gut environment. Among factors potentially affecting giardiasis outcomes the parasite infection with *Giardia lamblia virus* (GLV), a dsRNA non-enveloped virus of the family *Totiviridae,* must be considered. The aim of this study was to evaluate the impact of GLV infection on the interaction between *G. duodenalis* and enterocytes (IEC). We successfully adapted a previously developed *in vitro* co-culture model of intestinal epithelial barrier (CaCo2/TC7 and HT29) that was able to sustain up to 3 days interaction with *G. duodenalis WBC6 isolate.* Trans-epithelial electrical resistance (TEER), paracellular apparent permeability (Paap), trophozoite replication and cellular viability were evaluated every 24 hours within 3 days Localization of tight junction proteins was investigated by immunofluorescence microscopy, and expression of specific genes was evaluated by qPCR. We demonstrated that the model replicates certain pathogenic mechanisms of *giardiasis,* such as reduction of TEER, increase of Paap and disruption of tight junction. In contrast, GLV infection mitigates *G. duodenalis* induced intestinal barrier damages in a viral strain-dependent mode. Moreover, a differential response at gene level in enterocytes in relation to the GLV presence was observed. Our data provide the first experimental evidences pointing on GLV infection as a factor potentially attenuating giardiasis outcome by reducing *G. duodenalis*-induced alteration of the intestinal barrier.

This work was supported by Intramural grant "Ricerca indipendente ISS 2021-2023" (number ISS20-4389733b36a1)

Keywords: *Giardia duodenalis*, Giardiavirus, human enterocytes, intestinal barrier

OS – oral session

Session: [SE6] Host-parasite interactions (with microbiome relationship)

Using CRISPR to dissect *Toxoplasma*'s arsenal for host manipulation

Jeroen Saeij

Pathology, Microbiology and Immunology department, School of Veterinary Medicine, University of California Davis, Davis, CA, 95616, USA

Abstract

Toxoplasma gondii, an obligate intracellular parasite, manipulates host cellular mechanisms to ensure its survival and propagation. These manipulations are primarily mediated by secreted effector proteins from the parasite's rhoptries and dense granules. However, the exact mechanisms and genetic basis for these interactions remain incompletely understood. To address this, we employed CRISPR/Cas9 screens to systematically investigate the role of *Toxoplasma* effector proteins in host manipulation.

We performed genome-wide loss-of-function screens to pinpoint *Toxoplasma* genes critical for growth in IFNγ-activated cells. From these screens, we generated gene knockouts for the top hits and confirmed their roles in sustaining parasite growth under these conditions. Notably, several of the identified genes encode proteins that localize to dense granules and their functions will be discussed. In addition to identifying genes involved in immune evasion (GRA45, GRA57, GRA66, GRA70, GRA71, GRA72), we investigated the role of specific effectors in parasite dissemination. By using focused CRISPR libraries, we discovered that deletion of the gene TgWIP impairs the parasite's ability to reach distant organs, including the brain. TgWIP, secreted into the host cell upon invasion, interacts with the host WAVE regulatory complex and SHP2 phosphatase, influencing actin dynamics and enhancing the motility of parasitized dendritic cells. These studies underscore the utility of CRISPR/Cas9 in dissecting the complex interactions between Toxoplasma gondii and its host, providing insights into the molecular mechanisms underpinning parasite virulence and host manipulation. We are currently focused on the detailed characterization of these interactions, which could allow the development of potential therapeutic interventions targeting these effector proteins.

Keywords: *Toxoplasma*, CRISPR screens, effector proteins, dissemination, host-pathogen interactions

OS – oral session

Session: [SE6] Host-parasite interactions (with microbiome relationship)

Impact of reactive sulfur species on *Entamoeba histolytica*: modulating viability, motility, and biofilm degradation capacity

Jun Ye[1], Talal Salti[2], Eva Zanditenas[1], Meirav Trebicz-Geffen[1], Moran Benhar[2], Serge Ankri[1]

1 Department of Molecular Microbiology, Ruth and Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa 3525433, Israel

2 Department of Biochemistry, Ruth and Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa 3525433, Israel

Abstract

Reactive sulfur species (RSS) like hydrogen sulfide (H2S) and cysteine persulfide (Cys-SSH) emerged as key signaling molecules with diverse physiological roles in the body, depending on their concentration and the cellular environment. While it is known that H2S and Cys-SSH are produced by both colonocytes and by the gut microbiota through sulfur metabolism, it remains unknown how these RSS affect amebiasis caused by *Entamoeba histolytica*, a parasitic protozoan that can be present in the human gastrointestinal tract. This study investigates H2S and Cys-SSH's impact on *E. histolytica* physiology and explores potential therapeutic implications. Exposing trophozoites to the H2S donor, sodium sulfide (Na2S), or to Cys-SSH led to rapid cytotoxicity. A proteomic analysis of Cys-SSH-challenged trophozoites resulted in the identification of >500 S-sulfurated proteins, which are involved in diverse cellular processes. Functional assessments revealed inhibited protein synthesis, altered cytoskeletal dynamics, and reduced motility in trophozoites treated with Cys-SSH. Notably, cysteine proteases (CPs) were significantly inhibited by S-sulfuration, affecting their bacterial biofilm degradation capacity. Immunofluorescence microscopy confirmed alterations in actin dynamics, corroborating the proteomic findings. Thus, our study reveals how RSS perturbs critical cellular functions in *E. histolytica*, potentially influencing its pathogenicity and interactions within the gut microbiota. Understanding these molecular mechanisms offers novel insights into amebiasis pathogenesis and unveils potential therapeutic avenues targeting RSS-mediated modifications in parasitic infections.

Keywords: reactive sulfur species, hydrogen sulfide, cysteine persulfide, S-sulfuration, *Entamoeba histolytica*

OS – oral session

Session: [SE6] Host-parasite interactions (with microbiome relationship)

A novel faster method to isolate viral dsRNA from several organisms including parasites

Fernando Cardoso[1], Inês Pereira[1], Sofia Cortes[1], Isabel L Maurício[1], Olga Matos[1]

1 Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Rua da Junqueira 100, 1349-008 Lisboa, Portugal

Abstract

In recent years, several newly discovered viruses have been described that infect parasitic Cestoda, Trematoda, and Protozoa (*Leishmania spp., Trichomonas vaginalis, Giardia lamblia, Eimeria spp. and Babesia spp.*). The main genome of these viruses is ssRNA with a dsRNA replicative form or dsRNA. Some of these viruses, such as in *Leishmania spp.*, have been found to be implicated in parasite virulence. The purpose of this work was to develop and improve a new method to isolate viral dsRNA from these parasitic organisms. This new method involves a combination of phenol extraction at low pH, with a buffer containing ammonium sulfate. The dsRNA isolated using this novel method exhibits comparable quality to that obtained through the common cellulose purification method, and it is readily amenable to RT-PCR. Moreover, a single batch of yeast cell dsRNA isolation requires only 2-3 hours of hands-on time, thus simplifying and expediting the process significantly. This method was validated with *S. cerevisiae* and applied to *Leishmania* strains and helminth species for detection of viral dsRNA.

Keywords: Viral dsRNA, Phenol extraction, ammonium sulphate, sequencing, parasites

PS – poster session

Session: [SE6] Host-parasite interactions (with microbiome relationship)

Enterocin M and Durancin ED26E/7 from beneficial Enterococci modulate intestinal lymphocytes in mice infected with *Trichinella spiralis*

Emília Dvorožňáková[1], Miroslava Petrová[1], Viera Revajová[2], Andrea Lauková[3]

1 Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovak Republic
2 University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic
3 Institute of Animal Physiology, Centre of Biosciences, Slovak Academy of Sciences, Košice, Slovak Republic

Abstract

Probiotic/beneficial bacteria can successfully reduce the pathogenicity of many parasites. Their mode of action consists in competitive elimination of pathogens, enhancement of the gut epithelial barrier, secretion of active molecules, and immunomodulation. The whole probiotic bacteria can be replaced by their metabolites to achieve the beneficial activity. The immunomodulatory effect of enterocins and their producing strains in experimental trichinellosis were tested as a new therapeutic strategy.

Enterocin M and Durancin ED26E/7 and their producers *Enterococcus faecium* CCM8558 and *Enterococcus durans* ED26E/7 were daily administered to mice which were infected with *Trichinella spiralis* on 7th day of treatment. The changes in lymphocyte subpopulations in the epithelium (IEL) and *lamina propria* (LPL) of the small intestine were determined by flow cytometry.

T. spiralis infection significantly suppressed IEL and LPL CD4+, CD8+ and CD19+ during the intestinal phase of trichinellosis and their occurrence was normalized only during the muscle phase. The enterocins/enterococci significantly increased IEL CD4+ numbers on 18 dpi. The subpopulation of LPL CD4+ was significantly higher after treatment with *E. faecium* CCM8558 and Enterocin M (from 5 to 25 dpi). The subpopulation of LPL CD8+ in all groups with enterococci/enterocins was restored as early as 11. dpi. B cells (CD19+) inhibition after *T. spiralis* infection was not affected by enterocins/enterococci. T helper cells CD4+ initiate the host mechanisms (hypercontractility, mucin hypersecretion, inflammatory reactions) to eliminate worms from the intestine. The number of CD8+ cells after therapy was normalized from 11 dpi, at the beginning of massive newborn larvae migration. Cytotoxic CD8+ T cells may have been effective in destroying migrating *T. spiralis* larvae, resulting in a reducing effect on the number of muscle larvae in the treated groups.

The research was funded by the projects VEGA 2/0077/23 and APVV-17-0028.

Keywords: Enterocin M, Durancin ED26E/7, Enterococcus, *Trichinella spiralis*, lymphocytes

PS – poster session

Session: [SE6] Host-parasite interactions (with microbiome relationship)

T and B cell activity in the spleen of mice with experimental trichinellosis and enterocins/enterococci therapy

Emília Dvorožňáková[1], Miroslava Petrová[1], Viera Revajová[2], Andrea Lauková[3]

1 Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovak Republic
2 University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic
3 Institute of Animal Physiology, Centre of Biosciences, Slovak Academy of Sciences, Košice, Slovak Republic

Abstract

A protective role of probiotics in trichinellosis is known. *Enterococcus* strains produce various proteins=enterocins with immunomodulatory actions. The effect of enterocins and their producing strains on proliferation (MTT test) and differentiation (flow cytometry) of splenic lymphocytes was evaluated in mice with experimental trichinellosis.

Enterocins (Enterocin M, Durancin ED26E/7; dose of 50 µl of 2 mg/ml) and their enterococci (*E. faecium* CCM8558, *E. durans* ED26E/7; dose of 100 µl of 109 CFU/ml) were daily administered to mice which were challenged with *Trichinella spiralis* (400 larvae/mouse). The stimulatory effect of enterocins/enterococci on the proliferation of T and B lymphocytes was recorded in the intestinal phase (5-11 dpi.) of trichinellosis. *E. faecium* CCM8558 activated the T cell-proliferation even during the muscle phase. *T. spiralis* infection reduced CD4+T lymphocytes (5-25 dpi.) Enterocins/enterococci significantly stimulated the cells in the early phase of infection (5 dpi) and at the beginning of the muscle phase (18-25 dpi). The CD8+T suppression after *T. spiralis* infection was found during migration (11-25 dpi) of new born larvae (NBL). Enterocins/enterococci attenuated the inhibition and significantly increased CD8+T cell counts. CD8+T cells are effective in killing and lysing both NBL and muscle larvae. T helpers induce the B cell-proliferation to produce the antibodies to kill NBL. After *T. spiralis* infection, the presence of CD19+B cells increased until 11 dpi. in all groups. The B cells were inhibited by Enterocin M at the beginning of the NBL migration, *E. durans* ED26E/7 and Durancin ED26E/7 at the beginning of the muscle phase. The time shift of B cell suppression may be related to the antigenic stimulus, when Enterocin M more effectively eliminated adults from the intestine than *E. durans* ED26E/7 or Durancin ED26E/7.

The research was funded by the projects VEGA 2/0077/23 and APVV-17-0028.

Keywords: *Enterococcus*, Enterocin M, Durancín ED26E/7, *Trichinella spiralis*, T cells

PS – poster session

Session: [SE6] Host-parasite interactions (with microbiome relationship)

Plasmodium falciparum EBA-181 merozoite ligand - in search of its receptor on human erythrocytes

Marlena Jodłowska[1], Patrycja Burzyńska[1], Ewa Jaśkiewicz[1]

1 IITD PAN Wrocław

Abstract

Erythrocyte binding-like (EBL) ligands play a crucial role in the attachment of *Plasmodium falciparum* merozoites to human erythrocytes by binding to specific receptors. Four functional P. falciparum EBL proteins have been identified: EBL-1 which binds glycophorin B (GPB), EBA-175 and EBA-140 that target glycophorin A (GPA) and C (GPC), respectively, as well as EBA-181 which was the subject of our studies due to the fact that its receptor remained unidentified. Previously, it was shown that EBA-181 recognizes an unknown 33 kDa erythrocyte membrane protein, sensitive to chymotrypsin treatment, but it is not glycophorin B. In our studies, we used the far-western blotting method with erythrocyte membranes and the SPR technique to evaluate the Pf EBA-181 ligand specificity, usin a recombinant protein obtained in a bacterial expression system.

We have shown that the recombinant binding region of the EBA-181 ligand recognizes sialic acids: Neu5Ac and Neu5Gc, similarily to other EBA proteins. Moreover, we have identified Pf Rh2 merozoite protein as its co-ligand. We have not observed any binding of EBA-181 to erythrocyte membrane proteins, including: GPA, GPB, GPC and Band3.

Thus, the enigmatic sialylated erythrocyte receptor of EBA-181 ligand still remains unknown and further studies will be performed to identify it.

Keywords: EBL, erythrocyte, EBA-181 (JESEBL), malaria, *Plasmodium falciparum*

PS – poster session

Session: [SE6] Host-parasite interactions (with microbiome relationship)

Accumulation of metals by tapeworm parasite *Eubothrium* sp. in salmon *Salmo salar* from the southern Baltic Sea

Katarzyna Nadolna-Ałtyn, Lucyna Polak-Juszczak

National Marine Fisheries Research Institute

Abstract

Salmon *Salmo salar* is one of the most important elements in the Baltic Sea from ecological point of view and valuable fish species intended for human consumption. It has been found highly infected with intestinal tapeworm parasite *Eubothrium* sp. Metals occur naturally in aquatic ecosystems. Some of them are vital to physiological processes, but at high concentration represent both an ecological and human health concern. Fish may accumulate metals directly from the water they inhabit or from food sources. Fish parasites can also react to toxic compounds present in the aquatic environment. The aim of this study was to evaluate concentrations of metals (toxic metals As, Cd, Hg, Pb; macroelements Ca, K, Na, Mg, P; microelements Cr, Cu, Fe, Mn, Sr, Zn) in the tissues of salmon (liver and muscle) and its parasite *Eubothrium* sp. Samples from 40 individuals sampled in Polish marine waters were collected during standard ichthyological analysis in spring 2023. Concentrations of Ca, Fe, K,Mg, Mn, Na, Cr, Sr and Zn were determined by inductively coupled plasmaoptical emission spectrometry. Concentrations of Cu, Cd and Pb were determined with an atomic absorption spectrophotometer equipped with a graphite furnace. Mercury concentrations were determined using the cold vapor atomic absorption technique in a mercury analyzer. The relationships between metal concentrations in parasite and cod tissues were expressed as a bioconcentration factors (BCF). In the parasite - host (muscles) system: salmon parasites mainly accumulate microelements (Zn, Mn, Sr, Cu, Fe, Cr), which negatively affects the condition of fish. A high parasite - host (muscles) BCF ratio for Cd content should be considered as a positive factor protecting the fish against the toxic effects of this metal. In the parasite - host (liver) system: parasites absorb microelements (Sr, Zn, Mn) and macroelements and accumulate highly toxic Hg.

Keywords: salmon, *Eubothrium*, metal, toxic, Baltic

PS – poster session

Session: [SE6] Host-parasite interactions (with microbiome relationship)

Anisakidae nematodes as transporters of bacteria to the muscle tissue of cod *Gadus morhua* - the Baltic Sea case study

Agnieszka Pękala-Safińska[1], Katarzyna Nadolna-Ałtyn[2], Mirosław Różycki[1], Ewelina Iwan[3], Magdalena Podolska[2]

1 Poznań University of Life Sciences
2 National Marine Fisheries Research Institute
3 National Veterinary Research Institute - State Research Institute

Abstract

Increased abundance of Anisakidae nematodes has been observed in the cod *Gadus morhua* from Baltic Sea over the last decade. Parasites may migrate from the intestine into the flesh of the fish, even deeply into the fillets. The digestive tract of fish has a rich and diverse microbiota, including members of the specific spoilage bacteria of seafood. Nematodes migrating from the intestines to the muscle tissue of fish may transport high load of bacteria in their intestinal tract and on their surfaces. The aim of our studies was to investigate the role of muscle-invading larvae of the Anisakidae nematodes with respect to bacterial contamination of the flesh of cod from the southern Baltic. Fish were caught in spring

2019 in the Polish marine waters. On board standard analysis of fish were conducted and the fillets were investigated under white light transiluminator to reveal the presence of the nematodes. Parasites were gently removed from the fillet and washed in 70% ethanol to disinfect their surface. After the dissection of the parasites, a microbiological analysis was performed. Swabs from the intestines of parasite were collected and inoculated onto blood agar (BA) and tryptone-soya agar medium (TSA), and incubated at 25°C for 48h. The pure bacterial colonies were used to microbial identification based on their biochemical properties, sequencing were done on NextSeq (V2.5_2x150bp) and MinION. Assemblies were generated by Unicycler_v0.5.0. Genome characterization was done by CGE and Proksee platforms. The results of conducted studies revealed the presence of the following bacteria: *Aeromonas hydrophila* complex, *Acinetobacter lwoffii*, *Comamonas testosteroni*, *Pseudomonas fluoresens*, *Shewanella putrefaciens* group, *Shewanella galaciali piscicola*. It is worth to notice *Pseudomonas* and *Shewanella* bacteria are the types of microbe that play a crucial role in the spoilage process of seafood products. They may therefore pose a serious threat to the health of consumers.

Keywords: cod, bacteria, *Pseudomonas*, *Shewanella*, Baltic

PS – poster session

Session: [SE6] Host-parasite interactions (with microbiome relationship)

Dirofilaria immitis infection in *Aedes vexans* (Culicidae) is associated with changes in gut microbiome composition

Olena Nahimova[1], Natalia Volkova[2], Denys Hryhoriev[2], Miroslawa Dabert[3], Artur Trzebny[3]

1 Molecular Biology Techniques Laboratory, Faculty of Biology, Adam Mickiewicz University, Poznan, Poland; 2) Genetics and Cytology Department, School of Biology, V.N. Karazin Kharkiv National University, Kharkiv, Ukraine
2 Genetics and Cytology Department, School of Biology, V.N. Karazin Kharkiv National University, Kharkiv, Ukraine
3 Molecular Biology Techniques Laboratory, Faculty of Biology, Adam Mickiewicz University, Poznan, Poland

Abstract

Mosquitoes can act as vectors for many disease-causing viruses and parasites. Among them, more than 70 species are vectors of dogs, cats, and humans pathogenic *Dirofilaria immitis* and *D. repens*, which cause heart and lung failure or subcutaneous and connective tissue damage, respectively. Mosquito gut microbiota have important direct and indirect effects on parasite establishment. *Wolbachia* as a member of the microbiome can inhibit *Dirofilaria* transmission by upregulating the mosquito's innate immune system and inhibiting the development of filarial nematodes.

This study aimed to determine the gut microbiome response to *D. immitis* infection in *Aedes vexans,* the common *D. immitis* vector. A total of 153 female *Ae. vexans* were collected in August 2023 from the Kharkiv region, Ukraine. Metabarcoding based on group-specific primers of 12S rDNA and 16S rDNA was used to identify filarial and bacterial DNA, respectively.

A total of six *D. immitis* (3.92%) and one D. *repens* (0.65%) were identified among *Ae. vexans*. Microbiome analysis reveals dominance of Proteobacteria and Firmicutes in heartworm-infected mosquitoes. Additionally, gut microbiome of *Dirofilaria*-positive females was characterized by a high contribution of *Acinetobacter* sp. (Proteobacteria; uninfected: 0.02%; infected: 43%) and a low prevalence of *Sporosarcina* sp. (Firmicutes; uninfected: 88%; infected: 3%). Of the 14 *Wolbachia*-positive females (9.15%), two were hosts of *Dirofilaria,* one each of *D. immitis* and *D. repens*.

This study revealed a relatively low contribution of *Dirofilaria*-positive mosquitoes in the Kharkiv region, suggesting a low risk of infection. A high prevalence of *Acinetobacter* sp. in infected mosquitoes suggesting a critical role during infection. Furthermore, identification of *Dirofilaria* spp. in a few *Wolbachia*-positive individuals confirms that these bacteria probably prevent filarial proliferation.

This study was supported by the National Science Center, Poland Grant No.2020/37/N/NZ8/01735.

Keywords: *Aedes vexans*, *Dirofilaria immitis*, microbiota, mosquitoes, Ukraine

PS – poster session

[SE7]
Immunological aspects of host-parasite interactions (immunopathology and allergy)

Session: [SE7] Immunological aspects of host-parasite interactions (immunopathology and allergy)

Human immunodeficiency virus and *Toxoplasma gondii* coinfection is associated with exacerbated exhaustion

Kamila Caraballo Cortes[1], Renata Welc-Falęciak[2], Maria Urbanik[1], Małgorzata Bednarska[2], Agnieszka Pawełczyk[1]

1 Department of Immunopathology of Infectious and Parasitic Diseases, Medical University of Warsaw, Warsaw, Poland
2 Department of Parasitology, University of Warsaw, Warsaw, Poland

Abstract

Toxoplasmosis is one of the most common opportunistic diseases observed in the course of HIV/AIDS infection and a leading cause of mortality in this group of patients. T cell exhaustion is an immune dysfunction characteristic of chronic infections and cancer, resulting from constant antigenic stimulation and inflammatory signals. It is characterized by overexpression of inhibitory receptors (iRs), including programmed death receptor-1 (PD-1) and T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3). These receptors have also their corresponding soluble analogs in plasma (sPD-1, sTIM-3).

While exhaustion is well characterized in HIV-1 infection, it is largely unknown how *T. gondii* coinfection affects this phenomenon.

The aim of the study was to analyze the effect of *T. gondii* coinfection on sPD-1 and sTIM-3 in patients with chronic HIV-1 infection.

The study employed plasma from 24 patients with chronic HIV-1/*T. gondii* coinfection without clinical manifestation of toxoplasmosis. Controls comprised patients with HIV-1 (n=25), *T. gondii* (n=12) monoinfections as well as 16 blood donors with excluded HIV-1/*T. gondii*. Detection of *T. gondii* was performed by real-time PCR. Soluble sPD-1 and sTIM-3 in plasma were quantified by ELISA.

SPD-1 and sTim-3 were found to be significantly higher in coinfection than in *T. gondii* monoinfection ($P<0.0001$ and $P=0.0010$, respectively) and higher than in healthy controls ($P<0.0001$ and $P=0.0023$, respectively). Coinfection was also related to higher sTim-3 than HIV-1 monoinfection ($P= 0.0132$). This analogy was not observed in case of PD-1 (NS).

HIV-1/*T. gondii* coinfection may be related to exacerbated exhaustion and possible immune-related deficit, which instigates immune functions monitoring as well as molecular testing of *T. gondii*.

Keywords: HIV-1, *T.gondii*, coinfection, PD-1, Tim-3

OS – oral session

Session: [SE7] Immunological aspects of host-parasite interactions (immunopathology and allergy)

Pro-inflammatory properties of *Toxocara* excretory-secretory antigens

Janina Lekki-Jóźwiak[1], Magdalena E. Wysmołek[1], Justyna Karabowicz[1], Małgorzata Sobczak-Filipiak[2], Karolina Gregorczyk-Zboroch[1], Ewa Długosz[1]

1 Warsaw University of Life Sciences, Institute of Veterinary Medicine, Department of Preclinical Sciences, Poland
2 Warsaw University of Life Sciennces, Institute of Veterinary Medicine, Department of Pathology and Veterinary Diagnostics, Poland

Abstract

The ingestion of *Toxocara canis* eggs by paratenic hosts such as mice or humans leads to the development of toxocariasis, the symptoms of which depend on the final location of the parasite larvae. In case of larvae location in the lungs, symptoms and histopathological changes similar to those occurring in asthma are observed.

Inflammatory reactions occur due to both mechanical tissue injuries and the secretion of *Toxocara* excretory-secretory antigens (TES) by migrating larvae. Surprisingly, there has been no prior investigation into how exposure to TES alone, without the presence of the parasite, affects the host organism. Therefore, our research aimed to characterize the immune response in the lungs following intranasal treatment with TES in mice.

Female C57BL/6J mice received an intranasal treatment with TES dissolved in PBS for three consecutive days, while the control group received PBS alone. Animals were euthanized and lungs were collected 1 and 24 days post-inhalation. Histopathological examinations of the lungs were conducted, and a cytokine profile was created from lung lysates using the Proteome Profiler Mouse Cytokine Array.

Interestingly, intranasal administration of TES in the lungs does induce the typical Th2 response associated with parasitic infections. An upregulation in eotaxin level was noted, with increase in IL-5 and IL-4. Upon stimulation, there is also an increase in factors promoting fibrosis such as IGFBP-5, osteoprotegerin, and osteopontin. Additionally, the histopathological analysis revealed the infiltration of inflammatory cells and bronchiolar epithelium damage. These findings suggest that a 3 day intranasal stimulation with TES triggers a Th2-type inflammation, persisting for at least 24 days and causing tissue damage in the lungs. Additionally this inflammation can be an early fibrosis initiating event.

This study was supported by a research grant no. 2020/39/B/NZ6/02176 from the Polish National Science Center.

Keywords: *Toxocara canis*, lung pathology, IL-33, fibrosis

OS – oral session

Session: [SE7] Immunological aspects of host-parasite interactions (immunopathology and allergy)

Alpha-gal syndrome in patient infected with *A. lumbricoides*: a possible causal role of exposure to parasitic antigens for galactose-a-1,3-galactose sensitization and elicitation of clinical reactivity

Kacper Packi[1], Alicja Rudek[2]

1 Department of Nucleic Acid Biochemistry, Medical University of Lodz, Pomorska 251, 92-213 Lodz, Poland; AllerGen, Center of Personalized Medicine, 97-300 Piotrkow Trybunalski, Poland
2 AllerGen, Center of Personalized Medicine, 97-300 Piotrkow Trybunalski, Poland

Abstract

Alpha-gal syndrome is an IgE-mediated allergy to galactose-α-1,3-galactose (alpha-gal). It results in a delayed anaphylactic reaction to the consumption of red meat, offal and sometimes dairy products or gelatin. The development of sensitization is attributed to tick bites, but the possible role of other parasites has not been well studied.

We describe a case of alpha-gal syndrome diagnosed in patient infected with *A. lumbricoides* and analyze the impact of invasion on alpha-gal sensitization and the elicitation of clinical reactivity.

An 18-year-old woman experienced an anaphylactic reaction (T78.0). The patient was a vegetarian. In the late evening, patient ate a „fast food" dish containing, among others, mozzarella cheese and butter. Anaphylactic shock occurred at 6 *a.m.* - more than 6 hours after eating the last meal. Patient underwent a diagnostic workup for allergy, the following results were obtained: eosinophilia, total IgE - 2700 IU/ml, alpha-gal sIgE - 5 kUA/l (ImmunoCAP), dog/ cat lipocalin sIgE ~ 1 kUA/l (ALEX2). Additionally, patient had not been bitten by a tick in the last 2 years, and the results of serological tests for Lyme disease were also negative. Microscopic examination of the stool revealed *A. lumbricoides* eggs. Ascariasis was confirmed by serological tests (IgG - 12,7 NTU, sIgE 10,4 kU/l). Treatment for ascariasis was administered,

the patient is still a vegetarian, and symptoms have not recurred. The alpha-gal epitope was detected in *A. lumbricoides* glycoproteins at higher concentrations than in *A. hebraeum* and *R. evertsi* ticks. In patients with alpha-gal syndrome, the concentration of alpha-gal sIgE correlated with the level of *Ascaris* sIgE. The authors also demonstrated that the recombinant rABA-1 protein has the ability to activate basophils of patients with alpha-gal syndrome, indicating a possible causal role of exposure to *A. lumbricoides* in alpha-gal sensitization and elicitation of clinical reactivity.

Keywords: allergy, galactose-a-1,3-galactose, alpha-gal syndrome, *Ascaris lumbricoides*

OS – oral session

Session: [SE7] Immunological aspects of host-parasite interactions (immunopathology and allergy)

Deciphering the immunomodulatory role of *Fasciola hepatica* FABPs

Alicja Laskowska[1], Alicja Kalinowska[2], Mateusz Pękacz[1], Katarzyna Basałaj[2], Agnieszka Wesołowska[2], Bruno Guigas[3], Anna Zawistowska-Deniziak[1]

1 *Department of Immunology, Institute of Functional Biology and Ecology, Faculty of Biology, University of Warsaw, Poland*
2 *Museum and Institute of Zoology, Polish Academy of Sciences, Warsaw, Poland*
3 *Department of Parasitology, Leiden University Medical Center, Albinusdreef 2, 2333 ZA, Leiden, the Netherlands*

Abstract

In the intricate battle between host immune defenses and parasitic evasion, *Fasciola hepatica*, the liver fluke, emerges as a sophisticated foe. It utilizes a variety of immunomodulatory molecules to avoid immune detection, with Fatty Acid Binding Proteins (FABPs) playing a crucial role. These proteins are known for their anti-inflammatory properties. However, the specific landscape and functions of FABP isoforms in F. hepatica have remained largely unexplored. Previous research has shown that *F. hepatica* FABP1 induces a tolerogenic phenotype in human monocyte-derived dendritic cells (moDCs) and modulates T-cell polarization by enhancing dendritic cell (DC) secretion of Thrombospondin-1 (TSP-1). The signaling pathway for this process is not well understood.

Using a comprehensive methodological approach, we investigated the effects of recombinant *F. hepatica* FABPs, produced in *Pichia pastoris*, on moDCs. This included dendritic cell-allogenic T cell co-cultures and detailed DC phenotyping through transcriptomic, proteomic, and FACS analyses. Additionally, we explored the signaling pathways involved in the FABP phenomenon in more detail using the same model.

Our findings suggest that PPAR receptors and RXR are partially involved in the effect of FABP on TSP-1 expression, but not in the inhibitory effect on chemokines. Inhibiting PPAR receptors and RXR does not alter the effect of FABP on the maturation surface markers of DCs. Furthermore, FABP internalization into the cell is likely based on lipid rafts.

This detailed examination of FABPs enhances our understanding of *F. hepatica*'s immune evasion strategies and paves the way for targeted therapeutic interventions against fascioliasis. By uncovering the specific roles and interactions of FABP isoforms within the parasite's lifecycle, our study sets the stage for significant advancements in parasitology.

Financial support was provided by the National Science Center Poland 2021/43/D/NZ6/01555.

Keywords: *Fasciola hepatica*, FABP, innate immunity

OS – oral session

Session: [SE7] Immunological aspects of host-parasite interactions (immunopathology and allergy)

Characteristics of *Toxocara canis* induced lung inflammation in C57BL/6J mice

Janina Lekki-Jóźwiak[1], Magdalena E. Wysmołek[1], Justyna Karabowicz[1], Karolina Gregorczyk-Zboroch[1], Małgorzata Sobczak-Filipiak[2], Ewa Długosz[1]

1 *Warsaw University of Life Sciences, Institute of Veterinary Medicine, Department of Preclinical Sciences, Poland*
2 *Warsaw University of Life Sciences, Institute of Veterinary Medicine, Department of Pathology and Veterinary Diagnostics, Poland*

Abstract

Toxocara canis infection in mice results in severe lung pathology with asthma-like histopathological changes. Several studies showed that in the infection induces a strong systemic Th2-like response, however only few experiments focused on lung inflammation and proved that a mixed immune reaction may occur.

Female C57BL/6j mice were infected with 1000 embryonated *T. canis* eggs. Animals were euthanized and lungs were collected at 3, 5, 14 and 28 dpi. Uninfected mice served as controls. The presence of cytokines and chemokines in lung tissue homogenates was analysed using Proteome Profiler Mouse Cytokine Array. Different immune cell populations were analysed using flow cytometry and histopathological evaluation of lung tissue was performed.

We found that IL-33 was upregulated starting from 3 dpi and its highest level was noted at 28 dpi. This however did not result in a significant increase in IL-4 nor IL-5 and only in slight increase in IL-13 production. Th2-type chemokines, such as CCL11 and CCL17 were also noted throughout the course of infection. The numbers of immune cells reached the highest numbers at 3 and 5 dpi and then decreased with time, except for eosinophils, which remained significantly elevated even at 28 dpi.

Moreover, we noted that several molecules associated with pulmonary fibrosis and maladaptive tissue remodelling such as amphiregulin, IL-1α, osteopontin and matrix metalloproteinases were produced in lungs of infected mice with highest intensity at 28 dpi.

In conclusion, lung inflammation induced by *T. canis* larvae appears to be driven by an alarmin cytokine IL-33, which activates aTh2-type immune response and participates in the induction of wound healing what in a longer perspective may lead to fibrosis.

This study was supported by a research grant no. 2020/39/B/NZ6/02176 from the Polish National Science Center.

Keywords: *Toxocara canis*, lung pathology, IL-33, fibrosis

PS – poster session

Session: [SE7] Immunological aspects of host-parasite interactions (immunopathology and allergy)

Macrophage migration inhibitory factor from *Dirofilaria repens* (Dre-MIF-1) shows anti-inflammatory effects increasing IL-10 and decreasing TNF level in LPS activated macrophages

Justyna Karabowicz[1], Ewa Długosz[1], Piotr Bąska[2], Marcin Wiśniewski[1]

1 *Division of Parasitology and Parasitic Diseases, Department of Preclinical Sciences, Institute of Veterinary Medicine, Warsaw University of Life Sciences - SGGW, 02-786 Warsaw, Poland*
2 *Division of Pharmacology and Toxicology, Department of Preclinical Sciences, Institute of Veterinary Medicine, Warsaw University of Life Sciences - SGGW, 02-786 Warsaw, Poland*

Abstract

Dirofilaria repens is a parasite of canids, other carnivores and occasionally human. Adult filarial nematodes locate in the subcutaneous and intramuscular tissue. Dirofilariasis is a vector-borne parasitosis, an intermediate host is a mosquito from the genus *Aedes*, *Culex*, and *Anopheles. D. repens* is endemic in many countries of the Old World.

Orthologs of human MIF (macrophage migration inhibitory factor) have been found in numerous parasite species, including filarial nematodes. In mammals, MIF is a pro-inflammatory cytokine with pleiotropic functions and the key regulator of inflammatory immune response. The role of nematode MIF orthologs is not definitely understood, although several reports indicated that they have important role in immune evasion strategies and immunomodulation.

In this research, we obtained *Dre*-MIF-1 recombinant protein using a bacterial expression system, purified it using affinity nickel chromatography and investigated its impact on THP-1 macrophages *in vitro*. Our findings revealed that LPS pre-treated macrophages stimulated with r*Dre*-MIF-1 show increased production of IL-10 and downmodulate TNF production. This suggests that the protein may contribute to the reduction of inflammation. Moreover, the qPCR analysis also showed a decrease in expression of the pro-inflammatory marker iNOS.

The obtained results are consistent with the studies described in the literature concerning MIF orthologs in other nematode species and confirm the immunomodulatory properties of this molecule.

The study was financed by Science development fund of the Warsaw University of Life Sciences - SGGW

Keywords: Subcutaneous dirofilariasis, recombinant protein, MIF, immunomodulation, macrophage

PS – poster session

Session: [SE7] Immunological aspects of host-parasite interactions (immunopathology and allergy)

Identifying and evaluating short peptides for enhanced diagnosis of toxocariosis

Alicja Laskowska[1], Mateusz Pękacz[2], Ewa Długosz[2], Anna Zawistowska-Deniziak[1]

1 *Department of Immunology, Institute of Functional Biology and Ecology, Faculty of Biology, University of Warsaw, Poland*
2 *Division of Parasitology and Invasive Diseases, Department of Preclinical Sciences, Institute of Veterinary Medicine, Warsaw University of Life Sciences (SGGW), Poland*

Abstract

Toxocariasis, a neglected parasitic disease caused by species of *Toxocara* and *Toxascaris*, represents a significant global health concern. This zoonosis is prevalent in dogs and cats, but also affects humans, with an estimated 19% of the global human population seropositive for *Toxocara* larvae. In humans, toxocariasis often presents with nonspecific symptoms that vary based on the migration of larvae through the bloodstream to various organs, complicating both diagnosis and treatment. Current diagnostic methods, which include imaging and immunological techniques, have limitations that hinder their effectiveness. The objective of our study was to develop an innovative diagnostic approach for toxocariasis using short 12-mer peptides selected via Phage Display technology in conjunction with a mouse model. Through extensive rounds of prescreening and biopanning of a peptide library, we utilized antibodies from mice infected with *T. canis*, *T. cati*, and *T. leonine* to identify potential diagnostic markers. The selected peptides underwent rigorous bioinformatic analysis before being applied in phage ELISA tests with sera from both infected and non-infected mice. Our results demonstrated that these peptides specifically react with IgG and IgE antibodies in the sera from infected mice, highlighting their diagnostic potential. Additionally, some selected clones showed high specificity for *T. leonina*, enabling differentiation of the invasion. This study identifies a promising diagnostic strategy for toxocariasis and suggests a potential method for distinguishing between different causative agents. Future research will focus on identifying peptides specific to *T. canis* and *T. cati* to enhance diagnostic accuracy and specificity further.

Keywords: toxocariasis, phage display, ELISA

PS – poster session

Session: [SE7] Immunological aspects of host-parasite interactions (immunopathology and allergy)

A miRNA catalog from extracellular vesicles of *Anisakis simplex* provides new clues to the host-parasite relationship

Robert Stryiński[1], Ewa Fiedorowicz[1], Mateusz Maździarz[2], Łukasz Paukszto[2], Mónica Carrera[3], Elżbieta Łopieńska-Biernat[1]

1 *Department of Biochemistry, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Michała Oczapowskiego 1A, 10-719 Olsztyn, Poland*
2 *Department of Botany and Evolutionary Ecology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Plac Łódźki 1, 10-721 Olsztyn, Poland*
3 *Institute of Marine Research (IIM), Spanish National Research Council (CSIC), Eduardo Cabello 6, 36-208 Vigo, Spain*

Abstract

Anisakis simplex is a parasitic nematode of marine organisms. Humans can be an accidental host for this species. The discovery that parasitic nematodes can release extracellular vesicles (EVs) was groundbreaking. The secretion of EVs as signaling molecules by parasitic nematodes has been poorly studied. This prompted us to characterize the miRNAs contained in the cargo of EVs produced by *A. simplex* (Anis-EVs). Anis-EVs were isolated by ultracentrifugation and the transcriptomic cargo (miRNAs) of Anis-EVs was characterized using Illumina NovaSeq 6000. The study performed, in which the content of Anis-EVs was analyzed, revealed the presence of 75 miRNAs, including 16 highly expressed ones (e.g. miR-100a-5p, miR-9-5p, miR-71-5p, miR-5364-3p, lin-4-5p). These results are in agreement with those of the group working on the sibling species *A. pegreffii* (Cavallero et al. 2022; DOI: 10.1038/s41598-022-13594-3). The analysis of the host-parasite relationship showed, among other things, that the target for lin-4-5p is FREM1, a co-receptor of the IL-1 receptor family that contributes to the control of the activation of the inflammatory response. For miR-100a-5p, the target is TRIB2, which interacts with signal transduction pathways in physiological and pathological processes and modulates their activity. The results obtained should lead to a better understanding of the molecular processes underlying the development of *A. simplex* infection in humans and will expand the existing knowledge on the role of extracellular vesicles in host-parasite communication.

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Keywords: *Anisakis simplex*, extracellular vesicles, transcriptomics, miRNAs

PS – poster session

Session: [SE7] Immunological aspects of host-parasite interactions (immunopathology and allergy)

Characterization of the family of Fatty Acid-Binding Proteins (FABP) in *Fasciola hepatica*

Alicja Kalinowska[1], Mateusz Pękacz[2], Katarzyna Basałaj[1], Anna Zawistowska-Deniziak[2]

1 Museum and Institute of Zoology, Polish Academy of Sciences, Warsaw, Poland

2 Department of Immunology, Institute of Functional Biology and Ecology, Faculty of Biology, University of Warsaw, Poland

Abstract

Fatty acid-binding proteins (FABPs) play pivotal roles in facilitating the uptake and transport of fatty acids, which are crucial for parasitic helminths, including flatworms. These organisms have limited lipid metabolism and cannot synthesize fatty acids de novo. Studies suggest various functions for FABPs in parasitism, such as fatty acid uptake and transport. Additionally, FABPs are implicated in immune modulation through their interactions with fatty acid ligands, as well as in anthelmintic sequestration as a resistance mechanism, making them potential vaccine candidates against parasites. To date, seven isoforms of FABPs from *Fasciola hepatica* have been identified. We successfully cloned all seven isoforms of FABPs and achieved expression in yeast for six out of the seven FABPs. Quantitative analysis of FhFABPs gene expression across various developmental stages of *F. hepatica* showed distinct expression patterns, with the highest expression observed in the adult stage. Detailed analysis of FhFABPs isoform expression unveiled varying predominance across the developmental stages of fluke, emphasizing the dynamic regulation of FhFABPs isoform expression throughout the parasite's lifecycle. We also demonstrated differences in fatty acid binding by different FABPs isoforms, highlighting the potential roles of these proteins in adaptation and survival within diverse host environments. Furthermore, we investigated the diagnostic potential of purified FhFABPs by assessing specific IgG antibody responses in sheep experimentally infected with *Fasciola hepatica*.

Our findings contribute to a deeper understanding of the functions of FABPs in parasite biology, shedding light on their impact on parasite growth and development, as well as, their potential roles within host-parasite interactions

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Keywords: FABP, *Fasciola hepatica*, gene expression

PS – poster session

[SE8]

Novel perspectives for treatment and drug resistance

Session: [SE8] Novel perspectives for treatment and drug resistance

Treatment of cerebral malaria

Jacob Golenser[1], Ida Birman[2], Judith Waknine-Grinberg[3], Yechezkel Barenholz[4], Amir R Bagheri[5], Johanna Zech[6], Karsten Mäder[6], Andreas Greiner[5]

1 Department of Microbiology and Molecular Genetics, The Kuvin Centre for the Study of Infectious and Tropical Diseases, The Hebrew University of Jerusalem, Israel
2 Department of Microbiology and Molecular Genetics, The Kuvin Centre for the Study of Infectious and Tropical Diseases, The Hebrew University of Jerusalem
3 Department of Microbiology and Molecular Genetics, The Kuvin Centre for the Study of Infectious and Tropical Diseases and the Laboratory of Membrane and Liposome Research, Department of Biochemistry, The Hebrew University of Jerusalem, Israel
4 Laboratory of Membrane and Liposome Research, Department of Biochemistry, The Hebrew University of Jerusalem, Israel
5 Macromolecular Chemistry II and Bayreuth Centre for Colloid and Interfaces, University of Bayreuth, Germany
6 Department of Pharmaceutical Technology, Institute of Pharmacy, Martin-Luther-University Halle-Wittenberg, Germany

Abstract

Cerebral malaria (CM) is associated with mainly activation of immune responses that results in inflammation, endothelial damage, BBB destruction, cerebral edema, seizure and coma.

Several methods were examined in *P. berghei* ANKA infected mice for eliminating the parasites or ameliorating the immunopathology of CM: a. Formulation of liposome-encapsulated glucocorticoid, β-methasone hemisuccinate decreased BBB and CM immunopathology. It was accumulated in the brain of mice and reduced cerebral inflammation, hemorrhages and edema in parallel to CM. b. Rivoceranib an anti-VEGFReceptor-2 that blocks VEGF cascade prevented BBB damage and reduced CM as well. c. Artemisone (ART), an artemisinin derivative prevented severe disease. Few successful methods of its delivery were examined to overcome the need for repetitive injections: c1. release from subcutaneously injected gel, c2. use of a lipid-based ART microemulsion that was applied by gavage, intranasal or transdermal delivery. c3. Release from fibrous polymer nanocarriers (NFN). This goal was achieved by preparation of ART-loaded hydrophobic NFN and application in infusion system which unlocks the usage of hydrophobic drug eluting nanocarriers, a simple programmable system with reduced compliance complications.

Overall, combined immunomodulatory and anti-plasmodial therapy is suggested. The above-mentioned methods could be extended for treatment of other diseases.

We acknowledge the GIP-DFG for the financial support, Cipla for the donation of ART and Elevar Therapeutics for Rivoceranib.

Keywords: cerebral malaria, controlled release, treatment, Artemisone, Rivoceranib

OS – oral session

Session: [SE8] Novel perspectives for treatment and drug resistance

Efficacy of a feed phyto-additive in the prophylaxis of chicken coccidiosis

Adriana Györke[1], Mircea Coroian[1], Virginia Magdaș[1], Florica Ranga[1], Dan Vârban[1]

1 University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania

Abstract

Coccidiosis is a digestive protozoosis that affects the health and performance of chickens. It is recognized worldwide as one of the diseases with the highest economic impact in the poultry sector (over £10.7 billion annually in economic losses). The objective of this work was to evaluate the prophylactic efficacy of a phytoadditive in coccidiosis of chickens. The tested phytoadditive was represented by a mixture of *Artemisia annua, Origanum vulgare, Ocimum basilicum* and *Melissa officinalis* that was administered in feed ad libitum from the first day of the life of the chickens in a dose of 185 (dose 1) and 278 (dose 2) mg phenolic compounds/kg feed. The effectiveness of the phytoadditive was achieved by evaluating clinical, performance and parasitological parameters compared to a control group and a group to which a coccidiostatic was administered. The tested feed phytoadditive did not affect the general health of the chickens. The tested feed phytoadditive had a negative impact on weight gain and feed conversion rate during the first 21 days, more significantly in chickens administered 278 mg phenolic compounds/kg feed. The feed phytoadditive had coccidiostatic efficacy similar to coccidiostatic at dose 2 up to 21 days of age. These results are preliminary and weekly OPG value until the age of 42 days, the species of *Eimeria*, their abundance, and the structure of the intestinal microbiota by qPCR are to be evaluated. Additional research is required to identify the dose of phenolic compounds without impact on the zootechnical performances of chickens.

This work was supported by the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca through an internal grant, Solution, project number 24868/05.11.2021.

Keywords: coccidiosis, broilers, phytoadditive, prophylaxis

OS – oral session

Session: [SE8] Novel perspectives for treatment and drug resistance

Comparative proteomic analysis of metronidazole-sensitive and -resistant *Trichomonas vaginalis* suggests a novel mode of metronidazole action and resistance

Anna Lena Mayr[1], Ana Paunkov[2], Karin Hummel[3], Ebrahim Razzazi-Fazeli[4], David Leitsch[2]

1 VetCore Facility for Research, University of Veterinary Medicine,
2 Institute of Specific Prophylaxis and Tropical Medicine, Medical University of Vienna
3 VetCore Facility for Research, University of Veterinary Medicine
4 Karin.Hummel@vetmeduni.ac.at

Abstract

The single cell parasite *Trichomonas vaginalis* occurs worldwide and infects the urogenital tract of humans, especially in women. Because no vaccine is available the management of the disease is limited to administration of metronidazole and related drugs (5-nitroimidazoles). 5-nitroimidazoles are special because they are only toxic to anaerobic and microaerophilic microbes such as *T. vaginalis* rendering them safe for use in humans and animals. Resistance to metronidazole has remained comparably rare, but it occurs much more often in some parts of the world complicating treatment considerably.

In this study we compared the total protein expression profiles of metronidazole-susceptible and –resistant strains to identify proteins specifically associated with resistance. Surprisingly, the number of proteins found was very low, and when comparing the protein expression profiles of resistant clinical isolates and strains with resistance induced in the laboratory we only found one single protein to be downregulated in all data sets. We also found that this protein, flavin reductase 1 (FR1), reduces iron in the cell. Reduced iron, in turn, binds to proteins in the cytoplasm forming complexes which can react with metronidazole and render it toxic. Consequently, we found that in resistant *T. vaginalis*, FR1 is not expressed thereby keeping proteins safe from metronidazole.

Keywords: *Trichomonas vaginalis*, metronidazole, resistance, proteomics, iron

OS – oral session

Session: [SE8] Novel perspectives for treatment and drug resistance

Assessing the application potential of selected proteins of the *Trichinella* parasite

Anna Stachyra[1], Sylwia Grzelak[2], Anna Zawistowska-Deniziak[3], Katarzyna Basałaj[4], Justyna Bień-Kalinowska[5]

1 Department of General Biology and Parasitology, Medical University of Warsaw, Warsaw, Poland
2 Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Warsaw, Poland
3 Department of Immunology, Institute of functional Biology and Ecology, Faculty of Biology, University of Warsaw, Warsaw, Poland
4 Museum and Institute of Zoology, Polish Academy of Sciences, Warsaw, Poland
5 Maria Sklodowska-Curie Medical Academy, Warsaw, Poland

Abstract

Trichinella causes a disease called trichinellosis, which poses a significant threat to human health around the world. Source of infection include pork, horse and game meat, often consumed raw or undercooked. It is estimated that over 20 million people may be infected with these parasites, though this number may be underestimated due to the lack of experience among medical workers. During a series of proteomic analyses of *Trichinella* performed by our group, we investigated E-S products and crude extracts of muscle larvae and adult worms, identifying promising immunoreactive proteins. These proteins were further characterized bioinformatically for the presence of conserved domains, signal peptides, disulfide bonds, glycosylation sites, 3D structures and sequence similarity to other known proteins. The goal was to assess their protective, diagnostic and immunomodulatory potential. Identified immunoreactive proteins include: enzymes involved in basic cellular processes, protein folding and modification, metabolic and signaling pathways; proteases (serine proteases, aminopeptidases, cathepsins) and protease inhibitors; proteins of unknown function with no similarity to other proteins; others. Based on this analysis, seven proteins were selected for cloning and production in recombinant form to be used in subsequent analyses to reveal their application potential, i.e. improvements in diagnostics, vaccines and therapeutic purposes. They were suspected to play an important role in the parasitic infection process. The obtained recombinant proteins were then used in experiments, including immunization of mice, analysis of immune responses, stimulation of cells and ELISA-based detection of antibodies in animal sera. The results of the study are promising and show new possibilities for the application of selected recombinant *Trichinella* proteins.

Financial support was provided by the National Science Centre Poland (project no 2020/04/X/NZ6/00084 and 2015/18/E/NZ6/00502)

Keywords: *Trichinella*, recombinant proteins, immune respose, immunization, ELISA

OS – oral session

Session: [SE8] Novel perspectives for treatment and drug resistance

Searching for new chemical structures with potential nematicidal activity

Anna Bogucka-Kocka[1], Przemysław Kołodziej[1]

1 Chair and Department of Biology and Genetics, Medical University of Lublin, Poland

Abstract

Research in recent years shows that available antiparasitic drugs are becoming less and less effective and the phenomenon of multidrug resistance is increasing among parasites. Resistance to antiparasitic and nematicidal drugs poses a serious threat to animal and public health. Moreover, the lack of effective antiparasitic compounds may result in deterioration of animal welfare and large economic losses. Currently, there is a huge demand for new antiparasitic drugs around the world.

The aim of our research was to search for new structures and chemical compounds with potential nematicidal activity. The research was carried out, among others, on model nematodes of the genus *Rhabditis* sp. using a patented proprietary method (patent no. 232918, entitled: *Method for the culture of nematodes of Rhabditis sp. genus and determination of the nematocidal substances activity*). As part of the research, various synthetic chemical compounds were tested. The experiments were conducted in cooperation with many national research centers.

The research conducted by our team allowed us to obtain newly synthesized derivatives with significant nematicidal activity. In the future, the tested compounds may become potential candidates for nematicidal drugs used in the treatment of diseases caused by parasitic nematodes.

Keywords: nematicidal activity, new structures, antiparasitic drugs, *Rhabditis* sp.,

PS – poster session

Session: [SE8] Novel perspectives for treatment and drug resistance

Consequences of long-term usage of unilateral mosquito control in Hungary: Analyzing pyrethroid resistance in *Culex pipiens* and *Aedes albopictus*

Rebeka Csiba[1], Zsaklin Varga[2], Dorina Pásztor[1], Bianka Süle[3], Brigitta Zana[4], Gábor Kemenesi[1], Kornélia Kurucz[2]

1 1 National Laboratory of Virology, Szentágothai Research Centre, University of Pécs, Pécs, Hungary 2 Institute of Biology, Faculty of Sciences, University of Pécs, Pécs, Hungary
2 1 National Laboratory of Virology, Szentágothai Research Centre, University of Pécs, Pécs, Hungary 2 Institute of Biology, Faculty of Sciences, University of Pécs, Pécs, Hungary 3 National Laboratory of Health Security, University of Pécs, Pécs, Hungary
3 2 Institute of Biology, Faculty of Sciences, University of Pécs, Pécs, Hungary
4 1 National Laboratory of Virology, Szentágothai Research Centre, University of Pécs, Pécs, Hungary

Abstract

Mosquito-borne diseases pose a worldwide issue, typically managed with the use of pyrethroid-based adulticides, which are commonly utilized in Hungary. Although these substances are effective, their ecological harm and the potential for resistance development are concerning and underexplored. This study focuses on identifying pyrethroid resistance in local mosquito species, particularly through detecting knockdown mutation in *Culex pipiens* and *Aedes albopictus*, the main vectors of West Nile and Dengue viruses, respectively.

We examined 100 *Ae. albopictus* and 170 *Cx. pipiens* mosquitoes, collected in 2023 from three different regions of Hungary, using CO2 baiting traps. To identify whether mosquitoes carried the resistance and/or susceptible alleles, we used allele-specific PCR and gel electrophoresis.

The analysis indicated that 55% of the *Cx. pipiens* mosquitoes carried the allele linked to pyrethroid resistance, with 92% of these displaying a heterozygous genotype. Conversely, all *Ae. albopictus* samples showed only the susceptible genotype, no resistance alleles detected.

The absence of resistance alleles in *Ae. albopictus* in Hungary, despite their presence in neighboring countries, underscores the need for preventive strategies. Since *Ae. albopictus* introduced to Hungary only in 2014, it might not have had enough time to develop resistance. In contrast, the endemic *Cx. pipiens* has shown resistance, likely due to prolonged and frequent exposure to pyrethroids. Continued reliance on pyrethroid-based strategies could exacerbate environmental harm and prove ineffective in the control of mosquito populations. It is crucial to explore alternative mosquito management methods to ensure sustainable vector control in Hungary. Our further aim is to broaden resistance testing to include other vector species.

Keywords: adulticide resistance, mosquito management, common house mosquito, asian tiger mosquito

PS – poster session

Session: [SE8] Novel perspectives for treatment and drug resistance

The impact of glucuronides derived from common marigold (*Calendula officinalis*) on *Toxoplasma gondii* tachyzoites

Katarzyna Goździk[1], Elżbieta Scholz[1]

1 University of Warsaw, Faculty of Biology, Institute of Functional Biology and Ecology, Department of Parasitology, Warsaw, Poland

Abstract

Toxoplasmosis is a globally distributed zoonotic disease caused by protozoan parasite, *Toxoplasma gondii*. The disease is one of the most significant issues that continue to pose a serious threat to public health.

The use of pyrimethamine and sulphonamide in standard therapy have several limitations and may cause many side effects. Therefore, there is a definite need to identify novel active compounds that would act in the acute stage of the disease as well as during chronic infection.

Medicinal plant extracts contain different compounds that have been identified to possess a significant anti-bacterial, anti-viral and anti-parasitic activity.

Marigold (*Calendula officinalis*), is a well-known medicinal plant, widely used in traditional medicine, which contains significant amounts of oleanane saponins.

Saponins, especially glucuronides of oleanolic acid (GlcUAOA) of this plant, due to their detergent properties and ability to increase cell permeability, as well as their cytotoxic and cytostatic activities, seems to possess a great potential as therapeutic agents. The aim of the study was to assess the effect of glucuronides on the *T. gondii* tachyzoites in *in vitro* model.

Tachyzoites of *T. gondii* RH strain propagated in cell culture were harvested from culture and purified on Percoll gradient. Purified tachyzoites were then incubated in medium containing marigold oleanolic acid glucuronides (GlcUAOA) in different dilutions.

After incubation, tachyzoites were washed and introduced back to new HS-5 cells to determined anti-*T. gondii* activity of GlcUAOA. The cytotoxicity of GlcUAOA on tachyzoites was also determined using viability tests.

In the cell culture, a reduction in the ability of tachyzoites treated with GlcUAOA to enter HS-5 cells was observed. Additionally, an impact of glucuronides on the survival of *T. gondii* tachyzoites was observed.

The results suggest that glucuronides possess a promising potential for the development of new therapeutics against *T. gondii*.

COST Action CA21111 One Health Drugs against parasitic vector born diseases in Europe and beyond (OneHealthDrugs) is acknowledged.

Keywords: *Toxoplasma godnii*, toxoplasmosis, plant-derived therapeutics, saponins, glucuronides

PS – poster session

Session: [SE8] Novel perspectives for treatment and drug resistance

The ability to prefer blood in leeches of the *Hirudo* genus and the potential use of this preference in hirudotherapy

Maciej Grobelski[1], Bożena Sikora[1]

1 Department of Animal Morphology, Faculty of Biology, Adam Mickiewicz University in Poznań Uniwersytetu Poznańskiego 6, Poznań 61-712, Poland

Abstract

Leeches are aquatic organisms, amphibiotic, that take up food through predation or parasitism. It is well known that blood uptake is characterised by morphological adaptations such as the formation of suckers, the branching of the intestine into a series of small diverticula, and the secretion of an active substance that prevents the host blood from coagulating during uptake by leeches. However, there is a lack of detailed information in the literature on the ecological adaptations of leeches and their preference for the blood taken (relative to the host).

The aim project is to investigate whether there is, and if so, what the food preference of leeches of the genus Hirudo is for the blood they draw. To this end, two experiments are planned. In the first, leeches from sterile cultures will be subjected to food luring with blood from different animals, and in the second, different types of human blood obtained from a blood donation centre. A leech placed in the central test field will have a large but equal distance to the blood types. Once active, it will be caught before taking food and moved again to the centre of the test field to observe its chosen direction to the food 5-times. The results obtained, presented in the form of a poster, will answer the questions: do leeches of the genus Hirudo have the ability to recognise and prefer the blood of different animals; do they recognise and prefer any of the human blood types; and is there a difference between leech species in this respect.

The results of research into the feeding preferences of leeches will not only expand our knowledge of the biology of these parasites, but may open up new possibilities for their use in therapy. The discovery of specific feeding preferences for different leech species in relation to different types of human blood may contribute to improving the effectiveness of hirudotherapeutic measures and adapting therapies to individual patients' needs.

Funding source: ID-UB UAM 117/39/UAM/0027

Keywords: *Hirudo* spp., food preferences of leeches, hirudotherapy

PS – poster session

Session: [SE8] Novel perspectives for treatment and drug resistance

Effect of selected Indonesian plants on *Giardia intestinalis* in experimental *in vitro* model

Radka Pecková[1], Karel Doležal[2], Bohumil Sak[3], Dana Květoňová[3], Martin Kváč[4], Ivan Petřík[2], Wisnu Nucarhyo[5], Ivona Foitová[1]

1 Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic
2 Department of Chemical Biology and Genetics & Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University and Institute of Experimental Botany, Academy of Sciences of Cz
3 Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, v.v.i., České Budějovice, Czech Republic
4 Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, v.v.i., České Budějovice, Czech Republic; Faculty of Agriculture and Technology, University of South Bohemia in České Budějovice, Czech Republic
5 Department of Parasitology, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia

Abstract

Numerous gastrointestinal pathogens can have a serious impact on the health of an individual, just as the general health of an individual can affect their susceptibility to parasite infection. The investigation of interactions between parasites and hosts is necessary to understand how organisms fight diseases in their natural environment.

In vitro anti-giardial activity of four Indonesian plants with promising anti-parasitic activity, selected on the basis of collected behavioral data and ability to decrease parasite load in Sumatran orangutans was evaluated. Aqueous, methanol and methanol-tetrahydrofuran derived extracts of *Archidendron fagifolium*, *Diospyros sumatrana*, *Piper betle* and *Shorea multiflora* together with *Arabidopsis thaliana* as negative control were tested at different concentrations and compared with a standard giardicidal drug, metronidazole. *Giardia* cultures were counted in a hemocytometer using light microscopy and the anti-giardial effect was statistically evaluated.

We recorded a statistically significant decrease in *Giardia* trophozoites in both methanolic extract of *A. fagifolium* and the aqueous extract of *P. betle*, with the effective concentration 400 µg/ml and 100 µg/ml, respectively. No effect was observed in *D. sumatrana*, *Shorea multiflora* and *A. thaliana*. No evidence of cytotoxicity was shown.

The results of our study showed that extracts from *A. fagifolium* and *P. betle* could have a potential for an alternative treatment against *G. intestinalis*. These findings can serve as a basis for further research concerning their potential use in systemic therapy and/or prophylaxis of *G. intestinalis* infections, as well as the structure and activity of the active compounds.

This work was supported by the UMI – Saving of Pongidae Foundation project "Parasites and Natural Antiparasitics in the Orangutan", Czech Science Foundation (23-06571S) and Scholarship program (PřF_04_27.10.2006).

Keywords: *Giardia*, plant extracts, therapy

PS – poster session

Session: [SE8] Novel perspectives for treatment and drug resistance

Chemotherapy of trichinellosis caused by *T. britovi*

Dimitar Vuchev[1], Galya Popova[1]

1 Bulgaria

Abstract

On the eve of the New Year 2024, a trichinellosis outbreak was registered in a village in Pazardzhik Province (South Bulgaria). A total of 37 persons consumed homemade wild boar products without proper cooking. In 9 of the persons, we found a moderate form of trichinellosis (with fever, swelling and myalgia) and in 15 - mild cases (subfebrile, myalgia). They were treated as outpatient with albendazole for 10-15 days, antihistamines and antipyretics. The other 13 persons who were asymptomatic took chemotherapy with albendazole for 5 days. In all patients DBC was obtained and 10 of the them were tested serologically (ELISA). An infestation with *T. britovi* was proved by PCR. Outpatient observation of the patients continued until February, when some of them still had complaints of myalgia.

It was disscused the correlation of clinical manifestations and the quantity, way of preparation and frequency of consumption of infected products and parasite strain. Attention was drawn to the need for timely epidemiological investigation and preventive treatment of all persons who consumed contaminated products within the incubation period. We adhere to the opinion that coadministration of corticosteroids blocks the encapsulation of trichinella larvae and slows down the healing process, therefore we prefer treatment with antihistamine preparations in combination with albendazole. Follow-up of patients with trichinellosis is necessary, because eosinophilia can persist for several months, as well as late allergic symptoms can occur.

Keywords: trichinellosis, albendazole, outbreak

PS – poster session

Session: [SE8] Novel perspectives for treatment and drug resistance

Gastrointestinal parasites of Polish Primitive Horses with annotations on Benzimidazole-Resistant Strongyle Species

Kateryna Slivinska[1,5], Vitalii Demeshkant[2], Zbigniew Wróblewski[3], Jakub Gawor[4], Vitalii Kharchenko[5]

1 Museum and Institute of Zoology of Polish Academy of Sciences; I.I. Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine, B. Khmelnytskogo 15, 01030 Kyiv, Ukraine
2 Wrocław University of Environmental and Life Sciences
3 Veterinary Practice, Pisz, Mickiewicza 41, Poland
4 Polish Advisory Council for Parasitoses of Companion Animals ESCCAP Poland
5 I.I. Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine, B. Khmelnytskogo 15, 01030 Kyiv, Ukraine

Abstract

The study aim was to assess intestinal parasite occurrence in four main state breeding centers, i.e. Roztocze National Park (RNP), Popielno Research Station (PRS), Biebrza National Parks (BNP), „Lake of the Seven Islands" Nature Reserve (LNR) and to identify drug-resistant gastrointestinal parasites.

Ninety-one horses from stable and reserve breeding were examined. The identification methods used were coproscopy with the modified MacMaster (Herd, 1992) and the Baermann larvoscopy method with the cultivation and identification of L3 to species or genera (Norman D. Levine, 1980).

To detect and identify strongyle species resistant to benzimidazole drugs, 22 horses were dewormed twice with drugs with different active substances (fenbendazole 187.5 mg; ivermectin 18.7 mg + praziquantel 140.3 mg) during 14 days. After 24 and 48 hours after the second deworming, individual fecal samples were collected. A total of 1411 strongyles (Strongylidae) were isolated and identified based on a morphological key (Dvojnos, Kharchenko, 1994; Lichtenfels et al., 2008).

Nematodes from the Strongylidae family were found to infect the horses in all centers, with high prevalence and strongyle egg counts. *Parascaris* spp. infections were found common in horses, while *Eimeria* spp. oocysts were detected in Popielno Station. *Strongylus vulgaris* occurrence was confirmed through larval culture, with significant larvae counts. Cyathostominae presence was consistent across samples. Post-deworming, *Anoplocephala* spp. tapeworms and *Gasterophilus* spp. larvae were sporadically detected.

The dominant group of parasites in all horses examined were representatives of the Strongylidae family with identified *Strongylus vulgaris, S. edentatus, Triodontophorus* spp., and Cyathostominae. Seven benzimidazole-resistant Cyathostominae species were found: *Cyathostomum catinatum, Cylicocyclus nassatus, Coronocyclus coronatus, Cylicostephanus calicatus, C. minutus, C. longibursatus, C. goldi*.

The study was funded by the MSCA4Ukraine project (AvHID 1233593; https://cordis.europa.eu/project/id/101101923).

Keywords: gastrointestinal parasites, horses, benzimidazole-resistant Strongyle species, Poland

PS – poster session

[SE9]
Opportunistic and rare parasite infections

Session: [SE9] Opportunistic and rare parasite infections

Pneumocystis jirovecii in pregnant women

Vicente Friaza[1], Pilar Rojas[2], Rocio Salsoso[1], Elisa Garcia[2], Carmen De la Horra[3], Elena Garcia--Victori[2], Cristina Serradilla[2], Rubén Morilla[4], Francisco Medrano[5], Antonio Pavón[2], Enrique Calderón[6]

1 Instituto Biomedicina de Sevilla, España
2 Hospital Universitario Virgen del Rocío, España
3 Universidad Loyola, España
4 Universidad de Sevilla, España
5 Facultad de Medicina, Universidad de Sevilla, España
6 Facultad de Medicina, IBIs, Universidad de Sevilla, España

Abstract

Pregnancy could constitute a risk factor for colonization of *Pneumocystis jirovecii,* due to the mechanisms of immunological tolerance during this period. The objectives of this study were to understand the prevalence and clinical implications of *Pneumocystis* colonization in pregnant women.

A prospective observational study of pregnant women was conducted. Healthy non-pregnant women of childbearing age were included as controls. Identification of *Pneumocystis* colonization was performed by molecular.

A total of 82 women at the time of their delivery and their newborn children were included in the study. In addition, 36 non-pregnant women matched were studied. *Pneumocystis* DNA was identified in 39% of pregnant women *vs* 13.9% in non-pregnant women ($p = 0.007$). The prevalence of *pneumocystis* in newborn children was 29.7% showing a close correlation with the status of mothers. The mean duration of gestation in women colonized with *Pneumocystis* was 31.5 ± 5.5 weeks *vs* 34.4 ± 5.3 weeks in noncolonized mothers ($p = 0.017$). A linear regression analysis confirmed the effect of *Pneumocystis* colonization on gestational duration ($p = 0.01$) and showed that it was like the effect of diabetes mellitus.

Pregnancy is a risk factor for *P. jirovecii* colonization and could be a potential cause of previously unknown prematurity. Future studies are needed to further define the role of *Pneumocystis* colonization during pregnancy.

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Keywords: *Pneumocystis*, pregnancy, colonization

OS – oral session

Session: [SE9] Opportunistic and rare parasite infections

Cryptosporidium in birds: from parasite to disease

Nikola Holubová[1], Martin Kváč[2], Bohumil Sak[1]

1 Biology Centre of the Academy of Sciences of the Czech Republic, v.v.i., Institute of Parasitology, České Budějovice, Czech Republic
2 Biology Centre of the Academy of Sciences of the Czech Republic, v.v.i., Institute of Parasitology, České Budějovice, Czech Republic University of South Bohemia in České Budejovice, Faculty of Agriculture and Technology, České Budějovice, Czech Republic

Abstract

Cryptosporidium is classified in the phylum Apicomplexa, closely related to gregarines, infecting the gastrointestinal, respiratory and urogenital tracts of most vertebrates, including humans.

Currently, 51 valid species of *Cryptosporidium* parasitizing a wide range of hosts have been described. In addition to the validly described species, dozens of genotypes have been described for which there are no enough data to designate them as separate species.

Although the number of bird species greatly exceeds the number of representatives of the mammalian class, for which a high diversity of *Cryptosporidium* has been described, only six valid species of avian cryptosporidia have been described so far: *Cryptosporidium meleagridis*, *C. baileyi*, *C. galli*, *C. avium*, *C. proventriculi*, *C. ornithophilus*, and 20 genotypes for which we do not have enough data to designate them as separate species.

Cryptosporidia cause a disease called cryptosporidiosis, which is one of the major protozoan infections of birds. It manifests itself as a respiratory or digestive disease and affects a very large number of bird species on several continents.

Due to the limited number of studies conducted on avian hosts, many questions remain unanswered. Key questions include (i) is the species diversity of avian *Cryptosporidium* similar to that known for mammalian *Cryptosporidium*, (ii) are avian *Cryptosporidium* host-, age – and tissue-specific, (iii) what is the pathogenicity and course of infection of avian *Cryptosporidium*, or (iv) can birds be hosts of mammalian *Cryptosporidium*?

In this review, we aim to present complete information on the morphology, life cycle, host specificity, and species prevalence of *Cryptosporidium* infecting birds, as well as the epidemiology, clinical signs, pathology, immunology, diagnosis, and therapy of avian cryptosporidiosis.

Keywords: *Cryptosporidium*, birds, disease, review

OS – oral session

Session: [SE9] Opportunistic and rare parasite infections

Host adaptation of gastric cryptosporidia

Martin Kváč[1], Marie Křesťanová[2], Marie Marková[2], Hana Dudáková[2], Jana Tinavskám[1], Lenka Tůmová[2], František Kitzberger[3], Roman Konečný[2], Bohumil Sak[3]

1 Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic ; University of South Bohemia, České Budějovice, Czech Republic
2 University of South Bohemia, České Budějovice, Czech Republic
3 Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic

Abstract

Gastric cryptosporidia of mammals have adapted to life in the most unfriendly places in the host's body. While the adaptation of intestinal cryptosporidia has been widely studied, research and our knowledge of gastric cryptosporidia remains neglected and poor. We investigated the effects of infection and reinfection with *Cryptosporidium proliferans* in immunocompetent mice and mastomys. Infection with intestinal species is associated with watery diarrhoea, whereas infection with gastric species is asymptomatic. The results of studies have shown that during infection there are significant histopathological changes in the gastric tissue (enlarged mucosa, mucus production), an increased pH value of the gastric contents and a pronounced immune response. After the infection has subsided, the values return to their physiological state. The migration of immune cells is significant 7-14 days after infection. The host reacts to reinfection within the first few days after reinfection, although the functional and pathological changes are not as pronounced as in the initial infection. The intensity of the infection is significantly lower during reinfection than during the initial infection. The temperature and pH value of the environment have a considerable influence on the oocyst excystation. At temperatures below 32°C, less than 15% of oocysts excyst. The optimum temperature for the oocyst excystation of *C. proliferans* is 38°C, and increasing the temperature above 40°C has no effect on excystation. At a pH value of 3, which corresponds to the physiological values of the stomach, only 70% of the oocysts exist. At a pH value of 5-6, which corresponds to the peak of the infection, 85-90% of the oocysts are excysted. Newly formed *C. proliferans* oocysts are completely infectious to the host before they leave the host body and are protected from excystation in the host's digestive tract.

This work was supported by a grant from the Grant Agency of the Czech Republic (21-23773S).

Keywords: functional changes, stomach, excystation, infectivity, immune response

OS – oral session

Session: [SE9] Opportunistic and rare parasite infections

Novel marine compounds against *Naegleria fowleri*

Jacob Lorenzo-Morales[1], Iñigo Arberas-Jiménez[1], Javier Chao-Pellicer[1], Ines Sifaoui[1], Jose J Fernández[2], Ana R Díaz-Marrero[3], José E Piñero[1]

1 Instituto Universitario de Enfermedades Tropicales Y Salud Pública de Canarias (IUETSPC), Universidad de La Laguna (ULL), Canary Islands, Spain andCentro de Investigación Biomédica En Red (CIBER) de Enfermedades Infecciosas (CIBERINFEC), Inst. de Salud

2 Instituto Universitario de Bio-Orgánica Antonio González (IUBO AG), Universidad de La Laguna (ULL), Avenida Astrofísico Francisco Sánchez 2, 38206 La Laguna, Tenerife, Spain

3 Instituto de Productos Naturales y Agrobiología, Consejo Superior de Investigaciones Científicas, Avda. Fco. Sánchez 3, 38206 La Laguna, Tenerife, Islas Canarias, España.

Abstract

Naegleria fowleri is the causative agent of a fulminant disease affecting the central nervous system called primary amebic meningoencephalitis. The disease is fatal in more than 97% of reported cases, mainly affecting children and young people who reported having been involved in aquatic activities in fresh and warm non-treated water bodies that were contaminated by this pathogen. Currently, treatment of primary amoebic meningoencephalitis is based on a combination of different antibiotics and antifungals, which, however, are not entirely effective and lead to numerous side effects.

Therefore, the search for novel molecules with anti-*Naegleria* activity and low toxicity remains an urgent issue in the fight against PAM.

In our search for novel compounds, The red algae of the Laurencia genus have been proved to be an important source of bioactive molecules, including some parasites that belong to the free-living amoeba group. In this study, the anti-Naegleria activity of different molecules obtained from the Laurencia obtusa algae was evaluated. As a result, the chamigrenes (+)- elatol, (+)- obtusol and -(-) elatol were the most active molecules against *Naegleria fowleri*. Moreover, the type of cell death process that produces the (+)- elatol, the most active compound, was also determined, showing a programmed cell death induction in treated cells. Hence, (+)- elatol could be considered as a good candidate for the development of new treatments against the primary amoebic meningoencephalitis.

Keywords: *Naegleria fowleri*, PAM, treatment, Laurencia, PCD

OS – oral session

Session: [SE9] Opportunistic and rare parasite infections

Thirty-five years of *Acanthamoeba* infections in Austria

Julia Walochnik[1], Martina Köhsler[1], Ute Scheikl[1], Aline Lamien-Meda[1]

1 Medical University of Vienna, Austria

Abstract

Acanthamoeba spp. are ubiquitous free-living amoebae and do not need a host. They can however, cause disease upon accidental contact with a potential host. The most common *Acanthamoeba* infection in humans is *Acanthamoeba* keratitis (AK), but they can also cause different disseminating infections in immunocompromised individuals, eventually resulting in the extremely rare but often fatal granulomatous amoebic encephalitis (GAE).

Worldwide, the first case of AK was reported in the early seventies and in the mid-eighties the association between AK and contact lens wear was discovered. Today, acanthamoebae are among the most important causative agents of keratitis in contact lens wearers. AK often shows a severe progression, which is due to a lack of awareness but also to the lack of specific treatment. The dormant cysts pose a particular problem, often residing within the tissue and leading to reinfection after termination of treatment. In Austria, the first diagnosed case of AK was reported in 1989 and the first case of GAE occurred in 2004. Our institution is the Austrian reference laboratory for *Acanthamoeba* diagnostics. Altogether, we have had 377 AK cases and 6 GAE cases. Currently, we have an annual incidence of around 0.2 AK cases per 100,000 inhabitants, with >90% of cases occurring in contact lens wearers and the most affected age group being the 21-30-year-olds. In contrast to other countries, we do not see any seasonality and in contrast to the early years, today, males are equally affected. The vast majority of cases were unilateral and many cases had a prolonged progression, at times over one year. The by far predominant genotype was *Acanthamoeba* T4, other genotypes involved were T3, T5 and T6. The GAE cases diagnosed at our institution occurred mainly in young male patients who were either severely immunocompromised or had other underlying predisposing conditions and involved *Acanthamoeba* genotypes T2, T4 and T5.

Keywords: *Acanthamoeba*, keratitis, encephalitis, rare infections

OS – oral session

Session: [SE9] Opportunistic and rare parasite infections

Occurrence of free-living amoebae in a water reservoir in Wrocław and their molecular characterisation

Maria Wesołowska[1], Jolanta Piekarska[2], Michał Gorczykowski[2], Rusłan Sałamatin[3,4]

1 Department of Biology and Medical Parasitology, Wrocław Medical University, Wrocław, Poland

2 Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats, Wrocław University of Environmental and Life Sciences, Wrocław, Poland

3 Department of Microbiology and Parasitology, Faculty of Medicine. Collegium Medicum, Cardinal Stefan Wyszyński University in Warsaw, Warsaw, Poland

4 Department of General Biology and Parasitology, Medical University of Warsaw, Warsaw, Poland

Abstract

Free-living amoebae (FLA) are protozoa distributed worldwide, found in natural and artificial aquatic environments. They have been isolated also from many sources such as soils and the air. Pathogenic FLA such as *Balamuthia mandrillaris*, *Acanthamoeba* spp., *Naegleria fowleri* and recently recognized *Vermamoeba vermiformis* are the etiological agents of several human diseases. FLA play an important role in the transmission and dispersal of pathogenic microorganisms, called endocytobionts. The occurrence of FLA in the freshwater aquatic ecosystems of temperate climates is poorly known.

Aim of the study: Confirmation of the presence of free-living amoebae in water reservoirs in Wrocław.

Material and Methods: Surface water samples were collected in July 2022 and 2023 from 25 water bodies in Wrocław (8 fountains, 14 ponds in city parks and 3 bathing places/ swimming pools). The water temperature in the tested reservoirs ranged from 18.1°C to 26.4°C. NNA solid medium (1.5% non-nutrient agar, Difco) in petri dishes pre-coated with *Escherichia coli* was used for the isolation and culture of amoebae. Positive samples were subcultured and incubated at 42°C which allowed it to be assessed for its ability to thermotolerance. Species classification was performed using partial 18S-rDNA PCR-sequencing analysis.

Results: FLA were found in 44% (13/25) of the water bodies by NNA culture. Based on PCR detection Echinamoeba exundans and *Naegleria* spp., were identified in 24% (6/25), *Vermamoeba vermiformis* in 12 % (3/25), *Acanthamoeba* spp., and *Vannella* spp., in 4 % (1/25) of the samples from examined water bodies.

Conclusions: These pilot studies confirmed the presence of medically important FLA species in both natural and artificial water bodies in Wrocław and indicate the need for more extensive environmental water research into the presence of FLA, especially considering climate change and rising water temperatures during the summer months.

Keywords: Free-living amoebae (FLA), *Naegleria* spp., *Acanthamoeba* spp., *Vermamoeba vermiformis*

OS – oral session

Session: [SE9] Opportunistic and rare parasite infections

Chronic meningitis caused by the larval stage of *Taenia crassiceps* tapeworm in an immunocompetent woman, Slovenia

Jana Rejc Marko[1], Albin Gačnik[2], Manca Radež[3], Sandra Kolar[1], Timotej Petrijan[2], Barbara Šoba[3]

1 Department of Infectious Disease and Febrile Conditions, University Medical Centre Maribor, Ljubljanska ulica 5, 2000 Maribor, Slovenia
2 University Department of Neurology, University Medical Centre Maribor, Ljubljanska ulica 5, 2000 Maribor, Slovenia
3 Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Zaloška 4, 1000 Ljubljana, Slovenia

Abstract

Taenia crassiceps tapeworm is an intestinal parasite of carnivores, while rodents serve as natural intermediate hosts in whose body cavities or subcutaneous tissues cyst-like larvae proliferate by budding. Rarely, larval stages of this parasite can cause severe zoonosis. We describe a case of human *T. crassiceps* infection in a woman with chronic meningitis and progressive deterioration of neurological symptoms.

The 74-year-old patient has been managed by a neurologist since May 2023 for chronic aseptic meningitis. In December 2022, she noticed intermittent balance disturbances and subsequently developed urinary incontinence, mild cognitive decline and left limb incompetence. The condition progressively worsened and after a year the patient presented psychomotor slowing, tetraparesis, which was markedly left-sided, with moderate to severe haemiparesis. The patient underwent lumbar puncture three times during this period, which confirmed aseptic meningitis with intrathecal IgG, IgM and IgA formation. Extended microbiological tests of cerebrospinal fluid and blood were performed. All tests were negative, except blood serology for *Taenia solium* IgG, which was equivocal. Finally, the patient's CSF was tested for *T. solium* IgG, which was also equivocal. Based on this result, the CSF was molecularly tested by cestode-specific PCR amplifying the mitochondrial 12S rRNA gene, which was positive, and *T. crassiceps* was determined by sequencing. MRI of the brain was unchanged over the year and showed non-specific signal enhancement of 40 x 15 mm in the basal cisterns on the right and in the area of the pontocerebellar angle and the internal auditory meatus on the right. Minor changes were also visible in the left pontocerebellar angle. The diagnosis of *T. crassiceps* cysticercosis is challenging as no tests are commercially available. Equivocal results of serology tests for other helminthiases may indicate a possible infection and molecular tools may prove helpful.

Keywords: case report, chronic meningitis, *Taenia crassiceps*, molecular diagnostics

OS – oral session

Session: [SE9] Opportunistic and rare parasite infections

Cryptosporidium mortiferum: first case in human and synanthropic rodents as source of infection in the Czech Republic

Martin Kváč[1], Alena Konvalinová[2], Nikola Holubová[3], Zuzana Hůzová[4], Kritina Beranová[5], Marta Kicia[6], John McEvoy[7], Bohumil Sak[3]

1 Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic, University of South Bohemia, České Budějovice, Czech Republic
2 University of South Bohemia, České Budějovice, Czech Republic, Czech Union of Nature Conservationists animal rescue center, Vlašim, Czech Republic
3 Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic
4 Institute of Public Health based in Ústí nad Labem, Ústí nad Labem, Czech Republic
5 University of South Bohemia, České Budějovice, Czech Republic
6 Wroclaw Medical University, Wroclaw, Poland
7 North Dakota State University, Fargo, ND, USA

Abstract

Cryptosporidium, a globally distributed protist parasite, poses a health risk to both humans and various vertebrate hosts. While *C. parvum* and *C. hominis* are still the predominant pathogens in humans, there has been an increase in the number of rodent-borne *Cryptosporidium* infections, including those caused by *C. mortiferum*. Its first occurrence in Europe was documented in Eurasian red squirrels in Italy in 2008, and its presence was linked with the introduction of the eastern gray squirrel to Italy in the mid-20th century. Experimental studies reveal diverse clinical manifestations among different squirrel species, ranging from asymptomatic or mild signs in eastern gray squirrels to severe diarrhea, apathy, and mortality in Eurasian red squirrels.

Fecal samples were screened for the presence of oocyst/DNA *Cryptosporidium* spp. by specific staining and nested PCR. The intensity of infection was quantified by qPCR. Human samples were investigated by PCR and bacteriological examination for the presence of bacteria (*Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, and *Clostridium*) and parasitic protists (*Encephalitozoon* spp., *E. bieneusi*, *G. intestinalis*, *Blastocystis* sp. and *C. cayetanensis*).

A 42-year-old urban resident suffered from gastroenteritis, with molecular analysis confirming *C. mortiferum* and genotyping revealing subtype XIVaA20G1T1. Simultaneously, young Eurasian red squirrels exhibited fatal infections, while European ground squirrels remained asymptomatic, indicative of host-specific susceptibility. Genetic analyses demonstrated identical *C. mortiferum* subtypes in both humans and squirrels. Notably, subtype XIVaA20G2T1 was exclusive to Eurasian red squirrels and humans in Scandinavia and the Czech Republic, challenging previous assumptions about the role of eastern gray squirrels in infections.

This work was supported by grant from the Grant Agency of the Czech Republic (21-23773S).

Keywords: *Cryptosporidium*, human, infection, squirrels

PS – poster session

Session: [SE9] Opportunistic and rare parasite infections

Pneumocystis jirovecii calcineurin gene diversity in patients treated with calcineurin inhibitor

Gilles Nevez[1], Chloé Gautier[2], Nathan Le Nan[2], Claire Hoffmann[1], Solène Le Gal[1]

1 Brest University Hospital, France
2 University of Brest, France

Abstract

Pneumocystis jirovecii is an ascomycete responsible for severe pneumonia (*Pneumocystis* pneumonia [PCP]) in immunocompromised patients with or without HIV-infection. PCP incidence has increased in solid organ transplant (SOT) recipients. Immunosuppression induced by ciclosporin or tacrolimus regimens in this patient population represents a risk factor for PCP occurrence. Beyond this iatrogenic induced immunosuppression, we hypothesized that the two aforementioned inhibitors of human calcineurin may also target *P. jirovecii* calcineurin and exert selection pressure. Calcineurin is a heterodimer composed of a catalytic A (CNA) subunit and regulatory B (CNB) subunit. The objective of this study was to investigate the diversity of *P. jirovecii* cna and cnb genes, and therefore to identify mutations potentially associated with ciclosporin or tacrolimus treatments. Five patients with PCP, three exposed to ciclosporin or tacrolimus and two without exposure were retrospectively enrolled and their *P. jirovecii* specimens were analyzed. *P. jirovecii cna* and *cnb* genes were amplified and sequenced. Three nucleotide substitutions were observed. Two of these substitutions (T1587C and A1822G) were found on the *cna* gene of a patient not exposed to calcineurin inhibitors and one mutation (A511G) was found on the *cnb* gene of all five patients (exposed or not exposed to calcineurin inhibitors). The three aforementioned mutations are synonymous and silent, therefore without impact on CNA or CNB protein sequences. To the best of our knowledge, these data represent preliminary but original results of *P. jirovecii* calcineurin gene diversity in SOT recipients. Nonetheless, whether calcineurin inhibitors do not exert selection pressure on *P. jirovecii* organisms is still to be established through additional investigation.

Keywords: *Pneumocystis jirovecii*, calcineurin, tacrolimus, ciclosporin

PS – poster session

Session: [SE9] Opportunistic and rare parasite infections

Characterization of free-living amoebae in environmental samples of agricultural origin from the island of Tenerife.

Marco David Peña Prunell[1], Patricia Pérez Pérez[1], María Reyes Batlle[1], Carlos J. Bethencourt Estrella[1], Rubén L. Rodríguez Expósito[1], Javier Chao Pellicer[1], Ameni Gharbi[1], Meriem Ben Youssef[1], Suhib Muhsen Hasan Aldala'een[1], Atteneri López Arencibia[1], Ines Sifaoui[1], José E. Piñero Barroso[1], Jacob Lorenzo Morales[1]

1 Universidad de La Laguna

Abstract

Free-living amoeba (FLA) are globally distributed protozoa that inhabit water, soil or dust, without the need for a host, they feed on other microorganisms in the environment. Although generally not pathogenic, some can cause diseases such as keratitis, granulomatous amoebic encephalitis or dermatitis. Among the amoebae commonly responsible for these conditions we find the genus *Acanthamoeba* and the species *Vermamoeba vermiformis*, *Naegleria fowleri* and *Balamuthia mandrillaris*.

Considering their ubiquity and they can act as vehicles of bacteria; the aim of this study was to evaluate the presence of free-living amoebae in 25 agricultural soils of different municipalities of Tenerife using two techniques: qPCR multiplex and culture on non-nutrient agar plates to isolate the FLA present in the sample. Of the 25 soils processed, all of them showed evidence of FLA by either method, of which the genus *Acanthamoeba* and the species *V. vermiformis and B. mandrillaris* stand out. It is worth noting that this is the first report of *B. mandrillaris* in the Canary Islands. Based on the obtained results, it is to be reasonably expected that foodstuffs from these soils are contaminated with these FLA, representing a health risk.

Keywords: soil, Tenerife, *Acanthamoeba* spp., *Vermamoeba vermiformis*, *Balamuthia mandrillaris*

PS – poster session

Session: [SE9] Opportunistic and rare parasite infections

Indole based compounds as amoebicidal agents against *Acanthamoeba* spp. Elucidation of action mode

Ines Sifaoui[1], Rubén L Rodríguez-Expósito[1], María Reyes-Batlle[1], Atteneri López-Arencibia[1], Carlos J. Bethencourt-Estrella[1], Javier Chao-Pellicer[1], Patricia Pérez-Pérez[2], Meriem Ben youssef[3], Ameni Gharbi[3], Suhib Muhsen Hasan Aldala'een[2], Alejandra Dumpiérrez Ramos[4], Raquel Diana-Rivero[5], David Tejedor[5], José E Piñero[1], Jacob Lorenzo-Morales[1]

1 Instituto Universitario de Enfermedades Tropicales Y Salud Pública de Canarias (IUETSPC), Universidad de La Laguna (ULL), Canary Islands, Spain and Centro de Investigación Biomédica En Red (CIBER) de Enfermedades Infecciosas (CIBERINFEC), Inst. de Salud
2 Instituto Universitario de Enfermedades Tropicales Y Salud Pública de Canarias (IUETSPC), Universidad de La Laguna (ULL), Canary Islands, Spain
3 Instituto Universitario de Enfermedades Tropicales Y Salud Pública de Canarias (IUETSPC), Universidad de La Laguna (ULL), Canary Islands, Spain; University of Jendouba, Tunisia
4 Universidad de La Laguna, Spain
5 Instituto de Productos Naturales y Agrobiología, Consejo Superior de Investigaciones Científicas, La Laguna, Tenerife, Spain

Abstract

In drug discovery field, organic and synthetic chemistry play an important role to enhance the required activity and/or decrease the cytotoxicity towards the host cells. Indoles are natural alkaloids compounds that been used as base structures to develop new bioactive analogues. In the present work, the activity of 40 indoles derivatives were tested against *Acanthamoeba castellanii* Neff. 5 molecules were able to inhibit the present parasite with and IC50 < 20µM and a cytotoxicity towards murine macrophages with an CC50 > 500 µM. Using confocal microscopy, the indole methyl 6-chloro-1H-indole-3-carboxylate could damage the cytoskeletal network via the destruction of actin and tubulin assemblies. In *Acanthamoeba griffini*, the present analogue would trigger Programmed Cell Death through mitochondrial dysfunction and reactive oxygen species production. The structure-activity relationship (SAR) analysis revealed that the amoebicidal activity would increase with the presence of a carboxyl group in the C-3 position and a halogen element as chlorine or fluorine.

Keywords: *Acanthamoeba* spp., indoles, amoebicidal activity, PCD

PS – poster session

Session: [SE9] Opportunistic and rare parasite infections

In vivo evaluation of Sepigel-based meglumine antimoniate and amphotericin B for cutaneous leishmaniasis treatment

Atteneri López-Arencibia[1], Carlos J. Bethencourt-Estrella[1], Diana Berenguer[2], Ines Sifaoui[1], María Reyes-Batlle[1], Rubén L. Rodríguez-Expósito[1], Javier Chao-Pellicer[1], Patricia Pérez-Pérez[3], Meriam Ben Youssef[4], Ameni Omrani[4], Suhib Muhsin Hasan Aldalaeen[3], Angélica Domínguez-Barros[3], Elizabeth Córdoba-Lanús[1], Jacob Lorenzo-Morales[1], Cristina Riera[2], José E. Piñero[1]

1 Instituto Universitario de Enfermedades Tropicales Y Salud Pública de Canarias (IUETSPC), Universidad de La Laguna (ULL), Canary Islands, Spain and Centro de Investigación Biomédica En Red (CIBER) de Enfermedades Infecciosas (CIBERINFEC), Inst. de Salud
2 Department of Biology, Health and Environment, Laboratory of Parasitology, Faculty of Pharmacy and Food Sciences, University of Barcelona, 08028 Barcelona, Spain
3 Instituto Universitario de Enfermedades Tropicales Y Salud Pública de Canarias (IUETSPC) and Universidad de La Laguna (ULL), Canary Islands, Spain
4 Instituto Universitario de Enfermedades Tropicales Y Salud Pública de Canarias (IUETSPC), Universidad de La Laguna (ULL), Canary Islands, Spain and University of Jendouba

Abstract

Cutaneous leishmaniasis (CL) poses a significant public health concern in endemic regions, characterized by its increasing prevalence and detrimental impact on affected individuals. The disease, primarily caused by *Leishmania* protozoa transmitted through insect bites, manifests in a spectrum of symptoms ranging from self-healing lesions to severe disfigurement. Current treatments, often involving parenteral administration of antimonials, present challenges including poor compliance and adverse effects. This study explores the efficacy of topical formulations containing meglumine antimoniate (MA) and amphotericin B (AmB) using Sepigel as an excipient for CL treatment. For the *in vivo* study, five weeks after infection of BALB/c mice with *L. amazonensis*, when lesions appeared at the injection site, mice were divided into eight groups: untreated mice, mice treated with miltefosine orally, mice treated with intraperitoneal MA, mice treated with 15%, 22.5% and 30% of MA-Sepigel topically, and mice treated with AmB-Sepigel. The treatments were applied daily for two weeks. The results reveal significant reduction in lesion size and parasite burden following topical treatment, particularly with AmB-Sepigel formulations and 30% MA-Sepigel. Furthermore, the Sepigel-based treatment demonstrates improved patient compliance and reduced toxicity compared to systemic therapies. These findings highlight the potential of Sepigel-based formulations as a promising alternative for CL treatment in combined drug, offering enhanced efficacy and tolerability while mitigating systemic toxicity associated with conventional therapies.

Keywords: *Leishmania amazonensis*, *in vivo*, meglumine antimoniate, Amphotericin B, Sepigel

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Session: [SE9] Opportunistic and rare parasite infections

Blepharitis caused by *Demodex folliculorum*

Sylvia Vetrenska[1], Dimitar Vuchev[1], Galya Popova[1], Nina Vutova[1]

1 Bulgaria

Abstract

Demodex folliculorum is often found in people with blepharitis. It is a small mite, living in the lash follicules, where it feeds on sebum and follicular epithelial cells. We presented a case of blepharitis in a 40-year-old woman, caused by this ectoparasite. She complained of eyelid itching, tearing, lid margin redness and swelling as well as intermittent itching in the eyebrow area. The patient was diagnosed with *Demodex folliculorum* using tape, pressed to the eyelashes, and examined under a microscope. The woman was treated with topical ivermectin 1% along with a daily eyelid hygiene regimen with good therapeutic effect. Physicians should be aware of *Demodex* blepharitis and factors that can contribute to its clinical presentation.

Keywords: blepharitis, ectoparasite, ivermectin

PS – poster session

Session: [SE9] Opportunistic and rare parasite infections

Investigation of the possibility of migration of *Pneumocystis jirovecii* to lung tumor tissue

Magdalena Szydłowicz[1], Żaneta Zajączkowska[1], Mariusz Chabowski[2], Błażej Łukianowski[1,3], Paweł Gajdzisz[3,4], Antonina Lewicka[1], Marta Kicia[1]

1 Department of Biology and Medical Parasitology, Wroclaw Medical University, Wroclaw, Poland
2 Department of Surgery, 4th Military Clinical Hospital, Wroclaw, Poland
3 Department of Pathomorphology and Clinical Cytology, Wroclaw Medical University, Wroclaw, Poland
4 Department of Clinical and Experimental Pathology, Wroclaw Medical University, Wroclaw, Poland

Abstract

Pneumocystis jirovecii, a fungal species occurring in human lungs, is a causative agent of *Pneumocystis* pneumonia (PCP). Since it is an opportunistic respiratory pathogen, patients with lung cancer are at particular risk of infection. Due to the tendency for *Pneumocystis* to accumulate focally in the lungs, it may be assumed that in this group of patients such concentration may particularly concern tumor tissue.

The aim of the study was therefore to analyze the ability of *P. jirovecii* to migrate to lung tumor tissue. The study included 37 patients diagnosed with lung cancer, and various types of respiratory tract samples were collected from each of them: fragments of tumor tissue, margins and respiratory secretions. Genomic DNA was extracted from each sample, and a nested PCR protocol to amplify the *mtLSU* rRNA locus was used to detect pathogen DNA. *P. jirovecii* was detected in nine samples derived from six patients. The presence of the pathogen in the tumor tissue was demonstrated only in one patient, suggesting that the fungus actually has the ability to concentrate in this tissue type. However, since *P. jirovecii* was also present in the margins and secretions collected from the same participant, it cannot be concluded from this evidence whether migration to tumor tissue is preferential for this pathogen. This is confirmed by the results of other eight patients in whom fungal DNA was detected only in the margins and/or secretions, while the tumor tissue was not infected.

In conclusion, our preliminary results indicate that migration of *Pneumocystis* into lung cancer tissue is a possible but rare event and further consideration in larger numbers of patients is required.

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Keywords: *Pneumocystis jirovecii*; lung cancer; respiratory pathogen

PS – poster session

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Encephalitozoon spp. infection in patients with lung and colon cancer

Żaneta Zając̨zkowska[1], Magdalena Szydłowicz[1], Maciej Nowicki[2], Jakub Klekowski[3], Mariusz Chabowski[4], Błażej Łukianowski[5], Paweł Gajdzis[6], Martin Kváč[7], Bohumil Sak[8], Marta Kicia[1]

1 Department of Biology and Medical Parasitology, Wroclaw Medical University, Wroclaw, Poland
2 4th Military Clinical Hospital, Wrocław, Poland
3 4th Military Clinical Hospital, Wrocław, Poland; and Wroclaw Medical University, Wrocław, Poland
4 4th Military Clinical Hospital, Wrocław, Poland; and Wroclaw University of Science and Technology, Wrocław, Poland
5 Wroclaw Medical University, Wrocław, Poland; and 4th Military Clinical Hospital, Wrocław, Poland
6 4th Military Clinical Hospital, Wrocław, Poland; and Department of Clinical and Experimental Pathology, Wroclaw Medical University, Wrocław, Poland
7 Biology Centre of the Czech Academy of Sciences, v.v.i., Institute of Parasitology, České Budějovice, Czech Republic; University of South Bohemia, Faculty of Agriculture, České Budějovice, Czech Republic
8 Biology Centre of the Czech Academy of Sciences, v.v.i., Institute of Parasitology, České Budějovice, Czech Republic

Abstract

Microsporidia of the genus *Encephalitozoon* (*E. cuniculi*, *E. hellem*, and *E. intestinalis*) are opportunistic pathogens initially developing in the epithelium of the small intestine. In immunocompromised individuals, such as transplant recipients or persons living with HIV, severe symptoms and extraintestinal infections, especially urinary and respiratory, are observed. Besides, as another important group at risk of opportunistic infections oncological patients appear.

The aim of our research was designed to determine whether patients with lung or colon cancer are at risk of *Encephalitozoon* spp. infection and whether malignancy might be preferentially inhabited by these pathogens.

The study group consist of 37 patients with lung cancer and 49 patients with colon cancer being under the care of 4th Military Clinical Hospital in Wroclaw (Poland). Set of tissue biopsies (cancerous and tissue from the tumor margin) were screened from each patient. Additionally, the samples from intestinal tract (stool) or respiratory secretions were examined. Total DNA was extracted from samples and *Encephalitozoon*-specific nested PCR protocols were used to detect DNA of analyzed pathogens.

Overall, *Encephalitozoon* infection was confirmed in 16.2% (6/37) of patients with lung cancer. *Encephalitozoon* DNA was found both in tissue from lung tumor margin (one patient) and in respiratory secretions (five patients). No *Encephalitozoon* DNA was found in samples collected from patients with colon cancer.

Our preliminary results point that patients with lung cancer might be prone to *Encephalitozoon* respiratory infection, similarly as immunocompromised individuals.

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Keywords: *Encephalitozoon* spp., lung cancer, colon cancer,

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Pathophysiology and mechanisms of cerebral acanthamoebiasis

Natalia Łanocha-Arendarczyk[1], Karolina Kot[1], Irena Baranowska-Bosiacka[2], Maciej Tarnowski[3], Danuta Kosik-Bogacka[4]

1 Department of Biology and Medical Parasitology, Pomeranian Medical University in Szczecin
2 Department of Biochemistry, Pomeranian Medical University in Szczecin
3 3Department of Physiology in Health Sciences, Faculty of Health Sciences, Pomeranian Medical University in Szczecin
4 Independent Laboratory of Pharmaceutical Botany, Pomeranian Medical University in Szczecin

Abstract

Granulomatous amoebic encephalitis (GAE) caused by *Acanthamoeba* sp. is a rare opportunistic CNS infection. We still know little about the exact role of lipoxygenases (LOXs), matrix metalloproteinases (MMPs), neurotrophic factors (NTFs), in the development of *Acanthamoeba* sp. infection. The aim of the study was to check which mechanisms are involved in brain host response to amoebas in relation to the host immunological status. The experiments were performed *in vivo* and the *Acanthamoeba* sp. isolate belonging to the T16 genotype was used. Animals were divided into 4 groups: immunocompetent uninfected mice, immunocompetent *Acanthamoeba* sp. infected mice, immunosuppressed *Acanthamoeba* sp. infected mice, immunosuppressed uninfected mice. This study show the impact of free-living amoebas on the expression of gene lipoxygenase (*Alox5*) in the cerebral cortex and hippocampus during cerebral *Acanthamoeba* sp. infection. The increased *Alox5* expression in the hippocampus during acanthamoebiasis in immunocompetent host probably is associated with the presence of macrophages in which this lipoxygenase is expressed. We observed also the influence of the host's immune status on the expression of *Alox5*. Our research has shown that *Acanthamoeba* sp. infection can change the levels of MMPs and the tissue inhibitor of MMPs (TIMPs) in the central nervous system. We also found a heterogenicity in levels of neurotrophins in different stages of cerebral acanthamoebiasis according to host immunological status. Our results suggest that at the beginning of infection in immunocompetent hosts, brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) may reflect an endogenous attempt at neuroprotection against *Acanthamoeba* sp. infection. While in the immunosuppressed hosts, there is a pro-inflammatory effect of nerve growth factor (NGF) during acanthamoebiasis. This study may provide important new information for understanding the mechanisms of the cerebral acanthamoebiasis.

Keywords: *Acanthamoeba*, opportunistic parasite, brain

PS – poster session

[SE10]

Parasite infections in livestock and their control

Session: [SE10] Parasite infections in livestock and their control

Molecular identification of *Babesia*, *Theileria*, *Anaplasma*, and heamotropic Mycoplasma from aborted samples of domestic ruminants

Relja Beck[1], Daria Jurković Žilić[1], Ema Gagović[1], Šimun Naletilić[1], Irena Reil[1], Sanja Duvnjak[1], Maja Zdelar-Tuk[1], Silvio Špičić[1]

1 Croatian Veterinary Institute, Croatia

Abstract

A diverse range of infectious agents cause abortion in cattle and small ruminants, which is a significant source of economic losses in the global livestock industry.

Due to the fact that a number of the pathogens carried by ticks have not been thoroughly investigated as possible abortion agents, we did a molecular survey of organs from 336 cattle, 183 sheep, and 132 goat foetuses to look for piroplasms, members of the Anaplasmataceae family, and heamotropic mycoplasms. We previously tested samples as part of the regular national surveillance program to exclude *Brucella* spp., *Coxiella burnetti*, *Toxoplasma gondii*, and *Neospora caninum*. We found a single infection or co-infection in 73 cattle samples (21.7%). *Anaplasma* was present in 41 (11.2%) of the cases, with 34 *Anaplasma marginale* and seven *Anaplasma phagocytophilum*. We found *Theileria orientalis* in 16 samples, *Babesia canis* in three, *Babesia vulpes* in one, *Mycoplasma wenyonii* in seven, and *M. haematobos* in five. Four samples showed co-infection with *A. marginale* and *Th. orientalis*, while another four samples showed co-infection with *A. marginale* and *M. wenyonii*. One sample co-infected *B. marginale* with *B. canis*. 37 (20.3%) of the sheep's samples tested positive for DNA. We found a species of *Anaplasma* in 15 samples, or 8.1%: 12 *Anaplasma ovis* (6.5%), 2 *A. phagocytophilum* (1%), and one *A. marginale* (0.6%). We found eight *Theileria ovis* (6%), two *Babesia ovis* (1%), two *Babesia canis* (1%), three *Th. orientalis* (2%), and seven *M. ovis* (3.8%). In 11 goat samples (8.3%), three had *A. phagocytophilum*, one had *A. ovis*, two had *M. ovis*, and one had *M. hamatobos*. This study made it clear that tick-borne pathogens and hemotropic mycoplasms can cause abortions in ruminants, either on their own or as co-infections. However, the current acknowledgement of their significance is inadequate. This study specifically noted their importance in cattle, but found it to be less significant in goats.

Keywords: abortion, molecular, TBD, Mycoplasma, ruminants,

OS – oral session

Session: [SE10] Parasite infections in livestock and their control

Economic assessment of *Ostertagia ostertagi* and *Fasciola hepatica* infections in dairy cattle herds in Türkiye using ParaCalc®

Mahmut Sinan Erez[1], Esma Kozan[1], Tong Wang[2], Fatih Mehmet Birdane[1], Johannes Charlier[2]

1 Faculty of Veterinary Medicine, Afyon Kocatepe University, Türkiye
2 Kreavet, Belgium

Abstract

The aim of the current study was to estimate the economic costs of *Ostertagia ostertagi* and *Fasciola hepatica* infections in dairy cattle herds in Türkiye using the ParaCalc® model. Survey data were obtained from 564 farms across seven distinct geographic regions, followed by a questionnaire. On these farms, bulk tank milk (BTM) samples, along with ten fecal samples per farm from first-season grazing calves, were analyzed using a commercially available ELISA (Indical Bioscience, SVANOVIR, Germany) for *O. ostertagi* and *F. hepatica*, as well as the modified McMaster test. The BTM ELISA showed 31.91% (n=180) of samples were exposed ($0.5<\text{ODR-O}<0.8$) and 26.59% (n=150) were exposed with likely significant production losses ($0.8\leq\text{ODR-O}$) for *O. ostertagi* and 21.98% (n=124) were exposed ($0.3<\text{ODR-F}<0.6$) and 16.13% (n=91) were exposed with likely significant production losses ($0.6\leq\text{ODR-F}$) for *F. hepatica*. The median annual costs per farm due to gastrointestinal (GI) nematode and *Fasciola hepatica* infections were estimated to be €1,144 and €158, respectively. Decreases in milk yield in multiparous cows were the major reason for annual production losses due to GI nematodes (€41 per cow/year) and *F. hepatica* infections (€6 per cow/year). Median annual costs per cow for anthelmintic treatment against GI nematode infections in adult cows were estimated to be €5.51 This study is the first in Türkiye to quantify the economic losses resulting from GI nematode and *F. hepatica* infections using BTM ELISA tests. The study demonstrated that, given all required information, the ParaCalc® model can help identify production losses in dairy cattle herds caused by helminth infections and optimize farm economics in Türkiye.

Keywords: *Ostertagia ostertagi*, *Fasciola hepatica*, dairy cattle, production loss, Türkiye, ParaCalc®

OS – oral session

Session: [SE10] Parasite infections in livestock and their control

Equine tapeworms in Serbia: coprological, serological and molecular findings and control

Tijana Kukurić[1], Mihajlo Erdeljan[1], Aleksandra Uzelac[2], Jacqui Matthews[3], Kirsty Lightbody[3], Corrine Austin[3], Natalia Peczak[3], Stanislav Simin[1]

1 University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine (Serbia)
2 Institute for Medical Research, Institute of National Importance for the Republic of Serbia (Serbia)
3 Austin Davis Biologics Ltd, Great Addington (UK)

Abstract

Equine tapeworms are potential causes of colic in pasture kept horses worldwide. Of the three common tapeworm species, *Anoplocephala perfoliata*, which is pathogenic, is the most prevalent. Due to uneven distribution and small number of eggs in faeces, coprological diagnosis of *Anoplocephala* spp. is challenging, thus, ELISA-based antibody detection in serum or saliva is more advantageous. In Serbia, data on the occurrence of equine tapeworms is limited to a few studies based on coprology and therapeutics effective against tapeworms are not registered for horses. The aims of this study were to determine the prevalence of anoplocephalids using coprology and serology, identify the species by multiplex PCR designed to detect *A. perfoliata*, *A. magna* and *Anoplocephalides mamillana,* and finally, to develop and test an oral deworming paste. A total of 173 horses from over 20 localities throughout Serbia were included in the study, and owners were asked to complete a questionnaire on tapeworm control. A compound broad-spectrum ivermectin and praziquantel-based paste was developed and administered once to a subset of infected animals. Their feces were regularly examined over 8 weeks. Overall, anoplocephalid eggs were present in 11% of fecal samples (19/173), yet specific antibodies were detected in 35.3% of sera (61/173). Tapeworm eggs were detected in six seronegative horses, and their identification is underway. Preliminary PCR results confirmed *A. perfoliata* as the dominant species. Treatment was successful for all horses (n=11), and adverse effects of the paste were not observed. This study showed that *A. perfoliata* in horses in Serbia is prevalent, which warrants further surveillance and urgent registration of effective therapeutics. The questionnaire results showed that horse breeders are poorly informed on tapeworms, thus education and advocacy on treatment is necessary.

Keywords: Anoplocephala spp., equine tapeworm, diagnostics, praziquantel, Serbia

OS – oral session

Session: [SE10] Parasite infections in livestock and their control

Strongylids of domestic horses: influence of anthelmintic treatment on species composition and parasite community structure

Tetiana A. Kuzmina[1,2], Alzbeta Königová[1], Anatoliy Antipov[3], Vitaliy Kharchenko[2], Yaroslav Syrota[1,2]

1 *Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovak Republic*

2 *I. I. Schmalhausen Institute of Zoology NAS of Ukraine, Kyiv, Ukraine*

3 *Bila Tserkva National Agrarian University, Bila Tserkva, Ukraine*

Abstract

Strongylid nematodes are the most prevalent horse parasites worldwide; the main method of their control is using of anthelminthic drugs. We aimed to estimate the alterations in the species composition and structure of strongylid communities in domestic horses after two-decade use of highly effective anthelmintics.

The study was performed at two farms in Ukraine where almost no anthelmintic treatments were practiced before 2004, and regular dewormings with highly effective anthelmintics were applied during the last 10–15 years. Strongylid nematodes were collected after horse deworming with macrocyclic lactone anthelmintics in 2004 (from 22 horses) and 2023 (from 17 horses). In total, 18,999 strongylid specimens were collected and identified.

Twenty-one strongylid species were collected in horses in 2004 – six species of Strongylinae and 15 – of Cyathostominae. In 2023, only 13 cyathostomin species were collected. The similarity of the strongylid species compositions between farms was higher in 2023 (Jaccard index, J=0.66) than in 2004 (J=0.62). The similarity of species compositions between samples notably decreased from 2004 to 2023 (J=0.43). We observed that the rare species with a prevalence <20% (*C.elongatus, C.ultrajectinus, P.mettami, P.poculatum, C.bidentatus*) disappeared from the community during two decades; the prevalence of background species (*C.labiatus, C.labratus, C.insigne*) dramatically decreased. Only 10–12 most prevalent and abundant cyathostomin species survived regular dewormings with highly effective anthelmintics. Also, the dominance of one species, *C nassatus*, dramatically increased during two decades (Berger-Parker index=28.8 in 2004, and 74.4 in 2023). General structure of the strongylid community gradually transformed from multimodal to a bimodal (core–satellite mode) structure.

The study was funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V01-00015 and 09I03-03-V01-00046.

Keywords: Strongylids, parasite community structure, species diversity, anthelmintic treatment, macrocyclic lactones

OS – oral session

Session: [SE10] Parasite infections in livestock and their control

Non-invasive monitoring of parasite infections in beef cattle grazing nature conservation areas

Amalie Camilla Pedersen, Mita Eva Sengupta, Helena Mejer, Andrew Richard Williams, Stig Milan Thamsborg

Parasitology and Aquatic Pathobiology, University of Copenhagen, Denmark

Abstract

The use of cattle for nature conservation is increasing in Denmark and is often termed as "rewilding", which implies that the animals should be considered wild and interventions kept at a minimum. It is well known that parasite infections constitute a major health issue for grazing livestock, and with this increasing tendency of rewilding, it is debated how best to follow the health of animals over time.

To develop a non-invasive strategy for monitoring parasite infections in cattle by developing a method to identify individual cattle based on faecal samples.

A cohort of 45 *Angus* heifers (9-10 mos. at start) was monitored for two years. Blood and faecal samples were collected at turn-out. During the grazing seasons, 20 freshly deposited fecal droppings were collected monthly. Fecal egg counts (FEC) were done for gastrointestinal nematodes (GIN) and flukes. DNA was extracted from blood and feces of 12 individual heifers and genotyped using the Illumina BovineSNP50 chip. The genotypic concordance between blood (high-quality DNA) and feces was analyzed as a proof of concept to demonstrate that individual cattle could be identified using fecal DNA.

The GIN FEC peaked (mean 339 epg) one month after turn-out and declined during the 1st season and stayed low (< 50 epg) in the 2nd season. Fluke FEC levels showed a similar pattern for liver flukes while FEC for rumen flukes were persistently high with a prevalence of 95-100% at all times during the two years. The genotyping of blood and fecal DNA was able to identify 11 of 12 heifers with an adjusted call-rate of >90% using 30k SNPs.

The consistently high FEC and prevalence observed for the rumen flukes have not been observed in Denmark before and requires further investigation. This first attempt of genotyping cattle using SNP-analysis on faecal DNA showed promising results, and the method will be further validated for non-invasive monitoring of individual animals.

Keywords: cattle, parasite, genotype, livestock, fluke

OS – oral session

Session: [SE10] Parasite infections in livestock and their control

Longitudinal monitoring of parasite infections in two Danish organic pig farms with agroforestry

Amalie Camilla Pedersen[1], Isabella Jørgensen[1], Andrew Richard Williams[1], Anne Grete Kongsted[2], Mita Eva Sengupta[1], Helena Mejer[1], Stig Milan Thamsborg[1]

1 *Parasitology and Aquatic Pathobiology, University of Copenhagen, Denmark*

2 *Department of Agroecology, Aarhus University, Denmark / Senior Researcher*

Abstract

Production of pigs in an agroforestry system may contribute to increased animal welfare, less nutrient leaching and affect parasite levels. The trees used in these systems may be selected for potential anti-parasitic effects. To describe the occurrence of gastrointestinal nematodes (GIN) in pigs in farms with agroforestry systems.

Two organic Danish farms with outdoor farrowings in paddocks, each with a partial cover of 20-30% poplar trees (*Populus* spp.), were monitored quarterly for one year. Farm 1 had 650 sows and reared 11000 piglets annually; the piglets were weaned at 10 weeks and reared in the farrowing paddocks until housing at 12 weeks. Farm 2 had 350 sows and reared 6000 piglets annually; the piglets were weaned at 10 weeks and moved to stables with outdoor access. On both farms, 20 fecal droppings were collected from different groups (small weaners, large weaners, finishers, lactating sows and pregnant sows) at each visit and analyzed for GIN fecal egg count (FEC).

The weaners and finishers had low *Oesophagostomum* spp. FEC (means <20 epg), while pregnant and lactating sows had consistently high *Oesophagostomum* spp. FEC (means >3500 epg). The weaners had sporadic infections with low *A. suum* FEC, whereas the finishers were more infected (means of 1000-2000 epg). Pregnant and lactating sows had a lower *A. suum* FEC of 100-200 epg. Both farms routinely dewormed piglets at weaning and pregnant sows one week before farrowing. Ingestion of poplar bark, leaves and branches by sows, was observed.

The low FEC in the weaners is probably due to collection of samples a few weeks after deworming. A high transmission of *Oesophagostomum* spp. is observed in the farrowing paddocks and expressed as high FEC in the lactating sows despite deworming shortly before farrowing. The results did not indicate any immediate anti-parasitic effect of the poplar trees, as these results were similar to other outdoor pig productions.

Keywords: pigs, livestock, anti-parasitic, agroforestry, parasite

OS – oral session

Session: [SE10] Parasite infections in livestock and their control

Circulation of *Cryptosporidium* species in Mediterranean island livestock: the case of Corsica (France)

S. Grech-Angelini[1], V. Villier[2], J.M. Santini[3], M. Riera[3], A. Guillaume[4], C. Boulocher[1], L. Favennec[2], R. Razakandrainibe[2]

1 Collège vétérinaire, Institut Polytechnique UniLaSalle, Rouen, France
2 EA 7510 ESCAPE, Université de Rouen Normandie, France
3 Laboratoires de la Collectivité de Corse (Corse-du-Sud et Haute-Corse), France
4 GDS Corse, Ajaccio, France

Abstract

Corsica, a mountainous Mediterranean island, relies significantly on livestock farming for its economic and cultural sustenance. Cryptosporidiosis, a widely distributed parasitic protozoan disease with a broad host range, including humans, bovines, canines, and horses, poses a significant concern. Despite the favorable context for such infections, the circulation of *Cryptosporidium* species remains unknown in Corsica, both in humans and animals.

In this study, fecal samples were collected from various livestock animals on the island and analyzed using coproscopy and molecular biology techniques to identify *Cryptosporidium* species and subtypes. A total of 127 fecal samples from 109 livestock farms were examined, with 92% originating from small ruminant farms. The findings revealed that 96% of the animals were infected with at least one internal parasite, and *Cryptosporidium* spp. were identified in 8 fecal samples (6.3%) from 7 different farms (6.4% of the farms).

Two zoonotic species, *Cryptosporidium parvum* and *Cryptosporidium ubiquitum*, were detected. The parasite was more prevalent in South Corsica than in Upper Corsica, with goats being the most affected species, accounting for 88% (7/8) of the positive animals. Additionally, two subtypes of *C. parvum*, IIdA14G1 and IIdA17G1, were identified. These findings highlight the circulation of *Cryptosporidium* in island livestock farms and its potential health impact on young animals.

The identification of these zoonotic species underscores the exposure risk to human populations, despite the absence of reported human cases to date.

Keywords: Corsica, livestock, Cryptosporidiosis, *Cryptosporidium parvum*, zoonotic

OS – oral session

Session: [SE10] Parasite infections in livestock and their control

Ex-vivo efficacy of commercially available anthelmintics against blood feeding stomach worm *Haemonchus contortus* of ruminants

Mohammad Zahangir Alam[1], Shanaz Parvin[1], Nusrat Nowrin Shohana[1], Anita Rani Dey[1]

1 Department of Parasitology, Bangladesh Agricultural University, Bangladesh

Abstract

Haemonchus contortus is the most prevalent and pathogenic gastrointestinal nematodes (GINs) in ruminants causing extensive economic losses. It is essential to estimate the efficacy of common commercially available anthelmintics against *H. contortus* parasite. Here, we standardized an *ex-vivo* culture platform for *H. contortus* and evaluated the efficacy of commonly used anthelmintics namely, albendazole (ABZ), levamisole (LVM), ivermectin (IVM), closantel (CLS) and rafoxanide (RFX). Adult worms were collected from abomasa of slaughtered animals, cultured in MEM, DMEM, M199 or RPMI with or without 20% FBS for up to 72 h. Cultured worms were incubated with ABZ, LVM, IVM, RFX or CLS in DMEM supplemented with 20% FBS at different concentrations (0.5-50 µg/ml) in triplicates and examined at 0, 3, 6, 12, 24, 36 and 48 h post treatment. Of the culture conditions, DMEM supplemented with 20% FBS supported the survival of *H. contortus* for ($P<0.001$) longer period of time which was used in the evaluation of anthelmintics. The efficacy of CLS and RFX were significantly ($P<0.001$) higher than other drugs and 100% mortality was observed at 2 µg/ml of CLS and RFX within 12 h post treatment. However, ABZ, LVM, and IVM showed significant effect at the concentration of 50 µg/ml with 48, 36, and 24 h, respectively. Morphological changes included severe cuticle disruption around the buccal cavity, posterior region and vulva as well as loss of cuticle structure integrity coupled with expulsion and fragmentation of digestive components of parasites when treated with 50 µg/ml of ABZ, LVM, and IVM and 2 µg/ml of RFX and CLS. Collectively, DMEM supplemented with 20% FBS can be used as *ex-vivo* culture platform for maintenance of *H. contortus*, and RFX and CLS can be used as the promising drugs for the prevention, control and treatment of *H. contortus* infections.

Keywords: e*x-vivo*, *H. contortus*, culture media, anthelmintic, survivability

PS – poster session

Session: [SE10] Parasite infections in livestock and their control

Comparison of the first time detected *Oesophagostomum asperum* with *Oesophagostomum columbianum* in sheep and goats in Bangladesh based on the trinity: Morphology, morphometry and genetic diversity

Mohammad Zahangir Alam[1], Nusrat Nowrin Shohana[1], Anita Rani Dey[1], Sharmin Aqter Rony[1]

1 Department of Parasitology, Bangladesh Agricultural University, Bangladesh

Abstract

Oesophagostomum spp. (Family: Chabertiidae) is keeping a low profile in terms of severity in Bangladesh while maintaining economic loss through disguise within sheep and goats. The study was performed to identify prevalence, confirmation of species through morphology and morphometry followed by phylogeny using *ITS2* and *COX1* genes. In total 384 slaughterhouse-sourced small and large intestines were pooled from Mymensingh, Kishoreganj, Netrokona, Sherpur and Tangail districts of Mymensingh division. Followed by isolation, *O. columbianum* and *O. asperum* were identified following their key morphological features. Notably, *O. asperum* was first time detected in Bangladesh. The overall prevalence of *Oesophagostomum* spp. was found 60.93%. The prevalence of *O. columbianum* (64.95%) was almost double than that of *O. asperum* (35.04%). Among several characters, only the distance between anus to tail tip showed a significant morphological disparity in female. The Neighbor-joining (NJ) phylogenic trees based on *ITS*2 and *COX1* genes confirmed the study species. The first time identified *O. asperum* along with morphometry and phylogeny will add value to the fact that nematodes are invisibly present with high prevalence in this country. This study will help to draw specific attention to command a practical control strategy for intervening in economic loss.

Keywords: *Oesophagostomum*, *ITS2*, *COX1*, morphology, morphometry, phylogeny

PS – poster session

Session: [SE10] Parasite infections in livestock and their control

Frequency of resistance to benzimidazoles of *Haemonchus contortus* helminths from dairy sheep, goats, cattle and buffaloes in Greece

Konstantinos Arsenopoulos[1], Styliani Minoudi[2], Isaia Symeonidou[1], Alexandros Triantafyllidis[2], Angeliki Katsafadou[3], Daphne Lianou[3], George Fthenakis[3], Elias Papadopoulos[1]

1 Laboratory of Parasitology and Parasitic Diseases, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
2 Laboratory of Genetics, Development and Molecular Biology, School of Biology, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
3 Veterinary Faculty, University of Thessaly, 43100 Karditsa, Greece

Abstract

The study investigated the presence of resistance to benzimidazoles in *Haemonchus contortus* helminths from ruminant species in Greece through the detection of the Phe/Tyr polymorphism in the amino acid at position 200 of the β-tubulin protein. In total, 288 adult female *H. contortus* helminths collected from the abomasum of various ruminant animals in Greece were tested. Of these, 96 were collected from sheep, 96 from goats, 48 from cattle and 48 from buffaloes. The frequencies of the homozygous and heterozygous resistant genotypes at the position 200 of the β-tubulin gene of helminths recovered from sheep were 96.9% and 3.1%, respectively. The frequencies of the homozygous and heterozygous resistant genotypes, respectively, were 100.0% and 0.0% in helminths from goats, 25.0% and 75.0% in helminths from cattle and 8.3% and 91.7% in helminths from buffaloes. In all parasitic populations, no homozygous susceptible genotypes were detected. The present study highlighted, for the first time, the emergence of benzimidazole-resistant *H. contortus* in goats, cattle and buffaloes in Greece, using an allele-specific PCR. It is postulated that benzimidazole-resistant alleles were transferred from sheep or goats to cattle and buffaloes at the commonly grazing pastures in Greece.

Keywords: allele-specific PCR, benzimidazole resistance, *Haemonchus contortus*, ruminants

PS – poster session

Session: [SE10] Parasite infections in livestock and their control

Assessing the efficacy of macrocyclic lactones in goat herds - Injectable vs pour on administration

Michal Babják, Alžbeta Königová, Marián Várady

Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, Košice, Slovakia

Abstract

The macrocyclic lactone eprinomectin (EPN) is the only anthelmintic drug with a zero-day milk withdrawal period approved for use in goats. The main objective of this study was to compare the efficacy of pour on form of EPN with the injectable version of ivermectin (IVM) on goat farms in Slovakia. The goats on six farms were divided into two groups of 10 animals each. The first group received a topical form of EPN at the recommended dose for sheep and goats (1 mg/kg body weight) and the second group was treated with injectable IVM at 1.5 fold higher dose (0.3 mg/kg body weight) than the recommended dose for sheep. On the other two farms, the goats were treated only with EPN. The secondary objectives were to compare the *in vivo* results with the data from the *in vitro* larval development test (LDT) and to examine the morphology of the third stage (L3) larvae of the gastrointestinal nematodes before and after treatment in each goat farm.

The faecal egg count reduction test detected efficacy for IVM and EPN from 80.8 to 93.6% and 51.0 to 96.6% respectively. A comparison of the different routes of administration showed a substantial higher percentage reduction of 20 to 30% in the groups treated with injectable IVM in three farms. Minimal differences in the efficacy were found on the other three farms (1 - 3%). The results of the LDT were evaluated using the minimum inhibitory concentration (MIC). Infectious L3 larvae were observed at concentrations equal to or higher than MIC 21.6 ng/mL in all farms. *Teladorsagia circumcincta* was the predominant species in 75% of the farms before treatment. *Haemonchus contortus* was the dominant species detected after treatment with the both anthelmintics in all farms. *T. circumcincta* was identified after treatment with EPN from 3 to 5 % on two farms. This is the first case of reduced efficacy of EPN in goat herds in Slovakia.

The study was supported by Grant Agency VEGA, Grant No. 2/0090/22.

Keywords: Anthelmintic resistance, goats, eprinomectin, ivermectin, *Haemonchus contortus*

PS – poster session

Session: [SE10] Parasite infections in livestock and their control

Sarcocystis species identification in the blood samples of wildlife and livestock

Dovilė Laisvūnė Bagdonaitė, Dalius Butkauskas, Naglis Gudiškis, Laima Baltrūnaitė, Neringa Kitrytė, Petras Prakas

Nature Research Centre, Vilnius, Lithuania

Abstract

Sarcocystis (Apicomplexa) parasites use various hosts to complete their life cycle. Over 200 species of *Sarcocystis* are known, forming cysts in muscle tissues of intermediate hosts. In definitive hosts, consumption of mature cysts completes the life cycle in the intestines, illustrating the reliance on the prey-predator ecological relationship.

Meat containing visible cysts must be condemned under the laws of the European Union, causing monetary loss in the husbandry industry. Limited data exists on *Sarcocystis* spp. in wild rodents, but some *Sarcocystis* species that employ rodents as intermediate hosts are either pathogenic or increase the risk of predation of affected animals.

Sarcocystis parasites are examined post-mortem using light or electron microscopy; supplemented with molecular research methods, such as amplification of *cox1, ITS1, 18S rRNA* and *28S rRNA* sequences. Identification of *Sarcocystis* spp. in blood samples could improve research methodology and enable the detection of the parasite in living organisms.

In the current study blood samples of 47 sheep (*Ovis aries*), 143 bank voles (*Clethrionomys glareolus*) and 214 yellow-necked mice (*Apodemus flavicollis*) from Lithuania were examined for *Sarcocystis.* Molecular detection of *Sarcocystis* was performed using nested PCR for *cox1* in sheep blood and both *cox1* and *28S rRNA* in rodent blood samples. *Sarcocystis* species were identified using Sanger sequencing of amplified PCR products. Blood samples from 79.6% of sheep, 6.3% of bank voles and 0.9% of yellow-necked mice were found to contain *Sarcocystis* spp. *S. arieticanis, S. tenella, S. capracanis* and *S. morae* were identified in sheep; whereas *S. myodes*, *S. glareoli* and *Sarcocytis* sp. were confirmed in the rodents.

The obtained results are important in understanding the prevalence and species diversity of *Sarcocystis* spp. in wild animals and livestock, as well as further development of molecular identification of *Sarcocystis* parasites in live animals.

Keywords: *Sarcocystis*, blood, sheep, rodents, molecular analysis

PS – poster session

Session: [SE10] Parasite infections in livestock and their control

Effective natural remedies for treating digestive parasitosis in pigs

Mihai Horia Baies, Zsolt Boros, Vasile Cozma, Marina Spinu

University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Cluj-Napoca, Romania

Abstract

Parasitic diseases greatly affect pig production, causing economic losses through high morbidity and mortality, particularly challenging for smallholders and organic producers. Phytotherapeutics are available and used worldwide, however evidence of their antiparasitic efficacy is currently very limited.

The objective of this study, conducted in a free-range Transylvanian farm, was to evaluate the effectiveness of *Allium sativum* and *Artemisia absinthium*, native to Romania flora, in combating naturally occurring digestive parasites in pigs.

A total of 180 faecal samples were obtained from three age groups: weaners, fatteners, and sows. Parasite identification and quantification of parasitic load were conducted using flotation (Willis and McMaster method), modified Ziehl-Neelsen stained faecal smears, centrifugal sedimentation, modified Blagg technique, and faecal cultures.

The examination identified infections with *Ascaris suum*, *Oesophagostomum* spp., *Trichuris suis*, *Eimeria* spp., and *Balantioides coli*, categorized by age group. Administering 180 mg/kg bw/day of *Al. sativum* L. and 50 mg/kg bw/day of *Ar. absinthium* L. powders for 10 consecutive days demonstrated significant, taxonomy-specific antiprotozoal and antihelmintic effects.

In summary, our findings suggest that *Al. sativum* and *Ar. absinthium* are promising alternatives to commercially available antiparasitic drugs, offering natural options for treating digestive parasites in swine.

The research was supported by the PPILOW project. The PPILOW project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°816172.

Keywords: *Allium sativum*, *Artemisia absinthium*, free-range farm, antiparasitic effect

PS – poster session

Session: [SE10] Parasite infections in livestock and their control

Detection of *Sarcocystis* species infecting domestic animals in Lithuanian livestock farms

Agnė Baranauskaitė, Petras Prakas, Elena Servienė, Živilė Strazdaitė-Žielienė

Nature Research Centre, Lithuania

Abstract

Members of the genus *Sarcocystis* are single-celled protists found in the natural environment that infect vertebrates, including humans. Parasites can enter the animals' bodies with food or water contaminated with sporocysts, therefore their monitoring is not only relevant for economic losses but for public health too. However, little is known about the distribution of these parasites in the environment. The aim of the study was to evaluate the prevalence of *Sarcocystis* parasites infecting livestock from environmental samples using a molecular-based method.

During the summer of 2023, water, hay, and soil samples were collected from twelve farms located throughout Lithuania. Samples of water and hay were concentrated using filtration method prior genomic DNA extraction, while soil samples were used for direct isolation of genomic DNA. Eight species infecting domestic animals were selected for detection using nested PCR targeting *cox1* gene.

The highest prevalence of *Sarcocystis* spp. was found in the hay (35%) and water (33%) samples, while the lowest number was detected in samples from soil (11%). Even though three (42%) different species were usually identified in individual farms, as many as six different species were found in one of them (8%). The most common *Sarcocystis* species in livestock farms were cattle-infecting *S. cruzi* (64%), less frequently identified in samples were *S. bovifelis* (31%), and *S. capracanis* (31%). Meanwhile, sheep-infecting *S. tenella* (6%) was the least detected, and cattle-infecting *S. hirsuta* was not detected at all.

Considering the obtained results, these parasites seem to be extremely common on some farms, posing a risk to animals, and potential consumers. Therefore, routine examinations on livestock farms and the detection of *Sarcocystis* are important to prevent animals and humans from becoming infected with these parasites.

This project has received funding from the Research Council of Lithuania (LMTLT), agreement No S-MIP-23-7.

Keywords: *Sarcocystis*, environment, molecular methods

PS – poster session

Session: [SE10] Parasite infections in livestock and their control

Calendula officinalis and *Satureja hortensis*, effective natural remedies for treating digestive parasitosis in pigs (*Sus scrofa* ssp. *domesticus*)

Zsolt Boros, Mihai-horia Băieş, Marina Spinu, Vasile Cozma

University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca,

Abstract

Parasitic diseases are found all over the world in humans and animals. Parasitic infections affect pig production and cause economic losses, especially for farmers and organic producers.

The objective of this study was to evaluate the effectiveness of *Calendula officinalis* and *Satureja hortensis*, in combating naturally occurring digestive parasitosis in swine, from a free-range farm in Cluj county, Romania.

The experimental protocol lasted for 60 days. The mentioned plants were administered for ten consecutive days (100 mg/kg bw/day of *S. hortensis* and 140 mg/kg bw/day of *C. officinalis* powders), followed by sample collections on days 14th and 28th. In total of 180 fecal samples were obtained from weaners, fatteners, and sows. The parasitic burden and identification were obtained by using sedimentation, flotation, McMaster, modified Blagg, and modified Ziehl-Neelsen methods.

The examination of the samples resulted in the identification of *Eimeria spp.*, *Balantioides coli*, *Ascaris suum*, and *Trichuris suis* infections. The antiprotozoal and anthelmintic effects increased by day 14, and reached maximum therapeutic effect by day 28.

The findings of this study suggest that *C. officinalis* and *S. hortensis* are promising natural alternatives for antiparasitic medication for treating gastrointestinal parasites in swine.

The research was supported by the PPILOW project. The PPILOW project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°816172.

Keywords: *Calendula officinalis*, *Satureja hortensis*, Romania, free-range farm, antiparasitic effect

PS – poster session

Session: [SE10] Parasite infections in livestock and their control

Echinococcus spp. circulating in livestock in Serbia

Katarina Breka[1], Nikola Betić[2], Jelena Karanović[3], Ivana Klun[4], Aleksandra Uzelac[4]

1 Faculty of Biology, University of Belgrade
2 Institute of Meat Hygiene and Technology
3 Institute of Molecular Genetics and Genetic Engineering, University of Belgrade
4 Institute for Medical Research, University of Belgrade

Abstract

The echinococcosis case burden in the human population of Serbia is among the highest in Southeastern Europe, but comprehensive data on the circulating tapeworm species is scarce. To gain insight into the population genetics of *Echinococcus* spp. in Serbia, the project WORM_PROFILER will collect samples from livestock intended for human consumption at major abattoirs, slaughterhouses and homesteads over the next two years. During the first two months of sampling, 22 livers from pigs (n=15), lambs (n=5) and calves (n=2) with clearly identifiable cysts and/or cyst-like formations as well as scar tissue were collected from major abattoirs around the country. The cyst content and/or affected area was sampled by direct isolation using pipette tips and/or excision of the tissue and analyzed microscopically, followed by quick DNA extraction by boiling in 0.02 M NaOH for 15 min and amplification of the *cox1* gene using primers which can differentiate *E. granulosus*, *E. multilocularis* and *E. canadensis* via conventional PCR. Several large cysts were present only in the liver of one boar. Tapeworm larvae were microscopically confirmed in the cysts' content and later identified by PCR as *E. granulosus*. PCR analysis additionally revealed the presence of *E. canadensis* gDNA in a tissue sample from another pig from a different farm. Although these results indicate that most carcasses flagged for conditional use based on liver examination by abattoir veterinarians are not infected by *Echinococcus* spp., they nonetheless suggest that pigs may play an important role in its transmission in Serbia. Sequencing of the *cox1* and *nad1* genes from the samples in which *Echinococcus* spp. gDNA was detected and is currently underway.

This research was supported by the Science Fund of the Republic of Serbia, #10841, Worm Profiler: Surveillance and population genetics of Echinococcus in Serbia - WORM_PROFILER.

Keywords: *Echinococcus* spp., livestock, liver, population genetics

PS – poster session

Session: [SE10] Parasite infections in livestock and their control

Parasites in domestic horses: analysis of the regional, age, and seasonal distribution of occurrence of horse parasites in Slovakia

Alžbeta Königová[1], Ľudmila Burcáková[1,2], Tetiana A. Kuzmina[1,3], Michal Babják[1], Marián Várady[1]

1 Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovak Republic
2 University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic
3 I. I. Schmalhausen Institute of Zoology NAS of Ukraine, Kyiv, Ukraine

Abstract

Equids are parasitized by more than 90 helminth species; thus, regular examination of the horse infection is important. We aimed to examine analysis of the regional, age, and seasonal distribution of the main groups of parasites in domestic horses in Slovakia. Faecal samples from 735 horses from 45 farms throughout Slovakia were studied in 2021–2023. Coprological McMaster and Mini-FLOTAC methods were used to examine the infection level (EPG, egg/gram) with Strongylidae nematodes, *Parascaris* spp., *Oxyuris equi*, *Strongyloides westeri*, tapeworms *Anoplocephala* spp., and protozoan *Eimeria leuckarti*.

Strongylid nematodes were found to be the main group of parasites in domestic horses; their prevalence (P) was 60.8%. A low level of infection (<200 EPG) was detected in 19.9% of horses, moderate infection (<200-500 EPG) in 14%, and high infection (>500 EPG) in 26.9%. Parascarids were detected in 3.1% of horses presumably of <4 or >15 years old; *O. equi* and *S. westeri* were not detected by both coprological methods. *Anoplocephala* spp. was detected in 0.95% of horses. Cysts of *Eimeria leuckarti* were found in 0.3% of horses. Distribution of horse infection between regions revealed that horses from Western and Eastern regions were most infected with strongylids. No statistically significant differences between strongylid infection levels were observed for horses from Western, Central, and Eastern Slovakia ($p>0.05$). Young horses (<4 years) were the most infected with strongylids (P=74.7%; 759.4 EPG); foals (<1 year) were the most infected with parascarids (P=20.7%; 920.8 EPG); tapeworms *Anoplocephala* spp. were found only in horses >4 years old (P=0.95%; 50 EPG); *Eimeria* cysts were detected only in foals (P=3.5%; 125 cysts/g). Statistically significant differences in prevalence and levels of horse infections with strongylids and parascarids were observed between seasons ($p<0.05$).

The study was funded by EU NextGenerationEU No. 09I03-03-V01-00015 and VEGA 2/0090/22.

Keywords: horse, parasites, coprological methods, prevalence, seasonal distribution

PS – poster session

Session: [SE10] Parasite infections in livestock and their control

Anticoccidial effect of *Tanacetum vulgare* extract lyophilizate in naturally infected lambs with *Eimeria* spp.

Alīna Kļaviņa[1], Aīda Vanaga[2], Dace Keidāne[1], Ivars Lūsis[1], Aija Mālniece[2], Kristīne Ganola[1], Līga Kovaļčuka[2]

1 Latvia University of Life Sciences and Technologies, Faculty of Veterinary Medicine, Institute of Food and Environment Hygiene, Jelgava, Latvia
2 Latvia University of Life Sciences and Technologies, Faculty of Veterinary Medicine, Clinical Institute, Jelgava, Latvia

Abstract

Eimeriosis is one of the common parasitosis in young animals, especially lambs. It causes significant economic losses in sheep farming. In addition, the resistance of various drug groups, including anticoccidial drugs, is relevant nowadays. One of the another anticoccidial control strategy could be phytotherapy. The aim of the study is to find out the anticoccidial effect of *T. vulgare* extract lyophilizate in lambs.

A total of 24 Latvian dark head breed lambs aged 4-5 months, tested positive for *Eimeria* spp. infection were selected and randomly divided into four groups (A, B, C, D) of 6 animals in each group. Group A, B were administrated intraruminal boluses containing lyophilizate of *T. vulgare* extract in different compositions. Group C was received pallets of the same extract and group D was negative control. The research was conducted under the approval of the Committee for the Protection of Animals Used for Scientific Purposes of the Food and Veterinary Service of Republic of Latvia (No. 143/2023, 08.06.2023). Feacal oocyst counts were performed on 0 day, 14 days, 28 days, 42 days and 56 days and was calculated feacal oocyst count reduction.

The intraruminal bolus groups had stronger anticoccidial effect than the pallets group. The mean feacal oocyst reduction on 56

days were 85% (group A), 92% (group B), 72% (group C). On the other hand, the strongest efficiency was in group B, whose results are equivalent to the negative control group (93%).
The obtained results show that *T. vulgare* extract has no pronounced anticoccidial effect and it would be necessary to investigate the effect of the others factors on *Eimeria* spp. in this study.

The project is supported by Ministry of Agriculture of Latvia and the Rural Advisory Service of Latvia, project No. 22-00-A01612-000007 "Production of medication form of extract from tansy leaves, Latvian traditional medicinal herb, and its impact on microbiot of sheep digestive tract and antiparasitic control".

Keywords: anticoccidial, tansy, sheep

PS – poster session

Session: [SE10] Parasite infections in livestock and their control

Molecular detection of parasites in fecal DNA of Polish Konik horses

Agnieszka Perec-Matysiak[1], Katarzyna Buńkowska-Gawlik[1], Kateryna Slivinska[2,3], Joanna Hildebrand[1]

1 *University of Wrocław, Faculty of Biological Sciences, Department of Parasitology, Poland*
2 *Department of Parasitology Museum and Institute of Zoology Polish Academy of Sciences Twarda 51/55, 00-818 Warsaw, POLAND*
3 *I. I. Schmalhausen Institute of Zoology National Academy of Sciences of Ukraine Khmelnytskoho 15, 02000 Kyiv, UKRAINE*

Abstract

The Polish Konik horse is a breed of horse that originated as a result of a project initiated in Poland in the 1920s with the specific aim of recreating horses that resembled tarpans. The objective of this study was to estimate the prevalence of intestinal microparasites and nematodes in Polish Konik horses based on fecal samples using molecular methods. The study of Polish Konik horses was conducted at two stud farms with different management strategies in two provinces of Poland: the Warmian-Masurian province (Popielno Research Station) and the Podlasie province (Biebrza National Park). A total of 38 Polish Konik horses were included in this study during November-December 2022. All horses exhibited natural levels of parasite infections and had not received any anthelmintic treatment for at least seven months prior to the study. The classical PCR and nested PCR methods were employed in the study, and the resulting products were sequenced. Five species of small strongyles were identified in the material studied. The analysis of the sequences obtained demonstrated that *Cylicocyclus nassatus* was the most prevalent among the Cyatostominae. Among the microparasites, the presence of protozoans of *Cryptosporidium* spp., and the representatives of family Eimeriidae as well as the microsporidian *Encephalitozoon* spp. was confirmed. No *Giardia* spp. DNA was found in the samples tested. The significant prevalence of identified parasites in Polish Konik horses suggests the need of testing and routine monitoring of parasites in this host group.

The study was funded by the MSCA4Ukraine project (AvHID 1233593; https://cordis.europa.eu/project/id/101101923).

Keywords: Polish Konik horse, Cyatostominae, *Cryptosporidium* spp., *Encephalitozoon* spp.

PS – poster session

Session: [SE10] Parasite infections in livestock and their control

Benzimidazole-resistant *Haemonchus contortus* in captive European bison and alpacas in Poland; initial study

Anna Maria Pyziel[1], Marta Gałązka[1], Blanka Orłowska[1], Anna Rodo[2], Anna Trojakowska[3], Dagmara Sierociuk[4]

1 *Department of Food Hygiene and Public Health Protection, Institute of Veterinary Medicine, Warsaw University of Life Sciences – SGGW, Warsaw, Poland*
2 *Department of Pathology and Veterinary Diagnostics, Institute of Veterinary Medicine, Warsaw University of Life Sciences – SGGW, Warsaw, Poland*
3 *Veterinary Clinic, Poland*
4 *Alpawet Specialised Veterinary Clinic, Jabłonna, Poland*

Abstract

A blood-sucking abomasal nematode, *Haemonchus contortus*, is considered to be one of the great pathogenic and economic importance in ruminants worldwide. Heavily infected animals can suffer from haemorrhagic anaemia, which can lead to death. Moreover, *H. contortus* quickly develops resistance to anthelmintics.
The aim of the study was to test the worms isolated from the abomasa of death European bison and alpacas for benzimidazole resistance.
The PCR was used to obtain a product of ~ 750 bp from the isotype-1 β-tubulin gene of *H. contortus* and to compare the results with the GenBank data for benzimidazole-susceptible and benzimidazole-resistant isolates.
The worms were found in 5 of 12 dissected alpacas (prevalence = 41.6%; intensity of infection=1-22 worms). Additionally, worms isolated from 3 individuals of European bison were included into the study. Two of European bison were captive individuals (intensity of infection with *H. contortus*=45 and 51), whereas one of the animals was a free-roaming cow (intensity of infection with *H. contortus*=5).
The study revealed that all isolates of *H. contortus* from alpacas and captive European bison were resistant to benzimidazole, whereas the one isolated from free-roaming European bison was susceptible.

Keywords: *Haemonchus contortus*, benzimidazole-resistance, European bison, alpaca

PS – poster session

Session: [SE10] Parasite infections in livestock and their control

Antiparasitic effect of a tansy (*Tanacetum vulgare* L.) extract containing intraruminal bolus and pallets against gastrointestinal nematodes (Trichostrongylidae) in sheep *in-vivo.*

Aīda Vanaga[1], Alīna Kļaviņa[2], Dace Keidāne[2], Ivars Lūsis[2], Aija Mālniece[1], Kristīne Ganola[2], Līga Kovaļčuka[1]

1 *Latvia University of Life Sciences and Technologies, Faculty of Veterinary Medicine, Clinical Institute, Jelgava, Latvia*
2 *Latvia University of Life Sciences and Technologies, Faculty of Veterinary Medicine, Institute of Food and Environment Hygiene, Jelgava, Latvia*

Abstract

Small ruminants, especially sheep and goats are sensitive to various parasitic infections. Since antiparasitic resistance to synthetic deworming agents is relevant nowadays, it is essential to evaluate the use of natural remedies - traditional medicinal herb in the prevention of parasitic infections in animals.
30 Latvian dark head breed sheep of age 4-5 months was randomly divided in 5 (A-E) groups for experiment in controlled environment. On a day 0 of experiment - A and B group received a 3D printed intraruminal bolus filled with tansy extract lyophilizate in different composition, C group received tansy pallets for 40 days, D group - negative control and E group positive control sheep which was dewormed with levamisole 5mg/kg. On day 56, the study ended with humane euthanasia of the animals. The faecal egg count reduction test was applied to evaluate the effec-

tiveness of the tansy extract. The research was conducted under the approval of the Committee for the Protection of Animals Used for Scientific Purposes of the Food and Veterinary Service of Republic of Latvia (No. 143/2023, 08.06.2023).

The highest efficiency was observed in the E group – FECRT - 90%, in A, B, C groups efficiency was 74%, 71% and 78%, respectively. In clinical examination, blood hematological and biochemical examination no significant differences were observed. In this study tansy extract showed positive influence to decrease parasitic infection in sheep, especially in the form of pellets. Tansy extract can be used as an alternative to control parasitic infection in sheep, but more studies in naturally infected sheep in field conditions is needed.

The project is supported by the Ministry of Agriculture of Latvia and the Rural Advisory Service of Latvia, project no. 22-00-A01612-000007 " Production of medication form of extract from tansy leaves, Latvian traditional medicinal herb, and its impact on microbiot of sheep digestive tract and antiparasitic control".

Keywords: Trichostrongylidae, tansy, sheep

PS – poster session

Session: [SE10] Parasite infections in livestock and their control

Transgenic plants as a platform for obtaining virus like particle-assembled vaccine optimized for oral delivery for fasciolosis control

Kacper Karczmarzyk[1], Martyna Anna Przewoźnik[2], Emilia Mroczko[2], Jerzy Kozłowski[2], Hanna Pudelska[2], Tomasz Pniewski[2], Małgorzata Kęsik-Brodacka[1], Agnieszka Wesołowska[3]

1 National Medicines Institute, Warsaw, Poland
2 Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Poland
3 Museum and Institute of Zoology, Polish Academy of Sciences, Warszawa, Poland

Abstract

Many pathogens, including *Fasciola hepatica*, invade through mucosal surfaces. Hence, the best vaccination strategy is to target the mucosal surface, since it would reduce the pathogen's ability to establish infection. Mucosal delivery is often superior to injection in protecting mucosal sites.

Here, we developed a vaccine antigen that will be delivered orally to sheep. The vaccine antigen consists of plant-derived HBc-CPFhW, formed by cysteine proteinase of *F. hepatica* (CPFhW) fused to a carrier - Hepatitis B core Antigen (HBcAg). It assembles into virus-like particles (VLPs) which are known to enhance immune responses to displayed antigens. Moreover, VLPs are more resistant to digestion than soluble antigens in the intestine.

HBcCPFhW was expressed in lettuce using two vectors both optimized for codon usage in lettuce: (1) pLsBAR-gLsHBcCPFhW encoding HBcCPFhW and (2) pLsBAR-gLsHBcCPFhW-HBc encoding CPFhW fused to unaltered HBcAg. In the first case, transgenic lettuce generated HBcCPFhW in the form of ‚monoepitopic' VLPs, where each VLP monomer displayed attached CPFhW, while in the latter expressed heterodimers were assembled into both chimeric and mosaic VLPs, assumed to be more stable. Also, the reference VLPs formed by unaltered HBcAg were obtained.

The VLPs expression in plants varied from several hundred ng/g of fresh weight (FW) for HBcCPFhW-HBc, ≈1 µg/g FW for HBcCPFhW, to several µg/g FW for HBcAg depending on the protein complexity. Lyophilisation of plant material increased their concentration ≥ 10x. Hence, this platform is a viable alternative for vaccine antigens production.

The effectiveness of immunization with plant-associated and VLP-assembled HBcCPFhW and HBcCPFhWHBc optimized for oral delivery will be verified. The idea of mucosal vaccination against helminths is a relatively new concept. Any contribution in that field is highly awaited.

Research funded by National Science Centre, Poland, UMO-2019/35/B/NZ6/04002.

Keywords: *Fasciola hepatica*, oral vaccination, virus-like particles, livestock

PS – poster session

[SE11]
Parasite omics

Session: [SE11] Parasite omics

Evolutionary genomics of *Cryptosporidium parvum* in Europe

Simone Mario Caccio

Istituto Supeiore di Sanita, Rome, Italy

Abstract

The genus *Cryptosporidium* (Apicomplexa) encompasses over 40 different species, affecting multiple vertebrate hosts. In humans, *C. hominis* and *C. parvum* are the main pathogens, with a global distribution. While *C. hominis* is essentially adapted to humans, *C. parvum* infects both humans and ruminants. In recent years, many important aspects of the biology have been examined by the comparative analyses of whole genome sequence (WGS) data. Here, I focus on the evolution of *C. parvum* in Europe, based on WGS data from 123 human- and ruminant-derived isolates collected in 13 European countries, and included 72 additional data from Europe, Egypt, China and the USA (n=72) retrieved from published studies. Rigorous filters were applied to exclude mixed infections, resulting in a final dataset of 141 isolates. A total of 28,047 high-quality SNPs were identified and used to infer phylogenetic relationships, investigate the population structure, population genetics and recombination.

Three distinct and strongly supported populations were identified: population 1 (China and Egypt), population 2 (a minority of European isolates), and population 3 (most European isolates and all isolates from the USA). The common origin of all those populations dates back to around 10,000 years ago, thus overlapping with cattle domestication in the Middle East.

Population 3 has emerged more recently from population 2 and expanded throughout Europe and reached the USA, possibly after a single introduction event from the UK. As compared to population 2, population 3 is more frequently involved in epidemic outbreaks. Although the reason(s) for this successful spread of population 3 remain elusive, genes under selective pressure uniquely in this population were identified.

The results obtained highlight the role of cattle and other livestock as vehicles for the geographical spread of *C. parvum*, and possibly as determinants for its genetic diversification.

Keywords: *Cryptosporidium*, genomics, evolution, Europe

OS - poster session

Session: [SE11] Parasite omics

Deciphering the role of Calcium-Dependent Protein Kinases in the lifecycle dynamics of *Babesia divergens*

Ana Maria Filipe[1], Alper Dede[1], Adela Chlastakova[1], Viktoriya Levytska[2], Zuzana Cisarova[1], Tereza Zirovnicka[1], Tereza Kreckova[1], Daniel Sojka[2], Marie Jalovecka[1]

1 Department of Parasitology, Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic

2 Laboratory of Molecular Biology of Ticks, Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Ceske Budejovice, Czech Republic

Abstract

Babesia divergens, a tick-transmitted parasite found in Europe, is an economically important agent of bovine babesiosis and poses a serious threat to human health as a zoonosis. Its complex life cycle is coordinated by calcium signaling pathways, with Calcium-Dependent Protein Kinases (CDPKs) representing vital molecules orchestrating host-parasite-vector interactions. Due to the limited understanding of individual *B. divergens* CDPKs (BdCDPKs) roles in the parasite life cycle, our study aims to conduct an unbiased screen of yet unrevealed BdCDPKs and identify their association in fundamental biological processes crucial to the parasite's progression. We monitored dynamic expression profiles of individual BdCDPKs throughout the intra-erythrocytic and intra-tick stages of *B. divergens*, indicating distinct roles for BdCDPKs within each host environment. Particularly, our attention is drawn to BdCDPK5 due to its predominant expression during the parasite's passage within the tick, suggesting its involvement in regulating this phase of the parasite's development. Using a newly established platform of *B. divergens*-specific transgenic techniques, we successfully generated BdCDPK5 knock-out and knock-in *B. divergens* parasites and are currently in the process of generating the clonal lines. Both lineages will be used to characterize of this enzyme's role in the intra-erythrocytic and intra-tick life cycles, with particular emphasis on the transmission of the parasite to the vector.

This work was supported by the grant Czech Science Foundation (GA CR) project No. 21-11299S.

Keywords: *Babesia divergens*, Calcium-Dependent Protein Kinases, expression profile, life cycle, functional genomics

OS – oral session

Session: [SE11] Parasite omics

In-depth comparative proteomic analysis of *G. duodenalis* secreted vesicles

Marco Lalle[1], Sofia Moyano[2]

1 Department of Infectious Diseases, Istituto Superiore di Sanità, 00161 Rome, Italy

2 Instituto de Investigación Médica Mercedes y Martín Ferreyra, Consejo Nacional de Investigaciones Científicas y Técnicas (INIMEC-CONICET), Universidad Nacional de Córdoba, Córdoba 5016, Argentina

Abstract

The parasitic protist *Giardia duodenalis* causes in human and animal giardiasis, a diarrheal disease.Two *G.duodenalis* genetic groups (Assemblages A and B) cause human infection. High variability in the range of giardiasis symptoms is well documented and eventually linked to differences between and within Assemblages. Extracellular vesicles (EVs) operate as cargoes from cell to cell, for proteins and nucleic acids being implicated in physio-pathological processes. Here we characterize and compare EV proteomes from assemblage A and B. Released EVs, as well freely secreted proteins, were purified from spent medium by serial ultracentrifugation and morphologically and biochemically characterized by EM and DLS. EVs and secretome protein content were identified by HR-MS and datasets of relative abundance analyzed for protein-protein interaction networks and functional enrichment. Over 20% of *Giardia's* annotated proteome were identified extracellularly and ~4-fold more proteins than previous reports were quantifying. We unveiled a conserved extracellular proteome but with distinct signatures in assemblages A and B, consistent with variant pathophysiology and virulence. We mapped distinct protein signatures of *Giardia* EV sub-populations (i.e. exosomes and micro-vesicles) including (e.g. ribosome-mediated translation, cilium, vesicular trafficking, and exosome secretion). Mechanistic markers of ESCRT-dependent, ESCRT-independent, and Ectosomes pathways within the EV proteome were also identified. By exploring "conserved" and "eukaryotic-innovative" compositions in *Giardia* EV proteome relative to archaea EV proteome, we observed complex cellular processes providing insights into the origins of eukaryotic intercellular communication. Collectively, our findings advanced current comprehension of *Giardia's* protein release dynamics, establishing novel correlations between different *Giardia* Assemblages and diverse extracellular vesicle subtypes.

Keywords: *Giardia duodenalis*, Extracellular vesicles (EVs), High-Resolution Proteomics

OS – oral session

Session: [SE11] Parasite omics

First evidence of a quorum sensing mechanism in *Giardia duodenalis* linked to 14-3-3 protein polyglycylation level

Marco Lalle[1], Serena Camerini[2], Anna Maria Salzano[3], Melanie Marlow[4], Serena Cecchetti[2], Janet Yee[4]

1 Unit of Foodborne and Neglected Parasitic Diseases, Dep. Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy
2 Core Facilities Technical-Scientific Service, Istituto Superiore di Sanità, viale Regina Elena 299, Rome, Italy
3 Institute for the Animal Production System in the Mediterranean Environment, National Research Council, Naples, Italy;
4 Department of Biology, Trent University, Peterborough, Ontario, Canada

Abstract

The highly conserved dimeric 14-3-3s are a family of eukaryotic proteins that regulates a plethora of cellular processes by interacting with hundreds of client proteins via the recognition of conserved Ser/Thr phosphorylated binding motifs. The flagellated protozoan *Giardia duodenalis* encodes for a single 14-3-3 isoform constitutively phosphorylated at Thr214. In addition, the C-terminal Glu246 is subject to polyglycylation (polyGly), a polymodification consisting of the addition of multiple glycine residues to the γ-carboxyl group of the glutamate. PolyGly length affects the nuclear localization of g14-3-3 and encystations timing. Here we further explore how 14-3-3 and its PTMs levels change in *G. duodenalis* Assemblages A, B and E during parasite proliferation and cell-cycle. Variations of 14-3-3 polyglycine chain length was detected in *Giardia* trophozoites throughout 72 h of growth and confirmed by 14-3-3 affinity chromatography and MALDI-TOF analysis. PTMs alteration correlated with differential gene expression of the polyglycylase and deglycylase enzymes. Using counterflow centrifugal elutriation and verification by flow cytometry to separate cell cycle stages, no changes were detected in the level of the higher polyglycylated vs the lower polyglycylated form of 14-3-3 during cell cycle progression in log phase *Giardia* culture. Intriguingly, cell density, and to a less extent medium depletion, affected 14-3-3 expression and PTMs level. The results presented herein suggest that in *Giardia* trophozoites regulation of g14-3-3 polyglycylation might have an additional role being part of a not yet defined transduction mechanism integrating both nutrient and quorum sensing. Indeed, g14-3-3 with long polyglycine chain are present in cell approaching the stationary phase as well as cell growing at high density. Our observations clearly indicate that, as in bacteria and other protozoa, a quorum sensing mechanism might occur also in *Giardia* and deserve further studies.

Keywords: *G. duodenalis*, 14-3-3 proteins, polygycylation, quorum sensing, counterflow centrifugal elutriation

OS – oral session

Session: [SE11] Parasite omics

Canonical and non-canonical nuclear genetic codes: genomics of Blastocrithidiinae

Kristína Pašuthová[1], Zoltán Füssy[2], Amanda T.S. Albanaz[1], Anzhelika Butenko[1], Ambar Kachale[3], Natalya Kraeva[1], Arnau Galan[1], Jan Votýpka[3], Alexei Y. Kostygov[1], Leoš Shivaya Valášek[4], Zdeněk Paris[3], Julius Lukeš[3], Vyacheslav Yurchenko[1]

1 Life Science Research Centre, Faculty of Science, University of Ostrava, Ostrava, Czechia
2 Scripps Institution of Oceanography, University of California San Diego, La Jolla, USA
3 Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czechia
4 Institute of Microbiology, Czech Academy of Sciences, Prague, Czechia

Abstract

Trypanosomatids are one of the best studied groups of protists owing to their parasitic lifestyle infecting primarily insects, but humans, livestock, and plants as well. Recently, a departure from the canonical genetic code was described in a single trypanosomatid species. In *Blastocrithidia nonstop*, all three stop codons were reassigned to code for amino acids (UAA and UAG for glutamate, UGA for tryptophan), with UAA also specifying translation termination. UAA and UAG are decoded by cognate tRNAs that perfectly match the anticodon. On the other hand, UGA is recognized by a specific tRNATrpCCA with uniquely shortened anticodon stem from five to four base pairs increasing readthrough over this stop codon. To investigate the features of this genetic code, we sequenced genomes of three more *Blastocrithidia* species and four *Obscuromonas* species that represent a sister lineage to *Blastocrithidia* utilizing a canonical genetic code. The results show that the common ancestor of *Blastocrithidia* spp. already had a GC-poor genome with all three stop codons reassigned and only UAA employed as a genuine stop. Although the specific mechanism how the translation is terminated still remains unknown, UAA is significantly enriched after the genuine stop in open reading frame of the protein, while no such distribution was observed in *Obscuromonas* spp. This study allowed us to perform across-genome comparative analysis of trypanosomatids with the reassigned and canonical nuclear genetic code.

Keywords: genetic code, *Blastocrithidia*, trypanosomatids, in-frame stop codons, genomics

OS – oral session

Session: [SE11] Parasite omics

What can parasites tell us about their hosts: insight from population genomics of seal lice

Ludmila Sromek[1], Kevin P. Johnson[2], Mervi Kunnasranta[3], Eeva Ylinen[3], Stephany Virrueta Herrera[2], Elena Andrievskaya[4], Vyacheslav Alexeev[4], Olga Rusinek[5], Aqqalu Rosing-Asvid[6], Tommi Nyman[7]

1 University of Gdansk, Gdynia, Poland
2 University of Illinois, Champaign, USA
3 University of Eastern Finland, Joensuu, Finland
4 The Baltic Ringed Seal Foundation, St. Petersburg, Russia
5 Baikal Museum of Siberian Branch of the Russian Academy of Sciences, Listvyanka, Russia
6 Greenland Institute of Natural Resources, Nuuk, Greenland
7 Norwegian Institute of Bioeconomy Research, Svanvik, Norway

Abstract

Genetic analyses of host-specific parasites can be used as a unique marker to investigate the ecology and evolutionary history of their host. Here, we studied population genomics of seal louse *Echinophthirius horridus* parasitizing on different seals species and populations from the Arctic Ocean, the Baltic Sea, and relict landlocked ringed seal subspecies in lakes Saimaa and Ladoga. Our analysis revealed that the overall pattern of genome-wide variation within and among seal louse populations is strikingly similar to that found in their seal hosts, and that effective population sizes of lice reflect the census sizes of their respective seal host populations. At the same time, seal louse genomes offered improved resolution with respect to the timing and sequence of divergence events. Historical trajectories of population sizes differed markedly among louse populations associated with gray vs. ringed seals, with apparent links to Pleistocene and Holocene climatic variation as well as to the isolation histories of ringed seal subspecies. Interestingly, our analyses also revealed ancient gene flow between the lice of Baltic gray and ringed seals, demonstrating how parasite genomes can retain signs of alterations in distributions that do not result in hybridization in the hosts and are therefore impossible to detect using host genomics.

Keywords: Echinophthiriidae, marine mammals, host-

associated genetic differentiation, demographic history, phylogeography

OS – oral session

Session: [SE11] Parasite omics

Examples of the unravelling of genetic diversity in common intestinal parasitic protists of human and non-human hosts using amplicon-based next-generation sequencing

Christen Rune Stensvold

Department of Bacteria, Parasites and Fungi, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark

Abstract

Molecular characterisation of parasites is useful for epidemiological surveillance and for identification of lineages that differ in prevalence among healthy and diseased individuals and for identification of transmission patterns. Lately, the advantages of screening for genetic variation among common single-celled intestinal parasites using metabarcoding has proved its worth. Here, shorter fragments (a few hundred base pairs) of small subunit ribosomal RNA (SSU rRNA) genes are sequenced using next-generation sequencing, and consensus sequences can be generated from raw sequence outputs using DNA sequence clustering tools and be subject to phylogenetic analysis. The method can in some instances be sufficient for establishing new species/ribosomal lineages.

At Statens Serum Institut, amplicon-based NGS using three different primer pairs targeting nuclear ribosomal (18S) genes is regularly applied to faecal DNAs from human and non-human hosts. After ILLUMINA-based sequencing of partial 18S genes, data are automatically annotated to taxonomic level using the in-house software BION. Fasta files for all organisms detected can be retrieved manually and subject to analysis, including multiple sequence alignment and phylogeny.

By using this method, we have identified new subtypes/ribosomal lineages of most common intestinal parasites and we have also many examples of mixed subtype colonisation of e.g., *Blastocystis*—information that is easily obtained by NGS but that previously would require expensive and time-consuming Sanger sequencing of cloned PCR products.

Metabarcoding is a powerful tool for screening for genetic diversity in common intestinal parasites and for detection of mixed subtype colonisation. However, near-complete SSU rDNA sequences should be obtained for potentially new lineages whenever possible for more robust phylogenetic inferences.

Keywords: next-generation sequencing, microbiome, microbiota, 18S, gut, protists

OS – oral session

Session: [SE11] Parasite omics

Blastocrithidia nonstop – functional analysis of genomic predictions

Ingrid Sveráková[1], Kristína Záhonová[1], Vyacheslav Yurchenko[1], Katarína Benediková[2]

1 University of Ostrava, Czech Republic
2 Comenius University, Bratislava, Slovak Republic

Abstract

Blastocrithidia nonstop is a protist of the family Trypanosomatidae famous for the alteration of the canonical genetic code, i.e., it recoded it's all three stop codons to code for amino acids. Nevertheless, this genetic departure had no effect on glycosomal, mitochondrial, or cytosolic metabolism containing all typical features of these diverse and important parasites. The mitochondrial genome of *B. nonstop* is predicted to encode the same set of genes that is present in most other trypanosomatids. Moreover, the nucleus-encoded subunits of complex I (NADH:ubiquinone oxidoreductase), complex II (succinate dehydrogenase), complex III (ubiquinone:cytochrome c oxidoreductase), complex IV (cytochrome c oxidase), complex V (FoF1 ATPase) are all present, as well as cytochromes b, c, and c1. However, the close insight into function of biochemical pathways showed unique regulation of mitochondrial gene expression that leads to loss of respiratory complex I. Despite the predicted absence of enzymes involved in the biosynthesis of quinoid ring structure (UbiE, UbiF, UbiG and UbiH) of ubiquinone and its prenyl-side chain (solanesyl-diphosphate synthase), the substrate stimulated oxygen consumption appeared intact and sensitive to malonic acid (inhibitor of complex II), although the overall respiration is very low.

Keywords: Trypanosomatidae, genetic code, mitochondria

OS – oral session

Session: [SE11] Parasite omics

Fully resolved phylogeny of Trypanosomatidae

Vyacheslav Yurchenko, Alexei Kostygov

University of Ostrava, Czechia

Abstract

The number of sequenced trypanosomatid genomes has reached a critical point so that they are now available for almost all genera and subgenera. Based on this, we inferred a phylogenomic tree and propose it as a framework to study trait evolution together with some examples of how to do it.

Keywords: Trypanosomatidae, phylogenomics, trait evolution

OS – oral session

Session: [SE11] Parasite omics

The transcriptome profile of L3 larvae of *Anisakis simplex* s.s. with differentiated antihelminthic treatment

Elzbieta Lopienska-Biernat[1], Iwona Polak[1], Mateusz Mazdziarz[2], Lukasz Paukszto[2]

1 University of Warmia and Mazury in Olsztyn, Poland
2 Department of Botany and Evolutionary Ecology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Poland

Abstract

Significant changes in natural and social factors, changes in lifestyle and diet affect the spread of parasitic diseases and pose new challenges for the prevention, control, clinical diagnosis and treatment of these threats. Nematodes of the genus *Anisakis* parasitize marine mammals, and in humans the larvae cause a disease called anisakiasis. The main source of infection is food containing raw fish or shellfish. Due to the recently observed treatment resistance, there is a great need for new classes of antiparasitic agents with novel mechanisms of action. The aim of this study is to identify transcriptomic changes in L3 larvae of *A. simplex*, with particular emphasis on DEG and lncRNA under the influence of the drugs albendazole (A), ivermectin (I) and pyrantel (P). High-throughput sequencing (Illumina) and bioinformatics were used to achieve this goal. To understand the molecular functions of the identified DEGs (A66; I50; P30), enrichment analysis using Gene Ontology (GO) was performed, e.g. structural cuticle among A and I and metallopeptidase pathways upon treatment with P. Up-regulation of expression in A treatment: Kunitz-type putative protease inhibitor, in the I group, O-acyltransferase and metalloproteinase and BPI2 domain-containing protein in the P treatment. In addition, we identified lncRNAs (A10; I10; P7) that are mainly expressed to regulate the expression of other genes, especially in the A

treatment lncRNA that overlaps with the N-domain-containing protein of the collagen cuticle. The results obtained should lead to a better understanding of the molecular processes underlying the response of *A. simplex* to drugs and will add to the existing knowledge of the transcriptome changes of this parasite.

This work was supported by the National Science Centre of Poland, grants no. 2018/31/B/NZ9/01683 and 2020/37/N/NZ9/03312.

Keywords: Nematoda, transcriptome, lncRNA, anthelmintics

PS – poster session

Session: [SE11] Parasite omics

Transcriptomic plasticity of the Antarctic parasite *Contracaecum osculatum* D (Nematoda: Anisakidae) in response to thermal stress exposure

Marialetizia Palomba[1], Franco Liberati[1], Veronica Rodriguez Fernandez[2], Xavier Roca-Geronés[3], Armando Macali[1], Tiziana Castrignanò[1], Daniele Canestrelli[1], Simonetta Mattiucci[2]

1 Department of Ecological and Biological Sciences, Tuscia University, Largo dell'Università s.n.c. 00100 Viterbo, Italy

2 Department of Public Health and Infectious Diseases, Sapienza-University of Rome Piazzale Aldo Moro, 5 00185 Rome, Italy

3 Secció de Parasitologia, Departament de Biologia, Sanitat i Medi Ambient, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona, 08028 Barcelona, Spain

Abstract

Marine parasites, with complex life cycles, involving free-living larval stages, ectothermic intermediate hosts, and homeothermic definitive hosts, offer compelling systems for studying thermal adaptation (Wharton et al., 1999). Transcriptomic modulation is a key mechanism enabling these parasites to adjust gene expression and physiological processes in response to thermal variations (Pfenning et al., 2010). However, the interplay between molecular responses and environmental thermal stimuli in parasites remains poorly understood. *Contracaecum osculatum* sp. D, is an Antarctic marine parasite, whose life cycle involves free-living larval stages, L3 stages in ectothermic invertebrates and icefish hosts and, finally, adult stages in homeothermic in a pinniped host (*Leptonychotes weddellii*). This study explores the transcriptomic profile of *C. osculatum* sp. D to uncover molecular mechanisms enabling thermal tolerance. It aims to identify genes associated with thermal adaptation and investigate their expression patterns under different thermal conditions.

L3 of *C. osculatum* D were obtained from the ice fish in the Ross Sea during the XXXV Italian expedition. L3 were cultured *in vitro* at −2 °C, 1 °C, 37 °C (1min, 24h) to simulate temperature conditions experienced by parasites.

RNA was extracted and sequenced by NovaSeq 100PE. Bioinformatic analyses were performed.

A *de novo* transcriptome was generated. Differential expression analysis revealed genes linked to heat shock proteins, chaperones, and other stress responses, indicating activation of cellular defence pathways under thermal stress. Changes in metabolic gene expression suggested cellular reprogramming to cope with temperature fluctuations. These results provide new insights into the parasite's mechanisms for thermal adaptation and advance our understanding of how they manage temperature shifts during their life cycle.

Granted by Italian MUR-PNRA2019_125.

Keywords: *Contracaecum osculatum* sp. D, Antartic parasite, thermal stress, RNAseq

PS – poster session

Session: [SE11] Parasite omics

De Novo transcriptome assembly of the anisakid nematode *Contracaecum osculatum* (s.s.) from the Baltic Sea: implications in studies on its zoonotic role and ecological aspects

Marialetizia Palomba[1], Franco Liberati[1], Tiziana Castrignanò[1], Robert Strinsky[2], Daniele Canestrelli[3], Elżbieta Łopieńska-Biernat[2], Simonetta Mattiucci[4]

1 Department of Ecological and Biological Sciences, Tuscia University, Largo dell'Università s.n.c. 00100 Viterbo, Italy

2 Department of Biochemistry, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Oczapowskiego 1A Str., 10-719, Olsztyn, Poland

3 Department of Ecological and Biological Sciences, Tuscia University / Viterbo

4 Department of Public Health and Infectious Diseases, Sapienza-University of Rome - Piazzale Aldo Moro, 5 00185 Rome, Italy

Abstract

Marine metazoan parasites play pivotal roles in marine biodiversity, influencing various aspects of host biology and serving as ecological indicators of ecosystem health (Timi et al., 2020). Among these, the Baltic nematode parasite *Contracaecum osculatum* (s.s.) exhibits complex life cycle involving grey seals and fish species, particularly cod, making it a potential indicator of Baltic marine trophic web stability. Despite historical correlations between this parasite species density and fluctuations of hosts population (Buchmann, 2023), its role as an ecological indicator has been overshadowed by concerns over zoonotic risks. This study aims to provide, for the first time, the transcriptome data of the Baltic sibling species *C. osculatum* (s.s.), to enhance the knowledge on its zoonotic role, as well as exploring its utility as an ecological sentinel for the stability of the Baltic trophic web. Genetically identified *C. osculatum* (s.s.) third-stage larvae (L3) were extracted from the body cavity of the Baltic cod (*G. morhua callarias*) caught near Władysławowo, Poland. RNA was extracted and paired-end sequenced. Bioinformatic analyses were performed. The analysis generated 86830895 reads, which were assembled and then annotated. The high-quality assembly was validated, achieving over 88.6 mapping against Eukariota database and 71.1% against Nematoda. Orthogroup detection identified over 17% (7376) of transcript sequences as putative orthologs shared among *A. pegreffii*, *A. simplex* (s.s.), and *C. osculatum* sp. D. The transcriptome of this parasite will represent a valuable genomic resource for future studies aimed at exploring its zoonotic potential and evolutionary responses to environmental changes.

Keywords: *C. osculatum sensu stricto*, Baltic Sea, *De Novo* transcriptome

PS – poster session

Session: [SE11] Parasite omics

Comparative Whole Genome Sequencing of *Tritrichomonas foetus* isolates from different hosts reveals genetic differences and implications for transmission patterns

Joanna Dabrowska[1], Irene Keller[2], Jacek Karamon[1], Jacek Sroka[1], Weronika Piotrowska[1], Bruno Gottstein[3], Tomasz Cencek[1], Caroline F Frey[3], Norbert Müller[3]

1 Department of Parasitology and Invasive Diseases, National Veterinary Research Institute Partyzantów Avenue 57, 24-100, Puławy, Poland

2 Interfaculty Bioinformatics Unit, Baltzerstrasse 6, 3012 Bern, Switzerland

3 University of Bern, Länggassstrasse 122, 3012 Bern, Switzerland

Abstract

Tritrichomonas foetus is a protozoan parasite that colonizes the reproductive tract of cattle as well as the gastrointestinal tract of cats. Furthermore, *T. foetus* is known as an essentially apathogenic commensal located in the nasal cavity of pigs. Transmission of *T. foetus* between the different hosts has to be considered a realistic scenario that may have important implications for the epidemiology of infections and disease. In our study, we generated whole genome sequencing (WGS) data from bovine, feline and porcine *T. foetus* strains to investigate the genetic (dis)similarities among these diverse strains. As a reference, we used a previously released draft assembly from a bovine *T. foetus* strain K isolated from an infected bull in Brazil. In particular, we identified single nucleotide polymorphisms (SNPs) and the insertion-deletion (indel) variations within the genomes of the different strains. Interestingly, only a low degree of polymorphism (68 SNPs and indels) was found between the bovine and the porcine strains in terms. Conversely, however, a 964 times higher number of such differences was detected by comparing the feline with either the bovine (65,569) or the porcine (65,615) strain. These data clearly indicated a close phylogenetic relationship between bovine and porcine *T. foetus* but a remarkable genetic distinctness of these two strains from the feline strain. The latter observation was confirmed by PCR-based sequencing of 20 in silico-selected indel markers and five *in silico*-selected SNP markers that uniformly demonstrated a relatively distant phylogenetic relationship of three independent feline *T. foetus* isolates in comparison to the bovine and porcine strains investigated. In summary, our comparative genome sequencing approach provided further insights into the genetic diversity of *T. foetus* in relation to the different host origins of the parasite.

Keywords: genome *Tritrichomonas foetus*, hosts, sequencing, PCR

PS – poster session

Session: [SE11] Parasite omics

Identification of alternative splicing isoforms of genes in L3 and L4 larvae of *Anisakis simplex* s.s. under glucose conditions

Iwona Polak[1], Ewa Zaobidna[1], Robert Stryinski[1], Mateusz Mazdziarz[2], Lukarz Paukszto[2], Iwona Bogacka[3], Elzbieta Lopienska-Biernat[1]

1 Department of Biochemistry, Faculty of Biology and Biotechnology, University of Warmia and Mazury, M. Oczapowskiego St. 2, 10-719 Olsztyn, Poland

2 Department of Botany and Evolutionary Ecology, Faculty of Biology and Biotechnology, University of Warmia and Mazury, Plac Łódzki St. 1, 10-721 Olsztyn, Poland

3 Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, M. Oczapowskiego St. 2, 10-719 Olsztyn, Poland

Abstract

Alternative splicing (AS) of mRNA is an important mechanism for increasing genome complexity for the study of parasitic nematodes. Spliced isoforms of the same gene can have different molecular and biological functions in at different larval stages and under different conditions. The subject of the research is *Anisakis simplex*, a cosmopolitan nematode that parasitizes on marine organisms and has a complex life cycle. According to the European Food Safety Authority (EFSA), *A. simplex* is classified as a biohazardous organism. The development of *A. simplex* L3 and L4 takes place under anaerobic conditions, and it obtain most of their energy from saccharides. The aim of the present study was to identify different AS events of L3 and L4 under glucose exposure. Multivariate differential transcriptomic splicing analysis (rMATS v.3.2.5) was used to find potential alternative splice (AS) sites estimated from the differences between glucose-treated L3 and L4 larvae using BCFtools and R environment libraries. Differential alternative splicing events (DAS) between larval stages were statistically tested (FDR < 0.05). Moreover, DAS events were filtred according to differentia level of the ΔPSI > 0.1. DAS events were categorized into five subtypes (rMATS): alternative 5` splice site (A5SS), alternative 3 splice site (A3SS), mutually exclusive exons (MXE), retained intron (RI), alternative first exon (AF), alternative last exon (AL) and skipped exon (SE). The most common gene-splicing events such as SE (98), especially in protein methionine sulfoxide oxidase. The gene-splicing events in UDP-glucose: glycoprotein glucosyltransferase (AL), glucose transporter type 1 (RI) found in the comparisons in this study suggest the theory that genetic markers of the transport system can be well targeted in *A. simplex* larvae.

This work was supported by the National Science Centre of Poland, grant no. 2018/31/B/NZ9/01683.

Keywords: *Anisakis simplex*, alternative splicing, L3 and L4 larvae

PS – poster session

[SE12]
Parasites of wildlife

Session: [SE12] Parasites of wildlife

Red fox (*Vulpes vulpes*) and wolf (*Canis lupus familiaris*) as a reservoir of *Cryptosporidium* spp. and *Giardia intestinalis* in Poland

Dorota Dwużnik-Szarek[1], Ewa Julia Mierzejewska[1], Korneliusz Kurek[2], Mariia Topolnytska[1], Małgorzata Krokowska-Paluszak[3], Patrycja Opalińska[4], Łukasz Stańczak[4], Grzegorz Górecki[4], Anna Bajer[1]

1 *Department of Eco-epidemiology of Parasitic Diseases, Institute of Developmental Biology and Biomedical Sciences, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02 096 Warsaw, Poland*
2 *The Masurian Center for Biodiversity and Nature Education in Urwitałt, Urwitałt 1, 11-730 Mikołajki, Poland*
3 *Faculty of Agricultural and Forestry, University of Warmia and Mazury in Olsztyn, 10-719 Olsztyn, Poland*
4 *Department of Game Management and Forest Protection, University of Life Sciences, Poznań, Poland*

Abstract

In the recent years infections of zoonotic pathogens constitute an increasing problem in veterinary and human medicine. Among them, *Cryptosporidium* and *Giardia* protozoans are main cause of waterborne outbreaks worldwide. There is still limited data on wild carnivores, such as red fox and wolf that may act as reservoir hosts and suport transmission of these pathogens in the environment. The aim of the project is to analyse the prevalence of *Cryptosporidium*/ *Gardia* infection in foxes obtained from seven voivodeshpis and wolves from the warmińsko-mazurskie voivodeship, and a phylogenetic analysis of the detected parasites. For the detection of both parasites, the commercial immunofluorescent assay MeriFluor *Cryptosporidium*/*Giardia* was used. For *Cryptosporidium* detection modified Ziehl-Neelsen (ZN) staining of faecal smears and PCR amplification and sequencing of 18S rDNA were used. For *Giardia* detection, PCR amplification and sequencing of the glutamate dehydrogenase (*gdh*) gene were conducted.

In total 117 and and 69 fecal samples obtained from red foxes and wolves, respectively were screened for *Cryptoporidium*/*Giardia* presence. In red fox prevalence of *Cryptosporidium* was 37.6%. Prevalence of *Giardia* was much lower in comparison to *Cryptosporidium* and reached 15.4%. In wolves, prevalence of *Cryptosporidium* was 14.5%. Only one fecal sample from wolf was *Giardia* positive. *Cryptosporidium canis*, *C. hominis* and *Cryptosporidium* sp. rodent genotype were detected in red foxes, in wolves *C. hominis* and *C. parvum* were identified.

Presented results filled the knowledge on wild carnivores in the epidemiology of *Cryptosporidum* and *Gardia* in natural areas in Poland.

The study was financially supported by National Science Centre (NCN) Preludium grant no 2019/35/N/NZ7/01772.

Keywords: *Cryptosporidium*, *Giardia*, red fox, wolf, Poland

OS – oral session

Session: [SE12] Parasites of wildlife

Prevalence of *Cryptosporidium* spp. and *Giardia* in wild Scottish deer

Paul Bartley, Ross Bacchetti, Jackie Thomson, Renata Pimentel, Clare Hamilton, Tom McNeilly, Beth Wells, Frank Katzer

Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, Scotland EH26 0PZ

Abstract

Cryptosporidiosis and giardiasis are common zoonotic diseases affecting humans, domestic animals and wildlife species worldwide. Cryptosporidiosis in humans is predominantly caused by either *Cryptosporidium parvum* or *C. hominis* and is considered the second most common cause of infant human diarrhoeal disease and death in Asia and Africa. Human infections with *Giardia duodenalis* are usually associated with parasite Assemblages A and B, while Assemblages D and E are more common in dogs and ruminants respectively.

This study examined the prevalence of *Cryptosporidium* spp. and *G. duodenalis* in faeces collected from the four major species of wild deer found across Scotland. Faecal samples (n=991) were collected from Red deer (n=463), Roe deer (n=394), Sika deer (n=95) and Fallow deer (n=17). Samples were processed individually to concentrate oocysts/cysts. DNA was extracted and analysed by PCR. A total of 122 (12.3%) samples tested positive for *Cryptosporidium* 18S gene DNA. Three major *Cryptosporidium* species were identified; *C. ryanae* (n=77), *C. parvum* (n=22) and *C. ubiquitum* (n=13). The highest prevalence of *Cryptosporidium* spp. infection was seen in the Sika deer 23/95 (24.2%) positive. While infections in Roe and Red deer were comparable, being 52/394 (13.2%) and 47/463 (10.1%) respectively.

Overall, n=76 (7.9%) samples tested positive by *Giardia* PCR. An assemblage could be assigned to 73/76 samples; Assemblage A was most common (n=62); also identified were Assemblage B (n=4), Assemblage D (n=2) and Assemblage E (n=5). The highest prevalence of *Giardia* was seen in Roe deer 53/381 (13.9%), Fallow deer 1/17 (5.9%), Red deer 20/448 (4.7%) and Sika deer 2/94 (2.1%).

Co-infections with both *Cryptosporidium* and *Giardia* were only found in 14 (1.5%) animals.

The results show *Cryptosporidium* and/or *Giardia*-positive samples across the entire country and in all four species of deer, indicating that wild deer may pose a risk for the zoonotic transmission of both parasites.

Keywords: *Cryptosporidium*, *Giardia*, wild deer, Scotland

OS – oral session

Session: [SE12] Parasites of wildlife

An update on the prevalence of *Echinococcus multilocularis* in red foxes (*Vulpes vulpes*) in northern and north-eastern Poland

Paweł Gładysz[1], Anna Lass[1], Agnieszka Świątalska[2], Jacek Karamon[3], Małgorzata Samorek-Pieróg[3]

1 *Division of Tropical Parasitology, Institute of Maritime and Tropical Medicine, Medical University of Gdańsk, Powstania Styczniowego 9B, 81–519 Gdynia, Poland*
2 *Department of Veterinary Hygiene, Kaprów 10, 80–316 Gdańsk, Poland*
3 *National Veterinary Research Institute, Partyzantów 57, 24-100 Puławy, Poland*

Abstract

Knowledge of *Echinococcus multilocularis* prevalence in wild canids helps us assess the risk of zoonotic spillover. In this study, we updated the infection statistics among red foxes in northern and north-eastern Poland since the last monitoring effort at the district (Polish: powiat) level (2001-2004). We collected intestinal contents from 192 red foxes from the districts of Słupsk, Puck, Wejherowo, Kościerzyna and Kartuzy (Pomorskie Voivodship), 176 individuals from the districts of Bartoszyce, Kętrzyn, Gołdap and Iława (Warmińsko-Mazurskie Voivodship), and 47 individuals from the district of Augustów (Podlaskie Voivodship). Based on nested PCR confirmed by Sanger sequencing, we calculated the prevalence with a 95% confidence interval and compared the results to previous reports. We recorded the highest proportion of infected red foxes in Gołdap District (21/40, 52.5%, 95% CI: 37.5%-67.1%). The shared prevalence in Puck, Wejherowo, Kościerzyna and Kartuzy Districts was 10.4% (19/182, 95% CI: 6.7%-15.8%). One red fox tested positive in Iława District (1/48, 2.1%, 95% CI: -0.6%-11.9%). We found no statistically significant changes in prevalence in the sampled areas. Our results indicate that the epidemiological status of red foxes has remained stable over the past 20 years.

Keywords: alveolar echinococcosis, epidemiology, copro-PCR, nested PCR, 12S rDNA

OS – oral session

Session: [SE12] Parasites of wildlife

Grey wolves (*Canis lupus*) as a hosts of *Echinococcus multilocularis*, *E. granulosus* s.l. and other helminths – the new double zoonotic threat in Poland

Jacek Karamon, Małgorzata Samorek-Pieróg, Ewa Bilska-Zajac, Jacek Sroka, Weronika Korpysa-Dzirba, Jolanta Zdybel, Aneta Bełcik, Tomasz Cencek

National Veterinary Research instutute, Pulawy, Poland

Abstract

Grey wolf is a predator with increasing importance as a large predator In Europe. In Poland, since 2000, the population has increased approx. by 300% - it is one of the largest populations in Europe. Recently, our preliminary study showed the presence of *E. ortleppi* in wolf in south-eastern Poland. To continue that observations the aim of this study was to estimate the occurrence of *Echinococcus* spp. infections and other helminths in wolves in south-eastern Poland.

Overall, 74 samples of wolf feaces were examined with multiplex PCR and system of qPCRs methods to detect and identify *Echinococcus* spp. Twenty intestines were examined with SCT. Faeces were examined microscopically. Genetic analysis of fragments of mitochondrial genes of *Echinococcus* worms were performed.

E. multilocularis DNA was detected in 6.8% and *E. granulosus* s.l. (identified as *E. ortleppi*) in 4.1% faeces samples. DNA of *Taenia* spp. were found 43.2% and *Mesocestoides* in 4.1%.

Examination of intestines showed: *E. multilocularis* worms in 10%, *E. granulosus* s.l. (identified as *E. ortleppi*) -10%, *Taenia* spp – 100%, hookworms – 30%, *Alaria alata* – 20%, *Mesocestoides* sp. – 10%, *Trichuris vulpis* – 15%, *Molineus* sp. – 5%, *Euryhelmis* sp. – 5%. By coproscopy, Capillariidae eggs were found in 59% of faeces. Phylogenetic studies of *E. multilocularis* worms showed the presence of two European haplotypes previously described in Poland in foxes and pigs. Sequences of *E. ortleppi* worms showed full identity in relation to larvae from Polish human case.

In conclusion, our study showed the presence of *E. multilocularis* in wolves for the first time in Poland and confirmed our earlier observations on *E. ortleppi*. This „double" threat from *Echinococcus* in this wolf population should be taken into account when assessing the epidemiological risk. The study enriched the knowledge about other helminths found in wolves, also those (*Euryhelmis*) that were recorded for the first time in this species.

Keywords: wolf, *Canis lupus*, *Echinococcus*, helminths

OS – oral session

Session: [SE12] Parasites of wildlife

March or get infected: influence of winter ranging shaped by supplementary feeding on the spread of non-native nematode *Ashworthius sidemi* in European bison populations

Marta Kołodziej-Sobocińska[1], Aleksander W. Demiaszkiewicz[2], Katarzyna Filip-Hutsch[3], Tomasz Borowik[1], Rafał Kowalczyk[1]

1 *Mammal Research Institute, Polish Academy of Sciences, Białowieża, Poland*
2 *W. Stefański Institute of Parasitology, Polish Academy of Sciences, Warsaw, Poland*
3 *Department of Food Hygiene and Public Health Protection, Institute of Veterinary Medicine, University of Life Sciences (SGGW), Warsaw, Poland*

Abstract

Parasitic infections in wildlife are influenced by numerous factors, including those related to wildlife management. This includes supplementary feeding widespread in numerous ungulates, including European bison. We analysed the influence of supplementary feeding and winter ranging of European bison herds on the dynamics, prevalence and infection severity of the blood-sucking nematode *Ashworthius sidemi* in two areas in NE Poland: the Knyszyn Forest (KF) and the Białowieża Primeval Forest (BPF) with diversified management strategies. We found significant differences in *A. sidemi* abundance and intensity between European bison groups; supplementary fed European bison from the BPF had higher parasitic load (3020 parasites, on average), than non-fed individuals from the KF (1400) and from the BPF (770). The prevalence was relatively high in all groups (93-96%). In the KF the highest infection rate was observed 9 years after the first appearance of *A. sidemi*, with a maximum value of 8,620 nematodes; while in the BPF, after just 6 years with maximal load up to 44,310 *A. sidemi*. The most plausible mechanism behind the observed pattern is probably the winter ranging behaviour of differently managed herds. We found that increasing winter home range size of European bison was associated with a significant reduction in the *A. sidemi* abundance. Our study shows that different management strategies may have an impact on animal spatial behaviour and associated spread and dynamics of pathogens in mammalian populations, and stresses the importance of adaptive management in reducing threats to wildlife.

The study was financed by the National Science Centre project no. 2012/07/B/NZ8/00066 and LIFE Nature project „BISON LAND – European Bison Conservation in the Białowieża Forest, Poland".

Keywords: blood-sucking nematode, large herbivores, conservation management, space use

OS – oral session

Session: [SE12] Parasites of wildlife

Dark diversity of flea assemblages harboured by small mammalian hosts in the northern Palearctic: effects of environment, host traits, and host phylogeny

Boris Krasnov

Ben-Gurion University of the Negev, Israel

Abstract

An assemblage of species in a locality comprises two components, namely (a) species that are present (realized diversity) and (b) species from the regional pool that may potentially inhabit this locality, due to suitable ecological conditions, but that are absent (dark diversity). I investigated factors affecting the dark diversity of component communities of fleas parasitic on rodents, shrews and pikas in the northern Palearctic at two spatial scales. First, I considered the dark diversity of flea assemblages of the same host (for 13 host species) across regions and tested for the effects of environmental factors and the number of available host species on the dark diversity of within-region flea assemblages. Second, I considered the dark diversity of fleas across host species within a region (for 20 regions) and asked whether within-host dark diversity is associated with host phylogeny and/or traits. I found that the dark diversity of flea assemblages harboured by small mammals substantially varied (a) within the same host species across space (in 12 of 13 host species) and (b) between host species within a region (in eight of 20 regions). The size of the dark diversity of flea assemblages of the same host across regions was generally affected by environmental factors (mainly by the amount of green vegetation), whereas the size of the dark diversity of flea assemblages of a host species within a region was affected by host traits (mainly by the degree of host sociality and the structure of its shelter and, to a lesser degree, by its geographic range size) but was not associated with host phylogenetic affinities. I

conclude that application of the dark diversity concept to parasite communities across space or hosts allows a better understanding of the factors affecting the species richness and composition of these communities.

Keywords: dark diversity, fleas, mammals, community composition

OS – oral session

Session: [SE12] Parasites of wildlife

Exo-erythrocytic development of *Haemoproteus* parasites in the Thrush nightingale *Luscinia luscinia* with first report of abortive development

Tchoumbou Melanie Adele, Iezhova Tatjana, Hernández-lara Carolina, Duc Melanie, Valkiūnas Gediminas

Nature Research Centre

Abstract

Haemoproteus species (Haemosporida, Haemoproteidae) are cosmopolitan blood parasites that affect bird fitness and health. However for the majority of described species, the exo-erythrocytic development (tissue stages) remains unclear. This study aims to understand the exo-erythrocytic development of *Haemoproteus* parasites by investigating the organs of infected Thrush nightingales *Luscinia luscinia* (non-threatened species). Infections were confirmed in eight individuals by microscopic examination and PCR-based methods. Few birds were euthanized (permit Nr. AS-1932, 2021-03-12 by Environmental Protection Agency, Lithuania). Organs were examined for tissue stages using histology and chromogenic in situ hybridization methods. Exo-erythrocytic meronts of *Haemoproteus attenuatus* (lineage hROBIN1) were found and described for the first time in this avian host. Most meronts were seen in the lungs, with a few also present in the liver, heart, and pectoral muscle. Numerous megalomeronts were observed in the gizzard and the heart of one individual, which also presented infection with *H. attenuatus*. Based on the morphology, location in organs, and diagnostics using the lineage-specific probes, the megalomeronts were attributed to *Haemoproteus majoris* (lineage hWW2). Two empty capsule-like walls were seen in the gizzard, indicating that the megalomeronts at those positions were already ruptured and degenerated. However, the extensive microscopic examination did not reveal gametocytes of *H. majoris*, obviously indicating an abortive development. Abortive haemosporidian infections were speculated to occur in wildlife in many studies but have never been documented in naturally infected birds. This study recognised patterns in exo-erythrocytic development of *H. attenuatus* and also is the first proof of abortive *Haemoproteus* infection in naturally infected birds during exo-erythrocytic development.

This study was funded by the Research Council of Lithuania (S-PD-22-71).

Keywords: bird, *Haemoproteus*, meront, megalomeront, *in situ* hybridization

OS – oral session

Session: [SE12] Parasites of wildlife

Parasites and conservation medicine

Andrei Daniel Mihalca

USAMV Cluj-Napoca, Romania

Abstract

Conservation biology is a scientific discipline aimed to provide evidence-based data and approaches for the preservation of the interspecific and intraspecific biodiversity against various threats. Among these threats, certain pathogens and associated diseases, including parasitic ones, are able to induce more or less devastating declines in various hosts. Such studies and approaches are within the field of a relatively new and highly interspecific discipline known as conservation medicine. The current presentation aims to discuss the most important parasites which are able to produce diseases in endangered wildlife including the mechanism of spillover and spillback and the risk factors associated with such events. The most at risk wild animals which can be potentially affected by parasites are those more closely related to domestic hosts, such as ungulates and carnivores. However, other wild hosts such as endangered birds, reptiles or amphibians can be equally affected by threatening parasites due to factors such as invasion, habitat loss or climate change. Parasites which can pose a threat to endangered wild hosts include a variety of species in most larger parasitic taxa such as Apicomplexa, Nematoda, Arthropoda, and in the latter also associated vector-borne pathogens.

Keywords: wildlife, conservation medicine, endangered

OS – oral session

Session: [SE12] Parasites of wildlife

Taeniids in synanthropic rodents in Zealand, Denmark

Rosalina Rotovnik[1], Tatiana S. Lathrop[2], Jakob Skov[3], Pikka Jokelainen[4], Christian M.O. Kapel[5], Christen Rune Stensvold[6], Rebecca P. K. D. Berg[6]

1 Parasitology & Aquatic Pathobiology, University of Copenhagen, Denmark
2 DTU National Food Institute, Technical University of Denmark, Denmark
3 Vaccine Development Department, Statens Serum Institut, Denmark
4 Infectious Disease Preparedness, Statens Serum Institut, Denmark
5 Department for Plant and Environmental Sciences, University of Copenhagen, Denmark
6 Bacteria, Parasites & Fungi, Statens Serum Institut, Denmark

Abstract

Small rodents are intermediate hosts of several taeniid species, including zoonotic species, such as *Echinococcus multilocularis*. A decade has passed since the last survey of taeniid species in rodents in Denmark. In the current study, we screened rodents in Zealand, Denmark, for taeniid species, using molecular methods. As part of pest control, 99 rodents of 9 different species were collected around three farms and one private property in Zealand. Different livestock and companion animals were housed at the farms. Visible cysts were collected from the liver and peritoneal cavity of the rodents, postmortem. Cysts were subjected to DNA extraction on an automated platform and the acquired DNA was screened for cestode species using amplicon-based next generation sequencing (NGS). Selected samples were subjected to multiplex PCR and subsequent Sanger sequencing.

Cysts were collected from the liver of 16 rodents and from the peritoneal cavity of 9 rodents. Some rodents (n=3) harbored cysts in both locations. A total of 19 rodents tested positive for cestode species with amplicon-based NGS. Of these, 18 were confirmed with multiplex PCR and 13 were selected for Sanger sequencing. Three species of taeniids were identified molecularly. *Hydatigera taeniaeformis* was identified in liver cyst samples from four rodent species, including *Rattus norvegicus. Taenia polyacantha* and *Versteria mustelae* were identified in peritoneal- and liver cyst samples, respectively, from *Myodes glareolus*.

Amplicon-based NGS appears useful when screening cyst material for cestode species in small rodents. *H. taeniaeformis*, *T. polyacantha* and *V. mustelae* are present in small rodents in Zealand, Denmark. No rodents tested positive for *E. multilocularis*. To our knowledge, this is the first identification of *H. taeniaeformis* in *R. norvegicus* in Denmark.

Keywords: Rodentia, Taeniidae, molecular epidemiology, Denmark

OS – oral session

Session: [SE12] Parasites of wildlife

Echinococcus multilocularis: hotspot in red foxes in Denmark

Rosalina Rotovnik[1], Helena Mejer[1], Anne Sofie V. Hammer[2], Sussie Pagh[3], Maibritt T. Ovesen[2], Mia M. Jensen[2], Peter L. Hansen[2], Randi F. Petersen[4], Christen Rune Stensvold[4], Henrik V. Nielsen[4], Stig M. Thamsborg[1], Rebecca P. Berg[4]

1 Parasitology & Aquatic Pathobiology, University of Copenhagen, Denmark

2 Pathobiological Sciences, University of Copenhagen, Denmark

3 Environmental Biology Monitoring, Aalborg University, Denmark

4 Bacteria, Parasites & Fungi, Statens Serum Institut, Denmark

Abstract

Echinococcus multilocularis is an important zoonotic cestode. Infections in both humans and animals are notifiable in Denmark. A high local occurrence has previously been described in red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*) in Southern Denmark (years 2012-2015). Since then, there has been no targeted surveillance. A new survey of red foxes, utilizing optimized molecular methods, was therefore initiated in 2022.

Foxes included in the study are either found dead or regulated (hunted) in accordance with Danish laws. So far, 212 red foxes, collected across Denmark, have been examined. Of these, 61 were collected in Southern Denmark. Rectal contents from the foxes are subjected to DNA extraction based on magnetic capture. DNA is then analyzed by an in-house real-time PCR. Positive samples are confirmed by the sedimentation and counting technique as well as PCR and Sanger sequencing of DNA from rectal content and worms.

By spring 2024, 8 foxes (3.8%, 95% CI: 2-7%) have tested positive for *E. multilocularis*. Worm burdens ranged from 3 to 1252 (scolex count). All 8 foxes were collected within an area covering less than 120 km2 in Southern Denmark, close to the border of Germany. In this specific area, 26 foxes were collected, and the local estimated prevalence was 31% (95% CI: 17-50%).

Red foxes in Denmark continue to host *E. multilocularis* with one established, and apparently stable, hotspot in Southern Denmark. Further monitoring and investigation of the sylvatic cycle is needed. Updated knowledge can help increase awareness and aid in establishing targeted interventions to reduce the risk of human infection.

We thank the lab technicians at Statens Serum Institut and the University of Copenhagen for their excellent assistance. The project is funded by the Danish Veterinary and Food Administration and supported by the Danish Environmental Protection Agency.

Keywords: *Echinococcus multilocularis*, *Vulpes vulpes*, zoonosis, hotspot, Denmark

OS – oral session

Session: [SE12] Parasites of wildlife

Blood parasites of mouse-like rodents in the conditions of the Chornobyl Exclusion Zone

Vladyslava Storozhuk[1], Maryna Galat[1], Bita Shahi Barogh[2], Hans-Peter Fuehrer[2], Caroline F. Frey[3]

1 National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine

2 Institute of Parasitology, Department of Pathobiology, Veterinary University Vienna, Vienna, Austria

3 Institute of Parasitology, Vetsuisse Faculty, University of Bern, Bern, Switzerland

Abstract

Mouse-like rodents (Rodentia Muroidea) are a group of approximately 1400 species, such as rats, mice, voles, and others which have an almost worldwide distribution. A lot of species from this group are known as reservoir hosts for agents of potentially dangerous zoonotic diseases, including leptospirosis, plague, toxoplasmosis, bartonellosis, Lyme disease and many others. The conditions of climate change, urbanisation and influence of radioactive contamination may be favourable for wildlife habitats to move beyond their natural habitats. Since there is no data on blood parasites of mouse-like rodents under conditions of radioactive contamination, it was decided to study this issue.

During 2019-2020, we examined 119 mouse-like rodents that were captured at the landfills of the drained bed of the cooling reservoir of the Chornobyl Nuclear Power Plant. Among the species we studied were *Mus musculus*, *Apodemus agrarius*, *Myodes glareolus*, *Sylvaemus flavicollis*, and *Sylvaemus uralensis*. Traditional microscopic examination revealed the presence of *Trypanosoma* spp., *Hepatozoon* spp., *Bartonella* spp., *Ehrlichia* spp., *Rickettsia* spp., and *Babesia* spp. Further studies using morphometry, polymerase chain reaction and subsequent sequencing were used to clarify the preliminary results of the study. The species identified included *Bartonella taylorii, Bartonella grahamii, Trypanosoma grosi, Mycoplasma haemomuris* and others.

Keywords: Mouse-like rodents, Chornobyl Exclusion Zone, parasites, microscopy, PCR

OS – oral session

Session: [SE12] Parasites of wildlife

Spatial distribution and population structure of the seabird tick *Ixodes uriae* among Antarctic penguin colonies

Katarzyna Tołkacz, Ewa J. Mierzejewska, Stephen J. A. Jennings

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Abstract

Antarctic penguins serve as vital indicators of the environmental well-being of their habitat. Given their significance, it is imperative to monitor factors that could impact their fitness and survival. The seabird tick *Ixodes uriae*, prevalent across circumpolar regions, poses a potential threat to penguin hosts due to its heavy infestation. However, limited information exists regarding the distribution and prevalence of these ticks among Antarctic penguins. Here, we investigate the prevalence of seabird ticks in multiple colonies of three *Pygoscelis* penguin species (Adélie, gentoo, and chinstrap), along with the temporal dynamics of infestation during the breeding season.

Ticks were collected from four penguin colonies situated on King George Island, Antarctica. Underneath stones within the colonies served as primary sampling sites. Relative abundance was quantified by the ratio of stones harboring ticks to the total stones examined, with an approximate count of ticks per stone recorded. Each colony underwent inspection of 75-200 penguins, contingent upon colony size, to assess ectoparasite presence.

All developmental stages of ticks were detected across visited colonies. The highest prevalence was observed among gentoo (58%) and chinstrap (50%) penguins, whereas Adélie colonies exhibited lower prevalence (4%-28%). Tick abundance varied widely, ranging from 1 to approximately 2117 individuals per stone. Furthermore, prevalence, abundance, and population structure exhibited temporal fluctuations. Peak tick infestation on birds occurred during the late guard stage, affecting 48% of adult and 42% of chick gentoo penguins. Female ticks infested both adult birds and chicks, whereas nymphs and larvae predominantly parasitised chicks. Future investigations should delve into the implications of tick infestations on the health of Antarctic penguins.

This study received funding from the Polish National Science Centre (Grant No. 2022/44/C/NZ6/00142).

Keywords: Antarctica, *Ixodes uriae*, penguins, *Pygoscelis*, ticks

OS – oral session

Session: [SE12] Parasites of wildlife

Screening for blood-associated pathogens in the spleen of the wild raccoons (*Procyon lotor*) from Germany and phylogenetic analysis of detected *Mycoplasma* spp. and *Babesia* spp.

Maria Sophia Unterköfler[1], Aria Schwingshandl[1], Barbara Eigner[1], Josef Harl[2], Hans-peter Fuehrer[1], Peter Steinbach[3], Diana Jeschke[4], Michael Striese[4], Hermann Ansorge[5], Mike Heddergott[6]

1 Institute of Parasitology, Department of Pathobiology, University of Veterinary Medicine Vienna, Austria
2 Institute of Pathology, Department of Pathobiology, University of Veterinary Medicine Vienna, Austria
3 Faculty of Chemistry, University of Göttingen, Germany; Musée National d'Histoire Naturelle, Luxembourg
4 Senkenberg Museum of Natural History Görlitz, Germany
5 Senkenberg Museum of Natural History Görlitz, Germany; International Institute Zittau, Technical University Dresden, Zittau, Germany
6 Musée National d'Histoire Naturelle, Luxembourg

Abstract

Racoons (*Procyon lotor*) originate from North America and have been first released in the 1930s in Germany. Since then, they spread in Europe and are considered an invasive species. Due to their close contact to human settlements, they are important reservoirs for zoonotic disease, such as baylisascariosis. The relevance and prevalence of blood-associated pathogens, that are mostly transmitted by arthropod vectors, are not fully elucidated yet.

In this study we screened 285 spleen samples of raccoons from Germany collected in the years 2019 to 2022. Four different sampling areas in Thuringia (n=65), Saxony (n=144), Saxony-Anhalt (n=51) and Baden-Württemberg (n=23) were investigated. In two samples the origin was not known. To detect *Mycoplasma* spp., Anaplasmataceae, *Bartonella* spp.*, Babesia* spp.*, Rickettsia* spp.*, Dirofilaria* spp.*, Trypanosoma* spp. and *Hepatozoon* spp. PCR and if positive sequencing were performed. In total, 104 samples were positive for *Mycoplasma* spp. (36.49%), three for *Babesia* spp. (1.05%) and two for *Anaplasma phagocytophilum* (0.7%). The prevalence of *Mycoplasma* spp. was highest in Saxony-Anhalt (49.02%), followed by Thuringia (44.62%) and Baden-Württemberg (30.43%) and was lowest in Saxony (29.16%). A phylogenetic analysis was performed to illustrate the relationship to other species of *Mycoplasma* spp. and *Babesia* spp. Detected *Mycoplasma* spp. were all within one phylogenetic clade, which contrasts with *Mycoplasma* spp. found in *Procyon* spp. from the Americas. This is the first study to detect *Mycoplasma* spp. in raccoons outside of North America. Two different *Babesia* spp. were detected. One closely related to *B. canis* while the other is more closely related to *Babesia* spp. from ruminants. Except for *A. phagocytophilum*, which is an important pathogen in other animals and considered zoonotic, the impact of the detected pathogens on the health of raccoons or their role as reservoir for other wild or domestic animals is not clear to date.

Keywords: racoons, *Mycoplasma*, *Babesia*, vector-borne pathogens, haplotype network analysis

OS – oral session

Session: [SE12] Parasites of wildlife

Echinococcus spp.in golden jackals (*Canis aureus*)

Aleksandra Uzelac[1], Ivana Klun[1], Milica Kuručki[2], Ilija Pantelić[2], Katarina Breka[2], Tijana Kukurić[3], Aleksandra Penezić[2], Jelena Karanović[4], Duško Ćirović[2]

1 Institute for Medical Research, University of Belgrade
2 Faculty of Biology, University of Belgrade
3 Faculty of Agriculture, University of Novi Sad
4 Institute of Molecular Genetics and Genetic Engineering, University of Belgrade

Abstract

The golden jackal (*Canis aureus*) is a confirmed definitive host for tapeworms of the genus *Echinococcus.* As Serbia has one of Europe's largest resident populations of golden jackals, investigating their role in the transmission and distribution of *Echinococcus* spp. is of interest to public health. To analyze the population genetics of *Echinococcus* spp. circulating in golden jackals, the project WORM_PROFILER, which started in March of 2024, is collecting gastrointestinal (GI) tracts of legally hunted animals from different areas of Serbia. For this study, GI tracts of 33 animals were processed. After defrosting, the intestines were cut open longitudinally and feces were transferred into 50 mL centrifuge tubes. The mucosa was scraped and analyzed microscopically to isolate parasites. Approximately 3 g of feces was processed by ZnCl2 flotation and sequential mesh filtration to collect taeniid eggs. The DNA from adult *Echinococcus* spp. and taeniid eggs was extracted using quick boiling in 0.02 M NaOH and screened by multiplex PCR designed to detect *E. multilocularis*, *E. granulosus* and *E. canadensis.* Examination of the mucosa yielded several gravid *Echinococcus* tapeworms from the small intestine of one animal from western Serbia and eggs were collected from the feces. PCR identified the tapeworm species as *E. multilocularis.* Single egg picking and sequencing of the *cox1* and *nad1* genes is underway. Taeniid eggs were additionally collected from another jackal from the same area, but could not be identified as *Echinococcus* spp. by PCR. These early findings suggest that *E. multilocularis* is present in western Serbia and sampling in the same geographical area will be intensified. Processing of additional collected samples is ongoing.

This research was supported by the Science Fund of the Republic of Serbia, #10841, Worm Profiler: Surveillance and population genetics of Echinococcus in Serbia - WORM_PROFILER.

Keywords: *Echinococcus* spp., golden jackal, *E. multilocularis*, population genetics

OS – oral session

Session: [SE12] Parasites of wildlife

Small mammals – sentinels for *Toxoplasma gondii* in ecosystems with different levels of human activities in Slovakia

Daniela Antolová, Michal Stanko, Júlia Jarošová, Viktória Dandárová

Institute of Parasitology of the Slovak Academy of Sciences, Košice, Slovak Republic

Abstract

Toxoplasma gondii is a ubiquitous intracellular parasite with felids as definitive hosts and a broad range of warm blooded animals as intermediate hosts. Small mammals are considered suitable sentinels for prevalence studies of many infections, including toxoplasmosis. The study aimed to estimate the seroprevalence of *T. gondii* in small mammals from ecosystems with different level of human activities and to evaluate the correlation between the seropositivity and the species, age, and sex of the animals.

Altogether 1005 small mammals belonging to seven species were trapped and examined for the presence of antibodies to *T. gondii*. Seropositivity was recorded in 6.7% of animals. Antibodies to *T. gondii* were detected in six species, ranging from 0.0% in *Micromys minutus* to 7.7% in *A. flavicollis*. Seropositivity of animals from localities with different level of human activity showed statistically significant differences; higher positivity was detected in suburban and touristic areas (12.2%) than in agrocenoses, forests, hunting grounds and alluvia (5.5%). Females reached significantly higher seropositivity (9.7%) than males (3.8%), and adults were positive significantly more often (9.2%) than subadults (4.8%).

The results show that the occurrence of *T. gondii* varies significantly in rodents from habitats with varying environmental conditions and different levels of anthropic use. Several biological

and ecological factors, such as soil contamination, soil conditions, susceptibility of rodent species etc., may influence this variability.

Study was financially supported by the projects VEGA 2/0024/24 and APVV-21-0166.

Keywords: *Toxoplasma gondii,* small mammals, seropositivity, habitats

PS – poster session

Session: [SE12] Parasites of wildlife

Echinococcus multilocularis in red foxes (*Vulpes vulpes*) and hunting dogs in hunting areas of Pomeranian Province, Poland

Karolina Baranowicz[1], Joanna Nowicka[1], Paweł Gładysz[1], Agnieszka Świątalska[2], Anna Lass[1]

1 Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine, Medical University of Gdansk, Poland
2 Department of Veterinary Hygiene Gdańsk, Poland

Abstract

In Poland, the main definitive host of *Echinococcus multilocularis* is red fox (*Vulpes vulpes*) and its population is being regulated. Hunting often involves hunting dogs. Direct contact of the dogs with wildlife results in increased exposure to infectious agents, including tapeworms. The aim of this study was to compare the prevalence of *E. multilocularis* infection in dogs assisting rangers and hunters and red foxes in a hunting areas of Pomerania Voivodeship. Faecal samples were collected from 164 hunted red foxes and 72 dogs of hunting breeds. Fox faeces were collected during dissection directly from the intestine. Canine faecal samples were provided by owners along with a questionnaire describing the dog condition and behavioural habits. All faecal samples were examined microscopically using the flotation method and molecularly using nested PCR and Sanger sequencing. Tapeworms eggs of the family Taeniidae were detected in 4 (2.44%) faecal samples collected from foxes. In contrast, molecular study confirmed presence of *E. multilocularis* DNA in 19 (10.98%) and 6 (8.3%) fox and dogs faecal samples, respectively. According to questionnaire, all infected dogs had daily access to the forest and meadows where both foxes and rodents are seen, they were often let loose in all nearby areas and possibility of close contact with foxes and wildlife carcasses was indicated as high. Results of this preliminary study show that the prevalence of *E. multilocularis* infection in dogs accompanying hunters and rangers, with direct access to excreta and carcasses of wildlife, is at similar level to living in hunting districts red foxes. This indicates that hunting dogs may become as important reservoir of *Echinococcus* as foxes and wild canids are. In addition, a close co-habitation of humans with dogs, especially breeds used for hunting, makes it an important aspect of public health risk factor.

This work was supported by the National Science Centre grant no.2020/37/B/NZ7/03934.

Keywords: *Echinococcus multilocularis*, red fox, hunting dogs

PS – poster session

Session: [SE12] Parasites of wildlife

Mustelids as hosts of *Toxoplasma gondii* in Denmark

Rebecca Berg[1], Rosalina Rotovnik[1], Lee O'Brien Andersen[1], Henrik Vedel Nielsen[1], Morten Elmeros[2], Pikka Jokelainen[1]

1 Statens Serum Institut, Denmark
2 University of Aarhus, Denmark

Abstract

Denmark is home to seven native species of mustelids (family Mustelidae). The current knowledge on their exposure to pathogens is limited. Therefore, a collaborative study between the University of Aarhus (AU) and Statens Serum Institut (SSI) aims to investigate the exposure of mustelids to selected pathogens. The zoonotic protozoan parasite *Toxoplasma gondii* has a wide host range and a global distribution. High seroprevalence estimates as well as cases of clinical toxoplasmosis have been described in mustelids elsewhere. Here we present preliminary findings of *T. gondii* in mustelids collected in Denmark.

Mustelids covering all the native species were included in the study. The dead animals were delivered to AU by a network of volunteers. A total of 90 blood samples and 108 hearts were collected and sent to SSI for analyses. Blood samples were centrifuged and the supernatant was analysed for antibodies against *T. gondii* with a commercial ELISA kit. Small samples from the apex of the hearts were submitted to DNA extraction on an automated platform. The DNA samples were then analysed by real-time PCR for *T. gondii* DNA. A total of 90 blood samples and 71 of the heart samples have been analysed so far.

Antibodies against *T. gondii* were detected in 65 of the 90 analysed blood samples (72%; 95% confidence interval 62%–80%). Seropositive animals were found within all seven species. *Toxoplasma gondii* DNA was detected in nine of the 71 analysed heart apex samples (13%; 95% confidence interval 7%-22%) with Ct-values ranging from 27 to 39.

Our findings confirm mustelids as hosts of *T. gondii* in Denmark.

We thank the lab technicians at the Laboratory for Parasitology, SSI, for their excellent technical assistance. The project is funded by Jagttegnsmidlerne, granted by the Danish Environmental Protection Agency.

Keywords: zoonosis, Apicomplexa, wildlife, serology, Scandinavia

PS – poster session

Session: [SE12] Parasites of wildlife

The *Echinococcus multilocularis* cycle: first detection of intermediate host in a highly endemic region of Northeastern Italy

Salvatore Andrea Cafiero[1], Chiara Rossi[2], Lucia Cenni[1,2], Adriano Casulli[3], Heidi Christine Hauffe[2], Alessandro Massolo[4]

1 Ethology Unit, Department of Biology, University of Pisa, Pisa, Italy
2 Conservation Genomics Research Unit, Centre for Research and Innovation, Fondazione Edmund Mach, San Michele All'Adige, Italy
3 European Union Reference Laboratory for Parasites (EURLP), Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy and WHO Collaborating Centre for the Epidemiology, Detection and Control of Cystic and Alveolar Echinococcosis, Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy
4 Ethology Unit, Department of Biology, University of Pisa, Pisa, Italy; University of Calgary, Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, Calgary, AB, Canada; UMR CNRS 6249 Chrono-Environnement, Université Bourgogne Franche-Comte

Abstract

Echinococcus multilocularis (*Em*) is a Taeniidae cestode, distributed across the Northern hemisphere, and circulating among canids and voles as definitive and intermediate hosts, respectively (Romig et al., 2017. Adv. Parasitol. Part A, 95: 213-314). Humans can develop alveolar echinococcosis following egg ingestion (Conraths et al., 2017. PLOS NTD, 11: 1-15). In Italy, the first *Em*-positive foxes were found in the Trentino-Alto Adige region in an autochthonous and highly endemic focus (Manfredi et al., 2002. Vet. Rec., 150: 757; Casulli et al., 2005. Int J Par 35: 1079-1083; Obber et al., 2022. PLOS ONE, 17: e0268045). Recently,

the first autochthonous case of alveolar echinococcosis was confirmed in the same area (Tamarozzi et al., 2024. Emerg. Infect. Dis., 30: 350-353). However, the role of rodents as intermediate hosts had never been investigated. We aimed to fill this knowledge gap in the region.

Small mammals were collected from the stomachs of 148 legally culled foxes across the Province of Bolzano. For 142 prey items, species was identified from DNA extracted from tail or legs samples using PCR to amplify a 350 bp long fragment of *cytb* gene. A qPCR protocol was then used to screen for *Em* in liver and other organs for 98 rodents with QuantiNova Pathogen + IC Kit (Qiagen), following the manufacturer's instructions with minor modifications, and *Em*-specific primers and probe targeting a 69 bp long fragment of *cob* gene (Massolo et al., 2021. Acta Trop., 223: 106078).

Microtus arvalis (common vole) was the most frequent (20.83%) and abundant (116/142 individuals) prey species. Only 1/98 rodents was *Em*-positive: 1/38 *M. arvalis* livers. The infected vole was found in an *Em*-positive red fox near the border with Austria. We report the first finding of an *Em*-positive intermediate host in this endemic region which might help to explain why *Em* is distributed in the Southern Italian Alps. Phylogenetic analyses are planned soon.

Keywords: *Echinococcus multilocularis*, Italy, intermediate host, red fox, *Microtus arvalis*

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Session: [SE12] Parasites of wildlife

Comparison of helminth communities of wintering rooks, *Corvus frugilegus* (Aves, Corvidae) in the cities of Poltava and Kyiv, Ukraine

Oksana Greben, Valeriia Dupak, Olga Lisitsyna, Yuriy Kuzmin

I. I. Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, Kyiv, Ukraine

Abstract

In 2020–2021 and 2021–2022, 46 dead rooks were gathered in winter at roosting sites in Poltava (n=24) and Kyiv (n=22). The birds were examined and a total of 427 specimens (289 in Poltava and 138 in Kyiv) of 13 helminth species were collected. The number of helminth taxa (species and subspecies) was the same in the two examined samples: 10 in Poltava and 10 in Kyiv. The similarity between the samples based on the Sorensen index was 60%. The species richness in the helminth infracommunities was higher in the sample from Poltava (1–5 species; median 3) than in the sample from Kyiv (1–3 species, median 2); the difference was significant according to the Mann-Whitney test. The dissimilarity between the two samples was mostly influenced by the infection parameters of *Acuaria anthuris*, *Baruscapillaria resectum*, *Diplotriaena tricuspis*, *Microtetrameres* spp. (higher in the sample from Poltava), *Eucoleus frugilegi*, and *Spiniglans affinis* (higher in the sample from Kyiv).

In the helminth component community from Poltava, the evenness and diversity indices were comparatively higher than in the component community from Kyiv; *Microtetrameres* spp. was the predominating taxon. In the component community from Kyiv, *E. frugilegi* predominated by infection intensity and abundance. Based on this information, we assume that the summer diet of the rooks wintering in Poltava includes more orthopterans (intermediate hosts of *Microtetrameres* spp.), while earthworms (intermediate hosts of *Eucoleus frugilegi*) are the most substantial part of the summer diet of rooks wintering in Kyiv.

Keywords: rook, bird parasites, Ukraine, helminth communities

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Session: [SE12] Parasites of wildlife

Molecular detection of *Trypanosoma* spp. and *Bartonella* spp. in deer keds (*Lipoptena cervi*) from European moose (*Alces alces*) in Finland.

Joanna Werszko[1], Marek Asman[2], Magdalena Świsłocka[3], Petra Heikkinen[4], Katarzyna Filip-Hutsch[5], Antti Oksanen[4]

1 Department of General Biology and Parasitology, Medical University of Warsaw, Warsaw, Poland
2 Department of Medical and Molecular Biology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia, Katowice, Poland
3 Department of Zoology and Genetics, Faculty of Biology, University of Białystok, Ciołkowskiego 1J, 15-245 Białystok, Poland
4 Finnish Food Authority, (FINPAR), Elektroniikkatie 3, FI-90590, Oulu, Finland
5 Department of Food Hygiene and Public Health Protection, Institute of Veterinary Medicine, Warsaw University of Life Sciences–WULS, Nowoursynowska 159, 02-776, Warsaw, Poland.

Abstract

Deer keds *Lipoptena cervi* are blood-sucking insects that play an important role in the pathogen transmision to many wild and domestic mammals. The species is spread across Europe, eastern Siberia and northern China and was also introduced to the eastern United States. Rapid invasion of deer keds has been observed in Finland since 1960s and was probably associated with an increase of moose density. The aim of our study was to examine deer keds collected from European moose in Finland on the presence of *Trypanosoma* spp. and *Bartonella* spp.

In autumn 2022, moose hunters collected moose heads for Chronic Wasting Disease analysis, mostly from Central Finland. From 8 moose, 64 deer keds were collected in Finnish Food Authority laboratory. Species of flies was identified using morphometrical features. Isolation of DNA and nested PCR followed by sequencing and phylogenetic analysis were performed. Morphometrical measurements confirmed the fly species as *Lipoptena cervi*. DNA of trypanosomes was identified in 17 deer keds. *Bartonella* sp. was detected in 15 specimens.

As *Lipoptena cervi* is regarded one of the most important ectoparasites of moose in Finland, it is necessary to determine their role as potential vectors for transmissible infections and the risk of arthropod borne infections for Finnish moose. Current results do not confirm vectoring of either *Trypanosoma* spp. or *Bartonella* spp., as the flies had fed on moose blood, but the latter has earlier been demonstrated from deer ked pupae, while two different *Trypanosoma theileri* group parasites have been found in blood from Finnish moose.

Keywords: deer keds, moose, *Trypanosoma* spp., *Bartonella* spp.

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Session: [SE12] Parasites of wildlife

Molecular and morphological studies on obligatory myiasis in wild cervids from Croatia

Ema Gagović[1], Šimun Naletilić[1], Daria Jurković Žilić[1], Krunoslav Pintur[2], Relja Beck[1]

1 Croatian Veterinary Institute
2 Karlovac University of Applied Sciences

Abstract

Flies from the Family Osteridae are obligatory parasites of mammals with two subfamilies infecting cervids from Europe (Hypodermatinae and Osterinae). Larvae of bot flies (subfamily Osterine) develop in nasopharynx, while larvae of warble flies from subfamily Hypodermatinae penetrate host's skin and migrate within connective tissue. Most prevalent species of warble fly and bot fly in cervids in Europe are species from genera *Hypoderma*, *Cephenemyia* and *Pharingomia*. So far, molecular studies on warble flies and bot flies in Southeast Europe and Croatia are scarce. Based on morphological determination, *C. stimulator*, *C. ulrichii*, *P. picta* and *Hypoderma* sp. have been detected in roe deer and red deer in Croatia. In total, 122 larvae from 28 roe deer and 8 red deer were determined by morphological characteristics and genomic DNA was individually isolated from 117 larvae, latter molecularly analyzed by sequencing of portion of COI gene with LCO and HCO primers. Species of warble flies and bot flies were morphologically determined as *H. diana*, *C. stimulator* and *P. picta*. *H. diana* was morphologically confirmed from 8 roe deer and 6 red deer. All sequences from 61 parasites were identical. BLAST analysis revealed 90% similarity with sequences of several *Hypoderma* species yet not with *H. diana*. One sequence of *H. diana* in GenBank is not overlapping with current sequence and complete mitochondrial genome of *H. diana* is lacking. *C. stimulator* was found entirely in 19 roe deer and all 40 sequences were equivalent and confirmed morphological identification matching 100% with available sequences in GenBank. Larvae of 4 *P. picta* were morphologically identified in 1 red deer and were all confirmed after sequencing. To the best of our knowledge, this study confirmed in Croatia and Southeast Europe *H. diana*, *P. picta* and *C. stimulator* based on sequencing portion of *COI* gene sequences. Furthermore, it seems that there is lack of heterogeneity of *H. diana* and lack of host specificity.

Keywords: obligatory myiasis, molecular detection, roe deer, red deer, Croatia

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Session: [SE12] Parasites of wildlife

Blastocystis occurrence and subtype diversity in wild ungulates: the Iberian Peninsula scenario

Ana M. Figueiredo[1], Alejandro Dashti[2], Jenny G. Maloney[3], Pamela C. Köster[2], Begoña Bailo[2], Ana Sánchez-de-las-Matas[2], Miguel Angel Habela[4], Antonio Rivero-Juarez[5], Joaquín Vicente[6], Emmanuel Serrano[7], Maria C. Arnal[8], Daniel Fernández-de-Luco[8], Patrocinio Morrondo[9], José A. Armenteros[10], Ana Balseiro[11], Guillermo A. Cardona[12], Carlos Martínez-Carrasco[13], Rita T. Torres[14], Carlos Fonseca[15], Atle Mysterud[16], João Carvalho[14], Dário Hipólito[17], Joana Fernandes[18], Josman D. Palmeira[14], Rafael Calero-Bernal[19], Isabel Guadano Procesi[20], David González-Barrio[2], Sergio Sánchez[2], Mónica Santín[3], David Carmena[21]

1 Department of Biology and CESAM, University of Aveiro, Aveiro, Portugal; Centre for Ecological and Evolutionary Synthesis, Department of Bioscience, University of Oslo, Oslo, Norway
2 Parasitology Reference and Research Laboratory, Spanish National Centre for Microbiology, Health Institute Carlos III, Majadahonda, Madrid, Spain
3 Environmental Microbial and Food Safety Laboratory, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland, USA
4 Department of Animal Health, Veterinary Sciences Faculty, Extremadura University, Caceres, Spain
5 Infectious Diseases Unit, Maimonides Institute for Biomedical Research (IMIBIC), University Hospital Reina Sofía, University of Córdoba, Córdoba, Spain; Center for Biomedical Research Network in Infectious Diseases (CIBERINFEC), Health Institute Carlos II
6 SaBio Group, Institute for Game and Wildlife Research, IREC (UCLM-CSIC-JCCM), Ciudad Real, Spain
7 Wildlife Ecology & Health Group (WE&H), Wildlife Environmental Pathology Service (SEFaS), Department of Animal Medicine and Surgery, Autonomous University of Barcelona, Bellaterra, Spain
8 Department of Animal Pathology, Veterinary Faculty, University of Zaragoza, Zaragoza, Spain
9 INVESAGA Group, Department of Animal Pathology, Faculty of Veterinary, University of Santiago de Compostela, Lugo, Spain
10 Council of Development, Territory Planning and the Environment of the Principado de Asturias, Oviedo, Spain
11 Animal Health Department, Veterinary School, University of León, León, Spain; Animal Health Department, Mountain Livestock Institute (CSIC-University of León), León, Spain
12 Livestock Laboratory, Regional Government of Álava, Vitoria-Gasteiz, Spain
13 Animal Health Department, University of Murcia, Regional Campus of International Excellence "Campus Mare Nostrum", Espinardo, Murcia, Spain
14 Department of Biology and CESAM, University of Aveiro, Aveiro, Portugal
15 Department of Biology and CESAM, University of Aveiro, Aveiro, Portugal; ForestWISE – Collaborative Laboratory for Integrated Forest & Fire Management, Vila Real, Portugal
16 Centre for Ecological and Evolutionary Synthesis, Department of Bioscience, University of Oslo, Oslo, Norway
17 Department of Biology and CESAM, University of Aveiro, Aveiro, Portugal; Veterinary Biology Unit, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia
18 Department of Biology and CESAM, University of Aveiro, Aveiro, Portugal; Center for Evolutionary Hologenomics, The GLOBE Institute, University of Copenhagen, Copenhagen, Denmark
19 SALUVET, Department of Animal Health, Faculty of Veterinary, Complutense University of Madrid, Madrid, Spain
20 Department of Clinical Sciences and Translational Medicine, Faculty of Medicine, University of Rome "Tor Vergata", Rome, Italy
21 Parasitology Reference and Research Laboratory, Spanish National Centre for Microbiology, Health Institute Carlos III, Majadahonda, Madrid, Spain; Infectious Diseases Unit, Maimonides Institute for Biomedical Research (IMIBIC), University Hospital Reina

Abstract

The increase of wild ungulate populations across Europe has raised concerns about their role in the spread of infectious diseases, particularly those with zoonotic significance. *Blastocystis* is a microeukaryote capable of colonising the gastrointestinal tract of humans and animals worldwide. The lack of studies concerning wild ungulates has led us to investigate *Blastocystis* occurrence and subtype (ST) diversity in the Iberian Peninsula. Faecal samples (*n* = 1292) from wild ungulate species were collected across Spain (*n* = 1059) and Portugal (*n* = 233) between 1998 and 2021. Using conventional PCR and next-generation amplicon sequencing, *Blastocystis* was found in 14.2% (184/1292; 95% CI: 12.4–16.3) of the samples analysed, including 15.3% (70/459; 95% CI: 12.1–18.9) of wild boars and 13.7% (114/833; 95% CI: 11.4–16.2%) of wild ruminants, respectively. *Blastocystis* carriage was significantly more frequent in Portuguese hosts than in Spanish hosts for both wild boars (34.3%, 34/99 *vs.* 10.0%, 36/360) and wild ruminants (38.1%, 51/134 *vs.* 9.0%, 63/699). Sixteen *Blastocystis* STs (ST2, ST5, ST10a/b, ST13, ST14, ST15, ST21, ST23, ST24a/b/c, ST25, ST26, ST30, ST31, ST42b, ST43, and ST44) were identified among the surveyed wild ungulate populations, with a higher variability of STs found in Spanish wild ruminants and in Portuguese wild boars. Mixed infections were found in 32.2% (46/143) of all *Blastocystis*-positive samples. ST5 was found in all *Blastocystis*-positive wild boars, supporting the host preference of this subtype. These results improve considerably our current understanding of the *Blastocystis* epidemiology and ST diversity in wild ungulates from the Iberian Peninsula, providing molecular-based evidence of i) cross-species transmission at the sylvatic/domestic interface and ii) *Blastocystis* ST preference for certain host species.

Keywords: *Blastocystis*, Iberian Peninsula, wildlife, molecular epidemiology, subtypes

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Session: [SE12] Parasites of wildlife

Presence and molecular diversity of zoonotic microsporidia in spleen samples from wild micromammals in Spain

Maria Rebollo[1], Sergio Sánchez[1], Fátima Vioque[1], Mónica Santín[2], Pamela C. Köster[1], Jesus T. García[3], Begoña Bailo[1], Pedro P. Olea[4], Fernando Arce[5], Francisco Ruiz-Fons[3], Javier Viñuela[3], Isabel Guadano Procesi[6], Carmen Chicharro[1], Javier Nieto[1], David González-Barrio[1], David Carmena[1], Alejandro Dashti[1]

1 Parasitology Reference and Research Laboratory, Spanish National Centre for Microbiology, Health Institute Carlos III, 28222 Majadahonda, Spain
2 Environmental Microbial and Food Safety Laboratory, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland, USA
3 Research Institute of Hunting Resources IREC (CSIC-UCLM-JCCM), 13071 Ciudad Real, Spain
4 Department of Ecology, Autonomous University of Madrid (UAM), 28049 Madrid, Spain; Center for Research on Biodiversity and Global Change (CIBC-UAM), Autonomous University of Madrid (UAM), 28049 Madrid, Spain
5 School of Natural Sciences, University of Tasmania, Hobart, TAS 7004, Australia
6 Department of Clinical Sciences and Translational Medicine, Faculty of Medicine, University of Rome "Tor Vergata", 00133 Rome, Italy

Abstract

Microsporidia is a diverse group of emerging, intracellular and spore-forming parasites that infect a wide range of hosts. Although the role of micromammals as reservoirs of zoonotic pathogens is well stablished, little is known on the presence, molecular diversity, and zoonotic potential of microsporidia in wild micromammals, particularly in extra-intestinal locations. Individual spleen samples ($n = 398$) from 11 micromammal species captured across the five Spanish bioregions were tested for the presence of the microsporidia *Enterocytozoon bieneusi* and *Encephalitozoon* spp. using molecular (PCR and Sanger sequencing) methods. Overall, microsporidia DNA was detected in 7.0% (28/398; 95% CI: 4.7–10.0) of the spleen samples investigated. Mono-infections by *Encephalitozoon* spp. or *E. bieneusi* were detected in 6.0% (24/398; 95% CI: 3.9–8.8) and 0.8% (3/398; 95% CI: 0.2–2.2) if the samples. An additional sample harboured a co-infection by *Encephalitozoon* spp. + *E. bieneusi* (0.3%, 1/398; 95% CI: 0.01–1.4). According to host, *Encephalitozoon* spp. mono-infections were identified in members of the genera *Microtus* ($n = 22$) and *Apodemus* ($n = 2$), and *E. bieneusi* mono-infections in members of the genera *Microtus* (n =1) and *Apodemus* ($n = 2$). The sample co-infected by *Encephalitozoon* spp. + *E. bieneusi* belonged to *Microtus arvalis*. Sequences analyses confirmed all *Encephalitozoon* spp. isolates as *E. cuniculi*, whereas the zoonotic genotype C ($n = 2$) was identified within *E. bieneusi*. Genotyping analyses of the 24 *E. cuniculi* isolates are still in progress. These results indicate that wild micromammals are reservoirs of zoonotic microsporidia species/genotypes. Although the extra-intestinal dissemination ability of *Encephalitozoon* spp. is widely known, this is one of the very few molecular-based surveys demonstrating that *E. bieneusi* is also able to infect organs other than the intestinal tract.The biological and clinical implications of this finding warrants further investigation.

Keywords: *Encephalitozoon*, *Enterocytozoon*, micromammals, molecular epidemiology, Spain

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Session: [SE12] Parasites of wildlife

Of mice and men: the role of micromammals in the circulation of zoonotic parasites *Capillaria hepatica* and *Trichinella* spp.

Zuzana Hurníková[1], Petronela Komorová[1], Yaroslav Syrota[2], Martina Miterpáková[1]

1 Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, Košice, Slovak Republic
2 Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, Košice, Slovak Republic; I. I. Schmalhausen Institute of Zoology NAS of Ukraine, B. Khmelnytsky street, 15, Kyiv, Ukraine

Abstract

Climate change and alterations of the epidemiological environments increase the potential for human-parasite contact. Micromammals are hosts and reservoirs for numerous parasite species transmissible to humans and domestic animals. Our research focused on the distribution of two zoonotic parasites - *Capillaria hepatica* and *Trichinella* spp. in small mammal populations in the Tatra National Park (TANAP) and urban environment of Košice City in Slovakia. In total 715 individuals of 18 Micromammalia species, mostly found dead, road-killed, or deceased during the pest control operation, were collected from 2018 to 2023 under permit No. 498/2018-6.3. ME SR. The artificial digestion method was used to detect the presence of *Capillaria hepatica* eggs in the livers and *Trichinella* spp. larvae in the muscle tissue. The eggs and larvae found were identified by morphometry, and the *Trichinella* isolates were genotyped via PCR according to Zarlenga et al. (1999). Capillarid eggs were found in the livers of 23 individuals belonging to 7 species (*Apodemus flavicollis, Arvicola terestris, Clethrionomys glareolus, Microtus* spp., *Neomys fodiens, Rattus norvegicus, Sorex araenus*). The total prevalence of *C. hepatica* infection was 3.22 %, however, it was significantly higher in TANAP (4.09%) than in Košice (0.56%) (95% CI: 2,00%), where only a single *R. norvegicus* was infected. *Trichinella* spp. larvae were detected in two *A. flavicollis*, one *A. agrarius*, and one *C. glareolus* specimen (0.56%). Prevalence was the same in TANAP (3/538) and Košice (1/177). *T. pseudospiralis* was confirmed in two individuals of *A. flavicollis* from TANAP, *T. britovi* in *C. glareolus* from the same area, and *A. agrarius* from Košice City. Our research has confirmed the presence of *C. hepatica* and *Trichinella* species in communally living micromammals, posing a risk to human health both in natural ecosystems and in urban areas.

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Keywords: Micromammalia, *Capillaria hepatica*, *Trichinella pseudospiralis*, *T. britovi*

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Session: [SE12] Parasites of wildlife

First observations of buzzards (*Buteo*) as definitive hosts of *Sarcocystis* parasites forming cysts in the brain tissues of rodents in Lithuania

Evelina Juozaityte-Ngugu, Marius Jasiulionis, Tautvilė Šukytė, Vitalijus Stirkė, Linas Balčiauskas, Dalius Butkauskas, Petras Prakas

Nature Research Centre, Akademijos 2, LT-08412 Vilnius, Lithuania

Abstract

Protists of the genus *Sarcocystis* are cyst forming coccidians with an obligatory prey-predator two-host life cycle. Sarcocysts are formed in muscles of intermediate hosts (prey) and sporocysts develop in small intestines of definite hosts (predator). Two *Sarcocystis* spp., *S. glareoli* and *S. microti*, form cysts in the brains of rodents and are transmitted by the common buzzard (*Buteo buteo*). In the present study, brain samples of 694 small mammals caught in different regions of Lithuania were examined for *Sarcocystis* spp. In parallel, ten *B. buteo* and two rough-legged buzzards (*Buteo lagopus*) were tested for sporocysts of the analysed parasites. Eleven species of small mammals were tested for the presence of *Sarcocystis* spp. Based on 28S rRNA sequence comparison, *S. glareoli* was detected only in one small mammals species, the bank vole (*Clethrionomys glareolus*). Sarcocysts were detected in 34 out of 374 *C. glareolus* (9.1%, 95% CI = 6.4–12.5%). Merely, by molecular analysis *S. glareoli* was detected in the

intestines of fifty percent of common buzzard. In addition, two birds of *B. lagopus* were confirmed as potential definitive host for *Sarcocystis* sp. Rod3 and *Sarcocystis* sp. Rod4. Our findings highlight the need for more research on the rodent-bird cycle of *Sarcocystis* spp.

Keywords: *Sarcocystis*, small mammals, *Buteo*, 28S rRNA

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Session: [SE12] Parasites of wildlife

Differences in the structure of the juvenile stages of the common *Hoplopleura* lice (Psocodea: Anoplura) in Europe

Paulina Kozina, Joanna N. Izdebska

University of Gdańsk, Faculty of Biology, Department of Invertebrate Zoology and Parasitology, Wita Stwosza 59, 80-308 Gdańsk, Poland

Abstract

More than 540 species of sucking lice have been described worldwide. These obligate, wingless parasites are permanently attached to their hosts, either mono- or oligoxenically. Every change of host by a louse results in the transfer of microorganisms. While the potential of Anoplura as a disease vector has been studied previously, there is nevertheless a need for strict identification of hosts and parasites, including juvenile stages, for the mechanism to be properly understood. While lice associated with humans are well studied, those associated with small mammals have been overlooked because they were thought to present little threat to commercial animals or humans. However, by tracing the pathways of disease spread, small mammals are a significant source of microbial transmission to livestock or humans, through other parasites. With Anoplura as an important vector.

The most commonly-observed louse genus in small mammals is *Hoplopleura*. In Europe, five species are mostly recorded, with all described as juvenile stages. This paper aims to summarize the work related to the characterization of these nymphs and identify any differences that may be helpful for identification.

H. longula is easily distinguishable by its long PDHS. The other species have definitely shorter bristles. The juvenile stages of *H. longula* can be distinguished by the shape of the abdomen and the number of MAS. *H. affinis* and *H. captiosa* both have a distinctive heart-shaped abdomen: those of other species are round or barrel-shaped. The nymphs of *H. captiosa* have longer PDHS than those of *H. affinis;* also *H. captiosa* has a constant number of MAS, while those of *H. affinis* vary. The particular nymphal stages differ in the number of MAS, AcS and AnS. The greatest problems are provided by distinguishing between the nymphs of *H. acanthopus* and *H. edentula:* only small differences can be found, and it is advisable to gain confidence in identification by also finding adults nearby.

Keywords: sucking lice, Anoplura, *Hoplopleura*, juwenile stages, Europe

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Session: [SE12] Parasites of wildlife

Current data on the parasitofauna of the grey seal *Halichoerus grypus* (Carnivora: Phocidae) from the southern Baltic Sea

Leszek Rolbiecki[1], Joanna N. Izdebska[1], Paulina Kozina[1], Joanna Dzido[1], Tytus Kuczkowski[1], Sławomira Fryderyk[1], Iwona Pawliczka[2], Karolina Cierocka[1]

1 University of Gdańsk, Faculty of Biology, Department of Invertebrate Zoology and Parasitology, Wita Stwosza 59, 80-308 Gdańsk, Poland
2 University of Gdańsk, Faculty of Oceanography and Geography, Prof. Krzysztof Skóra Hel Marine Station, Morska 2, 84-150 Hel, Poland

Abstract

Most previous studies on the pinniped parasitofauna have been fragmentary, being concerned with selected groups or based on small samples. The Phocidae are well represented in the Baltic Sea by the grey seal, which provides an opportunity for more comprehensive analyses. The current study included 72 *Halichoerus grypus* from the Baltic Sea, obtained either by catch or found dead on shore. The seals were subjected to parasitological dissection, with the samples prepared using methods appropriate for particular groups of ecto-, meso- and endoparasites. So far, the findings indicate the presence of tapeworms of the genus *Diphyllobothrium*, the nematodes *Pseudoterranova decipiens, Contracaecum osculatum*, *Acanthocheilonema spirocauda*, Nematoda n.det., the acanthocephalans *Corynosoma semerme, C. strumosum, Corynosoma* sp. and unidentified Digenea. Arthropods were also found: the sucking louse *Echinophthirus horridus,* and the mites *Halarachne halichoeri* (from the respiratory tract) and *Demodex* sp., the latter of which may be new to science. This is a valuable discovery because only two species of the skin mite are known in pinnipeds, both from Demodecidae.

Although the parasites were common in the seals (total infection 100%), the level of infection varied with regard to individual parasite species and seasonal dynamics; it was also influenced by host categories (sex, age). The parasites showed clear topographic or topical preferences, optimally using environmental resources provided by the host's body. Thus, the lice preferred the head and front of the body, the mites *H. halichoeri* the front sections of the respiratory tract, the demodicids favored the hairy skin, and the acanthocephalans selected sections of the intestines. Comprehensive, long-term parasitological studies of seals are crucial both to determine the threats to their populations, and to fully understand the mechanisms of parasite circulation in the Baltic marine environment, which currently remains unclear.

Keywords: Baltic Sea, grey seal, helminths, *Halichoerus grypus*, parasitic arthropods

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Session: [SE12] Parasites of wildlife

Molecular analysis of vector-borne pathogens in Eurasian badgers (*Meles meles*) from Europe

Zoe Tess Lara Lindhorst[1], Sebastian Brandstetter[1], Maria Sophia Unterköfler[1], Barbara Eigner[1], Joachim Spergser[2], Marc Colyn[3], Peter Steinbach[4], Duško Ćirović[5], Nikica Sprem[6], Tomislav Dumić[7], Vincenzo Veneziano[8], Franz Müller[9], Georgiana Deak[10], Angela M. Ionica[11], Mike Heddergott[12], Hans-Peter Fuehrer[1]

1 Institute of Parasitology, Department of Biological Sciences and Pathobiology, University of Veterinary Medicine Vienna, Austria
2 Institute of Microbiology, Department of Biological Sciences and Pathobiology, University of Veterinary Medicine Vienna, Austria
3 Université de Rennes 1, CNRS, UMR 6553 Ecobio, Station Biologique, Paimpont, France
4 Department of Zoology, Musée National d'Histoire Naturelle, Luxembourg; Faculty of Chemistry, Georg-August University of Göttingen, Germany
5 Faculty of Biology, University of Belgrade, Belgrade, Serbia
6 Department of Fisheries, Apiculture, Wildlife Management and Special Zoology, Faculty of Agriculture, University of Zagreb, Zagreb, Croatia
7 Department of Wildlife Management and Nature Conservation, Karlovac University of Applied Sciences, Karlovac, Croatia
8 Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Naples, Italy
9 Wildlife Biology Working Group, Justus-Liebig-Universität Gießen, Giessen, Germany
10 Department of Parasitology and Parasitic Diseases, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca

11 Clinical Hospital of Infectious Diseases of Cluj-Napoca, Romania
12 Department of Zoology, Musée National d'Histoire Naturelle, Luxembourg

Abstract
Vector-borne pathogens (VBPs) are becoming increasingly important in both veterinary medicine and public health, with wildlife playing a crucial role in their transmission. To date there is limited data available on the prevalence of VBPs in Eurasian badgers (*Meles meles*). Our research aimed to investigate the presence of Anaplasmatacea, *Bartonella* spp., *Mycoplasma* spp., *Rickettsia* spp., piroplasms, Trypanosomatida and Filarioidea in Eurasian badgers. The results are used to assess the potential risk to domestic animals, other wildlife, and humans.

Between 2017 and 2021, we collected blood or spleen samples from 220 Eurasian badgers in nine European countries. For the identification of VBPs, we subjected the samples to PCR analysis and Sanger sequencing. Pathogens identified included *Babesia* sp. badger type A (54%), B (23%), and C (37%), *Trypanosoma pestanai* (56%), *Mycoplasma* sp. (34%), *Candidatus* Mycoplasma haematomelis (8%), *Candidatus* Mycoplasma haematominutum (0.5%), and *Ehrlichia* spp. (2%). However, *Rickettsia* spp., *Bartonella* spp., and filarioid nematodes were not detected in the samples tested. The highest prevalence of pathogens was found in Austria, Bosnia and Herzegovina, Croatia, Germany, and Hungary (each 100%), followed by Romania (94%), Serbia (92%), France (84%), and Italy (63%). The extensive sample size and diverse study populations in our research provide valuable insights into the distribution and epidemiology of the pathogens analyzed. Some of the vector-borne pathogens identified in our study show high similarity to those found in domestic animals. This finding suggests that badgers, as potential reservoirs for these pathogens, may pose a threat not only to other wildlife but also to domestic animals in close vicinity. Continuous surveillance is essential to monitor VBPs in wildlife and to facilitate the evaluation of their impact on other wildlife species, domestic animals, and human health.

Keywords: vector-borne pathogens, badger, *Babesia*, *Trypanosoma*, *Mycoplasma*

PS – poster session

Session: [SE12] Parasites of wildlife

First identification of multiple *Blastocystis* subtypes in marine turtles and cetaceans from Mediterranean Sea using amplicon-based next generation sequencing

Renato Aco-Alburqueque[1], Marialetizia Palomba[2], Veronica Rodriguez Fernandez[3], Tiziana Castrignanò[2], Meryam Carrus[2], Federica Marcer[4], Erika Marchiori[5], Mario Santoro[6], Simonetta Mattiucci[1]

1 Department of Public Health and Infectious Diseases, Section of Parasitology, Sapienza University of Rome / Rome, Italy
2 Department of Ecological and Biological Sciences, Tuscia University / Viterbo
3 Department of Public Health and Infectious Diseases, Sapienza-University of Rome Piazzale Aldo Moro, 5 00185 Rome, Italy
4 Department of Animal Medicine, Production and Health, School of Agricultural Sciences and Veterinary Medicine, University of Padua. Padua, Italy
5 Department of Animal Medicine, Production and Health, School of Agricultural Sciences and Veterinary Medicine, University of Padua. Padua, Italy.
6 Department of Integrative Marine Ecology, Stazione zoologica Anton Dohrn, Napoli

Abstract
Blastocystis is widespread in various terrestrial animal hosts, including humans with significant genetic heterogeneity, with 37 distinct subtypes (STs) detected to date. However, reports of Blastocystis in marine animals, such as fish and marine mammals, are rare. In the present study, we have characterized the subtype distribution of *Blastocystis* in 25 frozen stool samples collected from the rectum of 5 cetacean species: *Balaenoptera physalus* (n= 4), *Physeter macrocephalus* (n=3), *Grampus griseus* (n=2), *Stenella ceruleoalba* (n=3) and *Tursiops truncatus* (n=15). Additionally, 61 fecal samples were obtained from wild loggerhead sea turtles (*Caretta caretta*). Samples were collected from stranded individuals along the Mediterranean Sea coast between 2011 and 2021. A molecular survey was performed using a qPCR assay targeting the small subunit ribosomal RNA. PCR positive samples were then sequenced by next generation amplicon sequencing to determine the STs. Fourteen STs were detected with the following relative proportions: ST1 (2%), ST3 (4%), ST4 (57%), ST5 (4%), ST6 (5%), ST8 (6%), ST10 (16%), ST12 (8%), ST21 (8%), ST23 (28.6%), ST24 (12.2%), ST25 (4%), ST27 (2%), ST30 (2%). Among the samples, 72% of the marine turtles were colonized by 14 different STs, including 3 potential new STs. Regarding cetaceans, *B. physalus* was colonized by 4 different STs. Mixed infections were identified in most samples, indicating a high biodiversity of subtypes in both marine taxa. Individuals were found to be infected by STs common to humans and terrestrial farm animals. Additionally, there were subtypes overlap between different host species. This study represents the first molecular characterization of *Blastocystis* STs in cetaceans and marine turtles from Mediterranean Sea waters, using NGS sequencing. The presence of STs from humans and terrestrial, mainly farmed, animals suggest contamination of the marine ecosystem by freshwater runoff.

Granted by Latium Region-Italy

Keywords: *Blastocystis*, Marine turtles, marine mammals, Mediterranean Sea, NGS

PS – poster session

Session: [SE12] Parasites of wildlife

Helminths of *Sylvia* spp. collected during spring migration of birds on the south-eastern flyway

Izabella Rząd[1], Przemysław Busse[2], Simon Awad[3], Jiljí Sitko[4], Rusłan Sałamatin[5,6], Anna Okulewicz[7]

1 University of Szczecin, Institute of Marine and Environmental Sciences, Poland
2 Bird Migration Research Foundation, Choczewo, Poland
3 Environmental Education Center EEC/ELCJHL Jerusalem, Palestinian Territory
4 Comenius Museum, Moravian Ornithological Station, Přerov, Czech Republic
5 Department of Microbiology and Parasitology, Faculty of Medicine. Collegium Medicum, Cardinal Stefan Wyszyński University in Warsaw, Poland
6 Department of General Biology and Parasitology, Medical University of Warsaw, Warsaw, Poland
7 Department of Parasitology, Faculty of Biological Sciences, University of Wrocław, Poland

Abstract
The *Sylvia* species examined (Aves, Passeriformes) are migrating birds with a varied migratory lifestyle: *Sylvia borin* and *Sylvia communis* are long-distance migrants that winter in the eastern part of southern Africa. *Sylvia atricapilla* winters there as well, but some of these birds stop in the Mediterranean region in winter, including Palestine/Israel, where some of the research was carried out. *Sylvia curruca* winters in north-eastern Africa. The birds examined in the study belonged to populations nesting in Central and/or Northern Europe. Only *Sylvia hortensis* is found only in the Mediterranean region.The aim of the study was to obtain information on the occurrence of helminths in birds of the genus *Sylvia*. The birds included in the research, of the species *Sylvia atricapila*, *S. curruca*, *S. hortensis*, *S. communis*, and *S. borin*, were acquired in 2012–2015 in various locations – in Poland (Baltic coast, 58 birds) and Israel (Tulkarem, Jericho, and Talitha Kumi, 39 birds),

during the breeding season (Poland) and during spring migration. The birds had died due to various chance causes. Parasitological examination of the birds was performed. Helminths were fixed in 70–75% ethyl alcohol for further analysis. Cestodes (Cestoda), trematodes (Digenea), nematodes (Nematoda), and acanthocephalans (Acantocephala) were detected. Nematodes were located mainly in the body cavity and air sacs of the birds, trematodes in the intestine and hepatic ducts, and cestodes and acanthocephalans in the intestine. Knowledge of parasites of birds of the genus *Sylvia* is inadequate, and therefore examination of even single individuals obtained as a result of random events is justified.

Co-financed by the Minister of Science under the "Regional Excellence Initiative" Program for 2024-2027 (RID/SP/0045/2024/01.Material collected within SE European Bird Migration Network field work.

Keywords: *Sylvia*, helminths, migration

PS – poster session

Session: [SE12] Parasites of wildlife

Prevalence of lung nematodes *Eucoleus aerophilus* in red foxes in selected regions of Poland

Małgorzata Samorek-Pieróg[1], Tomasz Cencek[1], Ewa Bilska-Zając[1], Jacek Sroka[1], Paweł Gładysz[2], Anna Lass[2], Jacek Karamon[1]

1 National Veterinary Research Institute, Puławy, Poland
2 Medical University of Gdańsk, Poland

Abstract

Eucoleus aerophilus (syn. *Capillaria aerophila*) is a parasite with zoonotic potential and global distribution. Infection of this nematode is a causative factor of lung capillariosis of wild (e.g. foxes, wolves, coyotes) and domestic (dogs, cats) carnivores; moreover it has sporadically affected humans.

The aim of this study was to evaluate the prevalence of *E. aerophilus* in the red fox populations from selected regions of Poland. Moreover, sequence variation in partial *cox*1 gene of *E. aerophilus,* recovered from stool samples was described.

An investigation was carried out using 432 faeces samples of red foxes from different parts of Poland (2021-2023). DNA from faeces were investigated by semi-nested PCR to amplify the diagnostic region within *cox*1 gene of *E. aerophilus*. Obtained PCR products were sequenced and phylogenetic analyzes of received amplicons were performed in Geneious R11. Additionally, coproscopic study was conducted.

Amplification of partial sequences of *cox*1 gene was successful in case of 244 foxes. A comparison of the sequencing results of the obtained amplicons, using the GenBank database, confirmed DNA of *E. aerophilus* in 231 samples, mean prevalence in all investigated areas was 68%. In case of 14 amplicons, comparison of the sequencing results with the GenBank database revealed DNA of *E. boehmi*.

The molecular analysis of retrieved sequences revealed 22 haplotypes of *E. aerophilus*: EaPL1 – EaPL22 and 4 haplotypes of *E. boehmi:* EbPL1 – EbPL4.

Coproscopic examination revealed that the most prevalent in all regions were the eggs of Capillaridae family, with mean prevalence of 73%. Furthermore, there were detected eggs of: Taenidae, *Toxocara* spp., *Toxascaris leonina, Trichuris vulpis,* trematodes, hookworms and *Mesocestoides* spp.

This study provides an evaluation of the prevalence of *E. aerophilus* in the red fox population in selected regions of Poland and deeper insights of the genetic diversity of this parasite from investigated regions.

Keywords: *Eucoleus aerophilus*, haplotypes, genetic diversity, Poland

PS – poster session

Session: [SE12] Parasites of wildlife

Anisakidea nematodes in the grey seals (*Halichoerus grypus*) and fish of the Eastern Baltic Sea

Maija Seleznova[1], Maira Mateusa[1], Ēriks Krūze[1], Alma Magdalēna Zalcmane[2], Darja Hudjakova[2], Ivars Putnis[1], Gunita Deksne[1]

1 Institute of Food Safety, Animal Health and Environment "BIOR", Riga, Latvia
2 Faculty of Biology, University of Latvia, Riga, Latvia

Abstract

The grey seal (*Halichoerus grypus*) is a fish-eating marine mammal native to the Baltic Sea. It is the final host to Anisakidae nematodes, which use fish as paratenic and intermediate hosts and cause anisakidosis in humans, manifesting as gastrointestinal disease or allergies. The study aimed to observe the prevalence of Anisakidae nematodes and their species in grey seals and in five fish species in the Eastern part of the Baltic Sea.

During 2018-2023, a total of 59 by-caught grey seals from the Latvian commercial coastal fishery, 1946 whole Eastern Baltic cods (*Gadus morhua*) and livers, 1292 herrings (*Clupea harengus membras*), 891 sprats (*Sprattus sprattus*), 356 round gobies (*Neogobius melanostomus*) and 69 sea trouts (*Salmo trutta*) from ICES subdivisions SD26 and SD28 were collected. Samples were examined for the presence of Anisakidae nematodes by determining typical morphology and confirmed using molecular methods targeting ITS-1, 5.8S, ITS-2, 28S genes.

All seals were infected with Anisakidae nematodes, with the median infection intensity 299 (range 6-10090). Eastern Baltic cod had the highest Anisakidae prevalence – 30.9% with a median infection intensity of 3 (range 1-113). The infection prevalence in herring, sprats, and round gobies was in the range of 2.6-3.9% with a median infection intensity of 1 (range 1-2). No Anisakidea were found in sea trouts. Two Anisakidae species – *Contracaecum osculatum* and *Pseudoterranova decipiens* – were found in seals, whereas only *C. osculatum* larvae were identified in fish.

The results show that adult grey seals are a source of Anisakidae nematode infections in the Baltic Sea fish and this is of Public Health concern. The prevalence and parasite infection intensity varies in different fish species and may depend on the feeding habits and lifestyle of the fish.

Funding: Fundamental and applied research "Baltic cod (Gadus morhua) condition and health status in the changing ecosystem of Eastern Baltic: CODHEALTH" (lzp2021/1-0024).

Keywords: Anisakidae, *Contracaecum*, *Pseudoterranova*, cod, grey seal

PS – poster session

Session: [SE12] Parasites of wildlife

The helminth fauna of rodents in Slovakia: a historical retrospective

Yaroslav Syrota[1], Zuzana Hurníková[2], Vitalii Kharchenko[3], Martina Miterpáková[2]

1 Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, Košice, Slovak Republic; I. I. Schmalhausen Institute of Zoology NAS of Ukraine, B. Khmelnytsky street, 15, Kyiv, Ukraine
2 Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, Košice, Slovak Republic
3 I. I. Schmalhausen Institute of Zoology NAS of Ukraine, B. Khmelnytsky street, 15, Kyiv, Ukraine

Abstract

A search in scientometric databases has revealed that information about helminths of rodents in Slovakia available to the research community is very limited. However, a significant volume of data

was found by searching the final reports of the projects implemented at the Institute of Parasitology of Slovak Academy of Science and the study of regional publications not indexed in scientometric databases. The summarising of the collected data revealed that 80 species of helminths have been registered in Slovakia, found in 20 species of Rodentia. The registered parasites represent four groups: nematodes (30 species), cestodes (34 species), trematodes (15 species), and acanthocephalans (one species). Most data were collected in the second half of the 20th century, while modern research in the 21st century has been sporadic. Given the ecological importance of rodents and their role in the circulation of pathogens affecting humans and domestic animals, including the potential of some rodent helminths to be pathogenic for humans, new studies are necessary to update knowledge about this group of parasites in the region.

The research work was supported by the project APVV-21-0166 and the EUNextGenerationEU through the Recovery and Resilience Plan for Slovakia under project No 09I03-03-V01-00046.

Keywords: helminths, rodents, parasites, Slovak Republic

PS – poster session

Session: [SE12] Parasites of wildlife

First description of ectoparasitic mite *Hemipteroseius adleri* (Acari: Otopheidomenidae) on the red firebug *Pyrrhocoris apterus* (Heteroptera: Pyrrhocoridae) in Croatia

Iva Štimac[1], Franjo Martinković[1]

1 Faculty of Veterinary Medicine University of Zagreb

Abstract

Members of the mite family Otopheidomenidae (Acari: Mesostigmata) are ectoparasites of insects, primarily Hemiptera, Orthoptera, and Lepidoptera. *Hemipteroseius adleri* is the only mite species from the genus *Hemipteroseiu*s of the family Otopheidomenidae described in Europe (Poland, Lithuania, Czech Republic, Hungary, Slovakia, and Bulgaria) and in the Middle East (Israel) in association with the red firebug (*Pyrrhocoris apterus*). Given that there is no morphological data on *H. adleri* in the Republic of Croatia, the aim of this research was to identify mites found on *P. apterus* based on their morphological characteristics. The samples of red firebug adult males and females were collected in the park Maksimir (Zagreb), Republic of Croatia on one occasion in April 2020 year for the purpose of forming laboratory colonies. A total of 118 adults, 81 males and 37 females, were collected in two locations and kept in two separate plastic 5.89 liter containers given the location from which they were collected. Colonies were grown on sunflower and linden seeds. Water was available *ad libitum* in small ceramic dishes plugged with cotton wool and changed every other day. Mite infestation was first noticed during the examination of dead adult insects removed from the containers, using a lamp with magnifier and Dino-Lite Premier digital microscope. Mites were located under the wings, near the wing base. After several weeks, mites were observed on the surface of the wings and in some cases on the ventral side of the thorax of some living specimens. Collected mites were mounted on a microscope slides in lactophenol solution for detailed study. According to the keys and descriptions known so far in the scientific literature, different developmental stages of mite *H. adleri* were determined. By August 2020, all collected insect samples were examined and the prevalence of *H. adleri* infestation was found to be 41.5%.

Keywords: *Hemipteroseius adleri*, *Pyrrhocoris apterus*, insect parasites, Croatia

PS – poster session

Session: [SE12] Parasites of wildlife

Molecular based identification of *Accipiter gentilis*, *Accipiter nisus* and *Buteo buteo* as possible definitive hosts of numerous *Sarcocystis* spp.

Tautvilė Šukytė, Evelina Juozaitytė-Ngugu, Petras Prakas, Dalius Butkauskas

Nature Research Centre (Lithuania)

Abstract

Sarcocystis are apicomplexan parasites characterised by an obligatory prey-predator two-host life cycle. Sarcocysts are formed mainly in muscles of intermediate hosts, while sporocysts develop in small intestine of definitive host. In the period of 2016-2023, 16 northern goshawks (*Accipiter gentilis*), 16 Eurasian sparrowhawks (*Accipiter nisus*) and 30 common buzzards (*Buteo buteo*) were analysed for oocysts/sporocysts of *Sarcocystis* spp. Parasites were identified by DNA extraction from intestinal scrapings, species-specific nested PCR targeting the ITS1 region followed by Sanger sequencing.

Infection rates were found to be similar in goshawks (12/16, 75.0%) and sparrowhawks (11/16, 68.8%). Eight distinct *Sarcocystis* species, namely *S. calchasi*, *S. columbae*, *S. cornixi*, *S. halieti*, *S. kutkienae*, *S. lari*, *S. turdusi*, and *S. wobeseri* were identified, with the first-time detection of *S. calchasi* in Lithuania. Additionally, a genetically novel species, named as *Sarcocystis* spp. 23LTAcc, sharing close genetic relationship with *S. calchasi*, was identified in three specimens of goshawks. Moreover, the study represents the primary confirmation of *S. kutkienae* and *S. wobeseri* in *Accipiter* hawks.

Infection rate in common buzzards were lower than in hawks and reached 50.0% (15/30). Most of positive cases were due to *S. halieti* infection (36.7%, 11/30), followed by *S. wobeseri* (23.3%, 7/30), *S. kutkienae* (6.7%, 2/30), *S. turdusi* (3.3%, 1/30), and *S. cornixi* (3.3%, 1/30). These species were for the first time identified in common buzzards worldwide. Co-infections of different *Sarcocystis* species were observed in 16.6% (5/30) of buzzards in 18.8% (3/16) of sparrowhawks, and in 75.0% (12/16) of goshawks.

The current study shows the important role of bird of prey in the transmission of *Sarcocystis* parasites producing sarcocysts in muscles of birds. The use of specific primers provides a fast method for the identification of *Sarcocystis* species in intestines of predators.

Keywords: *Sarcocystis*, buzzard, gohawk, sparrowhawk, accipiter

PS – poster session

[SE13]
Parasitology research from One Health perspective

Session: [SE13] Parasitology research from One Health perspective

Primary cerebral cystic echinococcosis in a child from Roman countryside (source attribution and scoping review)

Adriano Casulli, Azzurra Santoro, Federica Santolamazza

ISTITUTO SUPERIORE DI SANITA; / EU Reference Lab Parasites / WHO CC Echinococcosis (Italy)

Abstract

Human cystic echinococcosis (CE) is a zoonotic parasitic infection caused by the larval stage of the species belonging to the *Echinococcus granulosus sensu lato* (*s.l.*) complex. Parasitic cysts causing human CE are mainly localized in the liver and in the lungs. In a smaller number of cases, larvae may establish in any organ or tissue, including the central nervous system (CNS). Cerebral CE (CCE) is rare but poses serious clinical challenges.

This study presents a case of CCE in a child living in the countryside near Rome (Italy), along with a comparative molecular analysis of the isolated cyst specimens from the patient and sheep of local farms. We also systematically searched the literature to summarize the most relevant epidemiological and clinical aspects of this uncommon localization.

The comparative molecular analysis confirmed that the infection was caused by *E. granulosus sensu stricto* (*s.s.*) (G3 genotype). The infection was most likely acquired in the family farm since data showed a clear and direct genetic relationship between the child's cyst and the cysts belonging to sheep of his own family's farm. In particular two sheep cyst sequences were identical (100%) to those of the child.

The literature search identified 2,238 cases of CCE. In 80.51% of cases, brain was the only localization and single CCE cysts were present in 84.07% of cases. Cyst rupture was reported in 12.96% and recurrence of CCE after treatment in 9.61% of cases. Permanent disability was reported in 7.86% of cases, while death occurred in 6.21%. When considering clinical centres reporting all anatomical sites of CE, liver represented 70%, lungs 19%, and unusual localizations 11% of all CE cases. In case series reporting all CE localization, CCE represented 1.5% of all CE cases.

The proportions of CE cases with uncommon localizations and with high impact on patients' lives have been globally neglected and should be included in the computation of the global burden of CE.

Keywords: *Echinococcus granulosus s.s.*, cerebral cystic echinococcosis, source attribution

OS – oral session

Session: [SE13] Parasitology research from One Health perspective

A multilocus genotyping scheme for *Cryptosporidium parvum* that meets the One Health challenge

Rachel Chalmers[1], Guy Robinson[1], Harriet Risby[1], Nastassya Chandra[2], Grace King[2]

1 Public Health Wales, UK
2 UK Health Security Agency, UK

Abstract

Cryptosporidium parvum causes about half of all *Cryptosporidium* outbreaks in England and Wales, and is the aetiological agent of a high proportion of outbreaks of gastrointestinal illness linked to open farms or on-farm events attended by the public. Outbreaks range in size and may be overlooked by disease surveillance if epidemiological and case exposure data are missing. Case-clustering by genetic analysis of infecting organisms is used to identify clusters and outbreaks of bacterial infections such as *Salmonella*, and may be a useful approach for *C. parvum*. Since March 2022, we have pioneered the use of Multilocus Variable Number of Tandem Repeats Analysis (MLVA) for this purpose with good effect. For example, in the first 18 months of the project in Wales and the North West of England, 1004 *C. parvum* case specimens were analysed. When ≥3 case-clusters were considered, 58 clusters involving 328 cases were identified. Some were household contacts or lived or worked on farms but one cluster signalled an outbreak subsequently found to involve nine laboratory confirmed cases. In six known outbreaks MLVA confirmed links or identified additional cases. MLVA is also used to investigate outbreaks and exceedances outside the pilot area, helping to define the extent of outbreaks and highlighting where public health action was needed. Sampling animal faeces at implicated premises has also resulted in molecular microbiological confirmation of a link between cryptosporidiosis cases and suspected sources. This research provided a One Health approach to the evidence for using MLVA typing of *C. parvum* in health protection activities.

Zoonoses team at the Public Health Wales Communicable Disease Surveillance Centre, Field Services UK Health Security Agency North West, and the members of the Outbreak Control Teams.

National Institute for Health Research (NIHR) Health Protection Research Unit in Gastrointestinal Infections.

Keywords: *Cryptosporidium parvum*, subtype, MLVA, outbreak

OS – oral session

Session: [SE13] Parasitology research from One Health perspective

Epidemiology of cryptosporidiosis in France and new insights on parasitic viruses - importance of one health approach

Damien Costa[1], Loic Favennec[1], Romy Razakandrainibe[1], Aurélie Chevillot[2], E Carvin[2], Pierrick Lucas[3], Yannick Blanchard[3], Houria Louifi[2], Razika Arab[2], Mohamed Mammeri[2], Myriam Thomas[2], Bruno Polack[2], Gregory Karadjian[2], Nolwenn Dheilly[4], Karim Adjou[5]

1 Université de Rouen, EA7510 ESCAPE, CHU de Rouen, CNR Cryptosporidioses, Microsporidies et Autres Protozooses digestives, France
2 Anses – Laboratoire de Ploufragan-Plounazé-Niort, Unité de Génétique virale et Biosécurité, Ploufragan, France.
3 Anses Laboratoire de Santé Animale, UMR1161 de Virologie, INRAE, Anses, ENVA, Maisons-Alfort, F-94700, France.
4 Anses Animal Health Laboratory, UMR1161 Virology, INRAE, Anses, ENVA, Maisons-Alfort, France.
5 UMR BIPAR, Ecole Nationale Vétérinaire d'Alfort, Anses, INRAE, Université Paris-Est, Maisons-Alfort, F-94700, France

Abstract

Cryptosporidium zoonotic transmission is important in countries where livestock farming is extensive. Transmission occurs by direct or indirect fecal-oral route. In France, cryptosporidiosis is not a notifiable disease but a national reference center was designated from 2017. From 2017 to 2023, the number of human reported cases increased from 151 to 1607 respectively. Cryptosporidiosis occurred predominantly in young children and in young adults, especially during late summer. Most patients were immunocompetent (80%), and deaths were reported only in immunocompromised patients. Main reported risk factors are water exposure (both consumption and exposition during recreative contact). *C. parvum* was predominant over *C. hominis* but the *C. hominis* IfA12G1 subtype is emerging. *C. parvum* GP60 subtypes IIa and IId were the most represented suggesting frequent zoonotic transmission. In France,among outbreaks with identified origins: water was predominant followed by consumption of contaminated food. Such contaminations are strongly linked to environmental contamination For outbreak investigations, new tools enable better detection of mix of species or discrimination of stools such as NGS or MLVA analysis. In addition, analysis of potential associations between strains of *Cryptosporidium* and parasitic viruses could be interesting. Studies have revealed that *Cryptosporidium* oocysts contain dsRNA classified in the *Partitiviridae* family and the *Cryspovirus* genus. The aim of the study in UMR Bipar was: i) to look for the presence of *Cryspovirus* in *Cryptosporidium* in the faeces of ruminants and humans. ii) to characterise *Cryspovirus* at molecular level. The data showed for the first time the presence of *Cryspovirus* in France in humans, calves, lambs and goat kids (prevalence >95%). Phylogenetic analysis showed the existence of several clades, suggesting that CSpV1 could be a molecular tool for tracing *C. parvum* at national level.

Keywords: *Cryptosporidium*, One Health, *Cryspovirus*, epidemiology

OS – oral session

Session: [SE13] Parasitology research from One Health perspective

Cattle and sheep as sources of environmental contamination with *Cryptosporidium parvum* oocysts: a risk for human infection

Frank Katzer, Paul M. Bartley

Moredun Research Institute

Abstract

Cryptosporidiosis is a significant disease in humans and in neonatal ruminants. Human cryptosporidiosis is mainly caused by either *C. hominis* or *C. parvum*. While there are several species of *Cryptosporidium* that infect ruminants, the only species that causes neonatal diarrhoea is *C. parvum*. *Cryptosporidium* infection is the most frequently diagnosed cause of neonatal enteritis in calves and sheep in the UK and can lead to mortality; reduced growth; and increased labour/veterinary costs.

Parasite transmission from animals to humans can occur: 1) directly by contact of people with infected animals or 2) indirectly, where animals contaminate the environment. Oocysts can be washed by rain into water courses potentially contaminating drinking or recreational water.

In the UK, human cases of *C. parvum* infection peak in spring, which coincides with lambing and calving. Adult cattle and sheep can also be infected with *C. parvum*. The oocyst shedding may be much lower in adult animals, but as few as 10 oocysts are sufficient to infect a person or a neonatal calf or lamb.

Catchment studies demonstrated that ruminants can contaminate surface waters with *C. parvum*. Interestingly we often found more *C. parvum* than other *Cryptosporidium* species/isolates, which may be due to the parasite's ability to infect multiple host species and infected hosts shedding larger numbers of *C. parvum* oocysts.

Currently there are two chemotherapeutic compounds licensed in Europe for the treatment of cryptosporidiosis in livestock. The drugs supress oocyst shedding during treatment, but re-infected animals can still shed significant oocyst numbers. A new vaccine against cryptosporidiosis in calves is being marketed, which claims to reduce the severity of the infection and reduces oocyst shedding ten-fold from about 1x1010 to 1x109 oocysts per infected calf. These treatments help the farmers but may only have a limited effect on the levels of environmental contamination with *C. parvum* oocysts by ruminants.

Keywords: *Cryptosporidium*, One Health, sheep, cattle

OS – oral session

Session: [SE13] Parasitology research from One Health perspective

Giardia duodenalis - has molecular biology convinced us whether or not this is a zoonotic parasite

Lucy Robertson

Norwegian University of Life Sciences, Norway

Abstract

The intestinal protozoan parasite, *Giardia duodenalis*, is currently divided into several (A-H) genotype groups, also known as assemblages, based on host specificity. Over a decade ago, in 2009, it was proposed that these morphologically identical, but genetically distinctive, assemblages should be re-described as individual species. However, for a variety of reasons this proposal has never been widely accepted, and, in general, publications refer to the species as being *Giardia duodenalis* and, where molecular characterisation has been performed, the assemblage or genotype. The latter may be at a single gene, but is more often multi-locus. Some researchers have even defined sub-groups of the main assemblages based on results from multi-locus results.

The finding of morphologically identical *Giardia* cysts in different animal species, and also in humans, led researchers to assume that this was a zoonotic parasite, and initial molecular work supported this to some extent. However, as molecular characterisation has matured and expanded, there is increasing evidence that zoonotic transmission of *Giardia* infection occurs, in general, rarely.

In this presentation, state-of-the-art evidence will be reviewed within the context of our previous understanding of *Giardia* transmission. The aim of the presentation will be to reach a consensual understanding of whether zoonotic transmission of *Giardia* occurs at all, and, if it does, the extent to which this happens.

Keywords: *Giardia*, molecular characterisation, transmission, zoonosision

OS – oral session

Session: [SE13] Parasitology research from One Health perspective

Molecular data of *Enterocytozoon bieneusi* from human and animal hosts in Italy

Isabel Guadano Procesi[1], Federica Berrilli[1], Laura Rinaldi[2], Antonio Bosco[2], Luciana Petrullo[3], Marianna Ascierto[3], Ilena Di Ieso[1], David Di Cave[1]

1 Department of Clinical Sciences and Translational Medicine, Faculty of Medicine, University of "Tor Vergata", Rome, Italy
2 Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy.
3 Azienda dei Colli of Naples, "D. Cotugno" Hospital, UOC Microbiology and Virology, Naples, Italy.

Abstract

Enterocytozoon bieneusi causes symptomatic and asymptomatic intestinal infections in immunocompetent and immunocompromised individuals. Faecal-oral transmission occurs in several hosts, although the zoonotic potential is still unclear. Few studies are available in Italy regarding *E. bieneusi* in humans, and no data on its genetic variability in animal hosts are so far reported. Through the ITS rRNA sequences analysis, we presented the first *E. bieneusi* molecular data from animal hosts and updated data from clinical patients in Italy.

Stool samples from 376 patients (Azienda Ospedaliera Universitaria Policlinico Tor Vergata, Rome, July 2023–March 2024) and one sample from Ospedale Cotugno, Azienda Ospedaliera dei Colli (Campania region) were screened through the commercial panel Novodiag® Stool Parasite and genomic DNA was extracted from *E. bieneusi*-positive samples.

Moreover, from September to December 2023, 34 stool samples from calves and sheep were collected and DNA extracted. Amplification of the ITS rRNA region was performed. Obtained sequences were compared to references and aligned to generate a Maximum Likelihood phylogenetic tree.

Three out of 377 human samples were positive to *E. bieneusi* qualitative detection (frequency of infection 0.8%). Isolates were assigned to genotype A and ITA-1, a new genotype identified in the present study.

Four calves tested positive (infection rate of 11.8%) and the genotypes detected were genotype A, genotype I and a genotype having 100% identity with GenBank sequences but not associated to a defined genotype. All genotypes observed in humans and animals belong to Phylogenetic Groups 1 and 2, which likely cause most zoonotic or cross-species infections.

This study updates *E. bieneusi* prevalence and genetic diversity data in humans and offers the first data from animal hosts in Italy. Additional research is required on national scale to fully comprehend *E. bieneusi* zoonotic potential and public health implications.

Keywords: Microsporidia, *Enterocytozoon bieneusi*, One Health

PS – poster session

Session: [SE13] Parasitology research from One Health perspective

Cryptosporidium spp. and *Giardia* spp. from One Health perspective in Latvia

Maira Mateusa, Dārta Paula Šveisberga, Maija Rozenfelde, Maija Selezņova, Margarita Terentjeva, Angelika Krūmiņa, Gunita Deksne

Institute of Food Safety, Animal Health and Environment "BIOR", Latvia

Abstract

Zoonotic pathogens *Cryptosporidium* and *Giardia* are focal points in One Health. They cause prolonged diarrhea, particularly in vulnerable human and animal populations, resulting in economic losses in animal and human healthcare. The aim of present study was to estimate the occurrence of these pathogens from One Health perspective in Latvia.

During 2022-2023, household (2260 humans, 373 domestic dogs), livestock (973 cattle), wildlife (219 red foxes, 78 raccoon dogs) and 1372 environmental (wastewater) samples were collected. All samples, after their respective pre-treatment, were processed using the flotation-centrifugation method and analyzed by fluorescent microscopy using Aqua-GloTM (Waterborne INC, USA). Further isolated DNA were analyzed targeting 18S RNA, *gp60* for *Cryptosporidium* species and sub-type differentiation and *beta-giardin* gene for *G. duodenalis* assemblage differentiation.

The highest *Cryptosporidium* spp. occurrence of 33.8% was observed in livestock (cattle), followed by 28.3% in wildlife (28.3% in foxes; 28.2% in raccoon dogs), 9.1% in the environment and 2.0% in households (0.7% in humans; 9.9% in dogs). High *Giardia* spp. prevalence of 43.6% was observed in the environment, followed by 28.3% in wildlife (27.4% in foxes; 30.8% in raccoon dogs), 8.4% in livestock (cattle) and 1.8% in households (0.4% in humans; 10.7% in dogs). In total, eight zoonotic *C. parvum* subtypes were identified, with IIaA15G2R1 and IIaA17G2R1 being the most prevalent. The zoonotic *G. duodenalis* assemblage A was also isolated.

From One Health perspective, both zoonotic parasites are widely spread among various hosts and in the environment. It is important to acknowledge the risks associated with both pathogens to minimize the exposure for both humans and animals.

Fundamental and applied research "Transmission of Foodborne Parasitic pathogens from animals to humans: TRANSPAR" (lzp-2021/1-0055).

Keywords: protozoans, cattle, canids, humans, wastewater

PS – poster session

Session: [SE13] Parasitology research from One Health perspective

Occurrence of *Blastocystis* in dogs and cats from animal shelters in a selected region of Poland (Pomeranian Voivodeship, north Poland)

Beata Szostakowska

Department of Tropical Parasitology, Medical University of Gdańsk, Poland

Abstract

Blastocystis is the most common intestinal protozoan found in humans and many animal species. Its pathogenicity is still the subject of many studies and discussions. In some subjects, its presence in the intestinal microbiome is asymptomatic, in others it causes various ailments, especially intestinal ones, including even irritable bowel syndrome.

To date, over 30 *Blastocystis* subtypes have been described, some of them detected in humans and animals, some only in animals. Infection with this protozoan occurs directly via the fecal-oral route (dirty hands disease), as well as through consumption of contaminated water and food. The existence of subtypes common to humans and animals suggests the possibility of transmission of the protozoan between them.

223 dogs from 6 shelters and 126 cats from 4 shelters for homeless animals in the Pomeranian Voivodeship (north Poland) were examined. The barcode method was used, i.e. PCR of SSrRNA region and sequencing of the obtained product.

Blastocystis was detected in 11 dogs from one shelter (prevalence 11%), all isolates represented ST3. The protozoan has not been detected in cats, even in the shelter where dogs infected with it were detected.

The low prevalence of *Blastocystis* in the examined animals confirms the suggestions of other researchers that carnivorous animals are not susceptible to colonization by this protozoan. On the other hand, its detection in animals from one shelter indicates a high probability of direct transmission between contacting individuals.

This work was supported by the project "Excellence Initiative – Research University" No 71-01415/0004618/61/772/772/2022 „Studies on the occurrence of Blastocystis in environmental samples and in selected animals".

COST European Cooperation in Science and Technology, Blastocystis under One Health (OneHealthBlastocystis), COST Action CA 21105 is acknowledged.

Keywords: *Blastocystis*, dogs, cats, shelters, Poland

PS – poster session

[SE14]

Recent trends in the epidemiology of leishmaniasis

Session: [SE14] Recent trends in the epidemiology of leishmaniasis

Unveiling the impact of Leishmania parasite viruses (LRV2) on pathogenesis: insights from parasitological perspective

Homa Hajjaran[1], Milad Rahmanipour[1], Zahra Mirabedini[1], Mehdi Mohebali[1], Elham Kazemirad[1], Mona Koosha[1], Hamed Mirjalali[2]

1 Department of Medical Parasitology and Mycology, Tehran University of Medical Sciences, Tehran, Iran
2 Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract
Leishmaniasis is caused by protozoan parasites of the genus *Leishmania*, resulting in varying disease outcomes from cutaneous (CL) to more severe mucocutaneous (MCL) and potentially lethal visceral leishmaniasis (VL). The infection's outcome depends on the Leishmania species, the host's immune response, and the impact of viral co-infections. Virus-like particles from the Totiviridae family have been found in Leishmania parasites, and studies indicate these viruses can alter cytokine expression in immune cells, increasing parasite activity and pathogenicity. This preliminary study compared the expression levels of pathogenicity-related genes in Leishmania major isolates with (LRV2+) and without (LRV2-) the virus.

We assessed RNA expression, focusing on several target genes, including Glycoprotein 63 (GP63), Heat Shock Proteins (HSP70 and HSP90), and Cysteine Protease b (Cpb), in LRV2 positive (LRV2+) and LRV2 negative (LRV2-) strains of *L. major* under in vitro conditions. THP-1 cells were exposed to promastigotes of both strains, and RNA was extracted at various time points (12, 24, and 36 hours) post-infection. Real-time qRT-PCR with specific primers for *Leishmania* above target genes was utilized to determine relative gene expression levels.

The findings showed that the presence of LRV2 in the *L. major* isolate significantly increased the RNA expression levels of the GP63, HSP70, and Cpb genes compared to the *L. major* LRV2-negative isolate ($P \leq 0.05$).

The findings demonstrated a significant increase in the expression of the studied genes in the presence of LRV2 in *L. major* ($P \leq 0.05$), indicating its potential role in enhancing the pathogenicity of *Leishmania* parasites. However, additional research is required to validate these findings.

Keywords: LRV2, L. major, GP63, HSP, qRT-PCR

OS – oral session

Session: [SE14] Recent trends in the epidemiology of leishmaniasis

Distribution patterns of *Leishmania infantum* vectors in Italy under climate change scenarios

Camila Gonzalez[1], Johan Manuel Calderón[1], Ana María López[1], Ilaria Bernardini[2], Luigi Gradoni[2], Marco Pombi[3], Gioia Bongiorno[2], Simona Gabrielli[3]

1 Centre for Research in Tropical Microbiology and Parasitology. Department of Biological Sciences. Faculty of Sciences. Universidad de Los Andes. Bogotá, Colombia
2 Department of Infectious Diseases, Vector-Borne Diseases Unit, Istituto Superiore di Sanità. Rome, Italy
3 Department of Public Health and Infectious Diseases. Sapienza University of Rome. Rome, Italy

Abstract
In Europe, *Leishmania infantum* is the primary agent of leishmaniasis in both animals and humans, with increasing incidence in Italy since the 1990s. In Italy, eight species of sand fly are reported, but only *Phlebotomus perniciosus* and *Phlebotomus perfiliewi* are major vectors, which are recently spreading geographically, raising public health concerns.

This study aims to evaluate the historical and current distribution of sand fly species in Italy and predict potential changes under climate and environmental scenarios using ecological niche modeling.

A comprehensive database was compiled, including original records and published data spanning from 1979 to 2013. From 866 records, 410 records of six species were used to build the models. The spatial variation was evaluated using RCP and CMIP5 models, producing binary outputs to predict presence/absence and expansion/reduction areas. Elevational changes were assessed for each species by evaluating suitable habitat percentages per elevation class.

Phlebotomus perniciosus and *Sergentomyia minuta* showed the broadest distributions, with the highest collection values in Latium, Sardinia, and Sicily. *Phlebotomus perniciosus* had the widest suitable area, while *Phlebotomus mascittii* had the most restricted, possibly due to variability in catching sites and techniques. Predictions indicated *P. perniciosus* and *S. minuta* would expand in central regions, *Phlebotomus neglectus* would predominate in northern and insular regions and coastal areas, *Phlebotomus papatasi* would find suitable habitats in all territories except in the Alps and Apennines, and *P. perfiliewi* would expand towards southern Apulia.

Our models identified suitable areas for future expansion of sand fly vectors in northern, central, and southern Italy. highlighting the potential role of prediction maps as useful tools for the implementation of *L. infantum* surveillance strategies.

Keywords: *Leishmania infantum*, sand fly vectors, ecological niche modeling, climate change, distribution patterns

OS – oral session

Session: [SE14] Recent trends in the epidemiology of leishmaniasis

Imported cutaneous leishmaniasis in a non-endemic country: impact of biodiversity on the management and prognosis

Florence Robert-Gangneux, Jean- Pierre Gangneux

Laboratory of Parasitology-Mycology, University Hospital of Rennes, France

Abstract
Cutaneous leishmaniasis is endemic in more than 87 countries and is now listed in the group of skin NTDs targeted by WHO. CL is a complex disease caused by over 17 species *Leishmania* showing different intrinsic drug susceptibilities. The management of CL is challenging as diagnosis can be difficult, identification of parasite species determines the choice of treatment, and the prognosis is variable.

While Northern France is not an endemic country for CL, the diagnosis is not rare, most often in travelers returning from endemics areas from South America, the Mediterranean area or Africa, in military staff or in migrants from the Middle East. Clinical presentation may differ according to the *Leishmania* species, and the prognosis as well. A good sample is required to make the diagnosis, which can rely on direct examination, culture and PCR adapted to Old World or New World species. Serology can also be useful in South America. MALDI-TOF mass spectrometry and sequencing are efficient tools to identify the species, which will guide treatment. The type, localization and number of lesions will also contribute to the choice of treatment, topical and/or systemic. The practitioner will have to evaluate the prognosis of lesions and define the follow-up scheme to detect potential relapses. An efficient management aims at minoring the impact of scars. Children and young women are particularly vulnerable to disfiguring and stigmatizing impacts of CL, which is a source of social rejection as for leprosy. We evaluated the long-term prognosis of patients

diagnosed in our hospital. Of 106 patients who participated to the survey, 36% had permanent disfiguring scars, mainly due to *L. major* and *L. guyanensis.*

Keywords: cutaneous leishmaniasis, non-endemic, diagnosis

OS – oral session

Session: [SE14] Recent trends in the epidemiology of leishmaniasis

Molecular identification of human cutaneous and canine visceral leishmaniasis agents in various regions of Iran via Internal Transcribed Spacer 1 PCR-RFLP

Homa Hajjaran[1], Farzad Akbar[2]

1 *Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
2 *Tehran Construction Engineering Organization*

Abstract

Leishmaniasis, a significant health concern, is prevalent in nearly half of Iran's 31 provinces. Our study aimed to identify cutaneous and visceral *Leishmania* spp. isolated from infected humans and domestic dogs across various regions in Iran.

We extracted DNA from 108 lesion exudate samples from suspected cutaneous leishmaniasis patients and nine liver and spleen aspirates from infected dogs. These samples were cultured in RPMI-1640 and amplified using partial sequences of the ITS1 gene. The resulting PCR amplicons underwent digestion with HaeIII endonuclease and were used in a restriction fragment length polymorphism (RFLP) assay. Subsequently, we sequenced 48 amplicons representing different hosts and compared them to sequences in GenBank using BLAST.

PCR-RFLP analysis revealed that 60 cutaneous leishmaniasis patients were infected by Leishmania tropica, while 48 patients were infected by L. major. The prevalence rates were 67% for L. major, 32% for L. tropica, and 99% for *L. infantum*.

The ITS1 region-based PCR-RFLP method effectively distinguishes three *Leishmania* spp.: L. major, *L. tropica*, and *L. infantum*. In intra-species comparisons of the ITS1 region, genotypic variations indicated that *L. tropica* isolates were more heterogeneous than L. major and *L. infantum* isolates.

Keywords: ITS1 gene; Iran; Leishmania; PCR-RFLP

PS - poster session

Session: [SE14] Recent trends in the epidemiology of leishmaniasis

Cross-reactivity of canine antibodies with *Leishmania infantum* and *Trypanosoma cruzi* antigen

Franjo Martinković[1], Daniela Heinz[2], Andreas Latz[2], Iva Štimac[1]

1 *Faculty of Veterinary Medicine University of Zagreb, Croatia*
2 *Gold Standard Diagnostics Frankfurt GmbH, Dietzenbach, Germany*

Abstract

Leishmanioses are human and animal diseases caused by flagellate parasite from *Leishmania* genus and transmitted with *Phlebotomus* and *Lutzomyia* sandflies. Most species are zoonotic. *L. infantum* (syn. *L. chagasi*) causes zoonotic disease endemic in the Mediterranean Region. Human leishmaniasis in Europe caused by *L. infantum* is an important public health problem with dogs as the main domestic reservoirs. To prevent the spread of parasite, rapid diagnosis of infected dogs is necessary.

Trypanosoma cruzi is the causative agent of Chagas disease transmitted with triatomine insect vectors with dogs serving as the main peridomestic reservoirs. Chagas disease was once endemic in the America, from south half of the United States down to Argentina but due to increased population mobility it has spread to other continents including Europe. International transport, trade and the breeding programs of dogs could increase the risk of importing Chagas disease into new areas.

L. infantum and *T. cruzi* belong to the same Trypanosomatidae family and their cross reactivity is a well known phenomenon. Presence of cross-reactive antibodies can complicate the serological testing results and final diagnosis of Chagas disease and canine leishmaniosis.

In Croatia the Chagas disease has never been reported, but canine leishmaniosis is enzootic. Therefore, the aim of our study was to comparatively examine canine sera with ELISA test based on *T. cruzi* and *L. infantum* antigen. Overall, 256 *Leishmania* positive and 20 *Leishmania* negative sera were tested with both antigens. Out of 20 *Leishmania* negative, none of them reacted positively with *T. cruzi* antigen. Out of 256 *Leishmania* positive, 54 (21,09%) reacted positive with *T. cruzi* antigen.

Although the Chagas disease is not enzootic in Croatia, without the knowledge that a dog originated from, traveled to, or lived in an endemic area, *T. cruzi* infection could be easily overlooked.

Keywords: *Leishmania infantum*, *Trypanosoma cruzi*, crossreactivity, Croatia

PS – poster session

Session: [SE14] Recent trends in the epidemiology of leishmaniasis

Seroprevalence of *Neospora*, *Babesia* and *Leishmania* in dogs in Istria, Croatia.

Franjo Martinković[1], Katja Močibob[2], Daniela Heinz[3], Andraes Latz[3], Samanta Kotiga[1]

1 *Faculty of Veterinary Medicine University of Zagreb, Zagreb, Croatia*
2 *Veterinary station Pazin, Pazin, Croatia*
3 *Gold Standard Diagnostics Frankfurt GmbH, Dietzenbach, Germany*

Abstract

Dogs are man's oldest companions and for thousands of years they have helped humans in various ways. Owing a dog can have numerous health benefits, including reduced stress, increased physical activity, and improved mental well being. As any animal or human being, they are susceptible to different kinds of diseases including parasitic diseases. Also, they can act as an reservoir of some parasites. The aim of our study was to analyze canine serum samples for the presence of antibodies against *Neospora*, *Babesia* and *Leishmania* in Istrian peninsula, Croatia.

Canine serum samples were collected during period 2022-2023 year, stored at -20°C, and subsequently analyzed using VetLine *Babesia canis*, *Leishmania infantum* and *Neospora caninum* ELISA tests. For detection of *Babesia* and *Leishmania* antibodies,164 sera were used while for *Neospora* 136. Out of all tested sera only one *Leishmania* positive and one *Neospora* positive sample was found while 14 samples tested positive and 11 equivocal for *Babesia*.

Babesiosis caused by *Babesia canis* is tick-borne disease transmitted with *Dermacentor reticulatus,* a vector currently absent in Istria. So many positive and doubtful samples could be the consequnce of traveling and trade from continental parts of Croatia, i. e. from *B. canis* enzootic region or crossreactivity with other *Babesia* species.

The *Leishmania* seropositive dog, as expected, was imported from enzootic region. Such cases are already known as the consequence of travelling or animal trade from enzootic region to non-enzootic region. Furthermore, it is important to state that although the vectors i.e. sandflies are present in Istria for a long time, luckily, there are still no autochtonous *Leishmania* cases.

Neospora caninum invasion was in the past proven in cattle and

sheep in Croatia, but never in dogs. This is to our knowledge first report about *Neospora* seropositive dog in Croatia.

Keywords: seroprevalence, *Babesia canis*, *Leishmania infantum*, *Neospora caninum*, Croatia

PS – poster session

Session: [SE14] Recent trends in the epidemiology of leishmaniasis

A candidate vaccine for *Leishmania major* base on Gp63, LACK, TSA, LmSTI1 and KMP11 antigens and evaluation in BALB/c mice

Sama Rashidi[1], Gholamreza Hatam[2], Bahram Kazemi[3]

1 Iran
2 Shiraz University Medical Science
3 Shahid Beheshti University of Medical Science

Abstract

Leishmaniasis is a significant health issue in tropical areas, lacking an effective vaccine. This research assesses the efficacy of Leish21, a multi-epitope DNA vaccine, against *Leishmania major*-induced cutaneous leishmaniasis in BALB/c mice. The vaccine, comprising *L. major* antigen epitopes, was transfected into eukaryotic cells, with expression verified by RT-PCR. Immunization with Leish21, with or without interleukin-12 (IL-12) adjuvant, notably reduced lesion size after *L. major* promastigote infection compared to controls.

The methodology involved creating the Leish21 construct with bioinformatics, *E. coli* transformation, expression confirmation in NIH-3T3 cells, and mouse immunization. Mice were immunized in five groups, receiving booster shots biweekly, then challenged with *L. major* promastigotes. Lesion progression and parasite burden were monitored.

Results showed vaccinated mice had smaller lesions and fewer parasites. IL-12 co-administration improved outcomes, increasing survival and reducing mortality in the Leish21+IL12 group. ELISA revealed higher IgG2a, IFN-γ, and TNFα in vaccinated mice, indicating a Th1 response, while controls had more IL-4, suggesting a Th2 response.

The study concludes Leish21 prompts a Th1 response, boosted by IL-12, providing substantial protection against *L. major*. These findings endorse DNA vaccines' potential for strong cellular immunity and underscore adjuvants' role in vaccine effectiveness. Further studies are needed to assess the vaccine's safety and efficacy in humans.

Acknowledgments go to the vice chancellor of research at Shiraz University of Medical Sciences and the Cellular and Molecular Biology Research Center at Shahid Beheshti University of Medical Sciences.

Keywords: DNA vaccine, *Leishmania major*, cutaneous leishmaniasis, multi-epitope vaccine, Leish21, IL-12, Th1 response, immunization, BALB/c mice

PS – poster session

Session: [SE14] Recent trends in the epidemiology of leishmaniasis

Evaluation of DNA extraction protocols for effective surveillance of *Leishmania*-infected Phlebotomine sand flies

Jorian Prudhomme, Aymeric Delabarre, Jean-Pierre Gangneux, Florence Robert-Gangneux

Univ Rennes, Irset Inserm U1085, Rennes, France

Abstract

Leishmaniasis is a disease caused by parasites of the genus *Leishmania*, transmitted through the bite of Phlebotomine sand flies. This disease is endemic around the Mediterranean basin and can cause a wide range of clinical symptoms. Despite being highly underreported, autochthonous canine leishmaniasis is expanding, with new foci emerging and old ones re-emerging. To monitor the spread of *Leishmania*-infected sand flies, standardized tools are needed to compare their distribution areas and infection prevalence. Furthermore, real-time PCR based on consistent amplification conditions and similar extraction protocols is essential for comparing results over time and across geographical areas. However, there is currently a lack of evaluation of extraction and qPCR techniques targeting *Leishmania*, which needs to be addressed. In this context, the objectives of this work were to analyze the performance of different extraction protocols for detecting *L. infantum* and *L. major*. We compared eight commercial kits from different companies, using Ph. perniciosus from well-adapted laboratory colonies and two *Leishmania* strains at five concentrations (10□, 10□, 10^3, 10^2, and 10^1). Our work revealed statistically significant differences in the performances of DNA extraction protocols for *Leishmania* amplification, with PK pre-treatment and DSP virus kit ® (Qiagen) using EZ1 robot (Qiagen) performing the best.

Our study highlighted the importance of standardized extraction protocols for monitoring *Leishmania*-infected sand flies, underscoring the need for careful selection of extraction methods to ensure accurate detection and comparison of *Leishmania* prevalence. By identifying the most effective procedures, this work will contribute to improved epidemiologic surveillance and will facilitate more reliable comparisons of data across different regions and time periods. This standardization is crucial for tracking the spread of leishmaniasis and implementing effective control measures.

Keywords: *Leishmania*, sand fly, vector surveillance, molecular diagnosis

PS – poster session

Session: [SE14] Recent trends in the epidemiology of leishmaniasis

Current status of *Leishmania infantum* prevalence in rural regions' dogs of Shabestar county, northwestern Iran

Seyed Masih Razavi[1], Mohammad Sadaghian[2]

1 Veterinary Medicine student, School of Veterinary Medicine, Shabestar branch, Islamic Azad University, Shabestar, Iran
2 Department of Pathobiology, School of Veterinary Medicine, Shabestar branch, Islamic Azad University, Shabestar, Iran

Abstract:

Visceral leishmaniasis (VL) caused by Leishmania infantum, is an emerging life-threatening parasitic disease which has been endemic in some areas of Iran since 1949, with dogs considered as the main reservoir of the parasite. New foci of infection are spreading as long as increase of stray dogs' population in all over of Iran, particularly metropolitan surrounding areas. Because of progressive prevalence of dogs' asymptomatic infections according to different serological surveys in neighboring areas, more sensitive diagnostic techniques are required about the rural regions, given the close contact of the rural dogs to human population. The survey aimed to evaluate the rate of area's rural dogs' infection for the first time, using PCR, to have the basic information for controlling the infection in Shabestar, Northwestern Iran. Blood samples of 102 rural dogs were randomly collected from the area. The samples' DNA were extracted by commercial kits and subjected to the 2 step- conventional PCR targeting kinetoplast minicircle DNA and internal transcribed spacer 1, 5.8S ribosomal RNA genes and the products examined by gel electrophoresis.

According to the results, 18 out of the 102 (17.6%) examined samples, were found to be positive for Leishmania spp, where 10 out of 102 (9.8%) examined dogs were PCR positive for the Leishma-

nia infantum. Among these, 12.2% of male dogs and 7.5% of female dogs were PCR-positive (P > 0.05). Also, the results showed that all of PCR-positive cases belonged to more than 18-month age groups' dogs (12.3%). Two out of 9 (22.2%) of herding dogs and 8 out of 87 (9.2%) of stray dogs was PCR-positive whereas no case of positivity was observed about guard dogs (P>0.05). Furthermore, 11.1% of the examined dogs with CVL related signs versus 9.7% of dogs without infection related signs were positive (P>0.05).

Considering the first data about the area, the results emphasize the continuous monitoring of infection in both human inhabitants and wild reservoirs.

Keywords: *Leishmania infantum*, dog, PCR, Shabestar, Iran

PS – poster session

Session: [SE14] Recent trends in the epidemiology of leishmaniasis

Patient-centred approach using telemedicine for remote monitoring of cutaneous *Leishmania infantum* leishmaniasis

Črt Loboda[1], Jasna Černoša[1], Janina Tamše[1], Barbara Šoba[2], Tadeja Kotar[1]

1 Department of Infectious Diseases, University Medical Centre Ljubljana, Japljeva 2, 1000 Ljubljana, Slovenia
2 Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Zaloška 4, 1000 Ljubljana, Slovenia

Abstract

A 49-year-old male patient with a history of atopic dermatitis sought medical attention due to a persistent ulceration over his left shoulder four months after an insect bite while on holiday on the island of Šolta, Croatia. A skin biopsy revealed the presence of *Leishmania* amastigotes in the tissue. *L. infantum* was identified by PCR and sequencing. The laboratory tests showed: leukocytes, 5.4×10^9/L (63% neutrophils, 25% lymphocytes, 10% monocytes); haemoglobin, 150 g/L; platelets, 196×10^9/L; creatinine, 86 mmol/L; CRP, < 5 g/L. The patient was serologically positive for *Leishmania*-specific antibodies, which was still the case after 9 months, while *Leishmania* DNA was never detected in his blood. Abdominal ultrasound revealed no abnormalities, and ultrasound of the axilla showed no enlarged nodes. Following discussion with the patient, it was decided not to start antiparasitic treatment but to wait and watch. Telemedicine was used for remote monitoring. After one month, the lesion was more superficial. Three months after the initial examination, the primary skin lesion was less intense. Four months after the initial examination, the lesion had completely dried up and healed, no new lesions were observed. Six months post-healing, the patient remains without any new lesions, at the site of primary lesion small scar can be seen.

This case illustrates a patient-centred approach using telemedicine for remote monitoring of progress resulting in spontaneous resolution of cutaneous leishmaniasis caused by *L. infantum*. In mild cases of cutaneous leishmaniasis, a wait-and-see approach may be considered, as lesions may heal without intervention. In Slovenia, there are no autochthonous cases of leishmaniasis in humans, but a case of probable autochthonous canine leishmaniasis was recently reported. Given the ongoing global warming altering ecological conditions and facilitating expansion of leishmaniasis vectors in Europe, we expect more cases of leishmaniasis in the future.

Keywords: case report, telemedicine, cutaneous leishmaniasis, *Leishmania infantum*, Slovenia

PS – poster session

[SE15]

Update on *Toxoplasma* infection epidemiology and diagnosis

Session: [SE15] Update on *Toxoplasma* infection epidemiology and diagnosis

Toward an effective toxoplasmosis vaccine

Maciej Chyb[1], Bartłomiej Ferra[2], Malwina Kawka[3], Justyna Gatkowska[3]

1 Department of Molecular Microbiology, Institute of Microbiology, Biotechnology and Immunology, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland. The Bio-Med-Chem Doctoral School of the University of

2 Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine in Gdynia, Medical University of Gdansk, 9b Powstania Styczniowego Str., 81-519, Gdynia, Poland.

3 Department of Molecular Microbiology, Institute of Microbiology, Biotechnology and Immunology, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland.

Abstract

Toxoplasma gondii is an opportunistic, cosmopolitan protozoan that causes toxoplasmosis in humans and animals, one of the most common parasitoses in the world. This protozoan invades and multiplies asexually in all warm-blooded animals. For immunocompetent people *T. gondii* invasion is mainly asymptomatic. People with a weakened immune system are at risk of severe toxoplasmosis that might lead to neurotoxoplasmosis or pneumonitis. Lack of efficient and safe immunoprophilaxis motivates scientists to search for new solutions to overcome this problem. The only available vaccine is Toxovax containing an attenuated S48 strain of *T. gondii*, which can only be used in animals to reduce the rate of miscarriages but not to prevent horizontal transmission of the parasite. One of approaches to development of immunoprophylaxis is to design recombinant *T. gondii* antigens, based on selected, most immunogenic, important in life cycle of *Toxoplasma* proteins. Researchers are focused on testing those antigens as protein or genetic vaccines.

The aim of our research is design, production, and assessment of immunogenic potential of recombinant *T. gondii* antigens *in vitro* and *in vivo* as well as determination of their immunoprotective capacity *in vivo*. To evaluate their efficacy *in vivo* the model of experimental toxoplasmosis in C3H/HeOuJ mice is used. Mice are immunized three times, with two-week intervals and after immunization selected parameters of specific humoral and cellular responses developed during vaccination are determined. Also, vaccinated mice are challenged with the DX strain of *T. gondii* to assess the degree of inhibition of infection and thus the effectiveness of vaccination.

Further understanding of the impact of vaccine antigen composition on vaccine efficacy brings us closer to development of the best performing vaccine candidate against *T. gondii* invasion.

This work was funded by Polish National Science Centre (grant number: UMO-2018/31/D/NZ6/02839).

Keywords: *T. gondii*, vaccine

OS – oral session

Session: [SE15] Update on *Toxoplasma* infection epidemiology and diagnosis

Computational image analysis of *Toxoplasma gondii* in Vero cells: ToxoReTREAT project

Olivera Lijeskić[1], Neda Bauman[1], Andjelija Ilić[2], Jelena Trajković[2], Đorđe Zlatković[1], Jelena Srbljanović[1], Dragana Vujić[3], Vladimir Dobričić[4], Branko Bobić[1], Tijana Štajner[1]

1 National Reference Laboratory for Toxoplasmosis, Group for Microbiology and Parasitology, Centre of Excellence for Food- and Vector-borne Zoonoses, Institute for Medical Research, University of Belgrade, Belgrade, Serbia

2 Institute of Physics Belgrade, University of Belgrade, Belgrade-Zemun, Serbia

3 Faculty of Medicine, University of Belgrade, Serbia

4 Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia

Abstract

Nearly one-third of the world's population is infected with the protozoan parasite *Toxoplasma gondii*. Although acute infection with this parasite in immunocompetent adults is often non-symptomatic, reactivation of toxoplasmosis in chronically infected, severely immunocompromised individuals, such as patients undergoing hematopoietic stem cell transplantation (HSCT), can be fatal. Stage conversion of slow-dividing bradyzoites to the fast-replicating tachyzoites, a crucial step during reactivated toxoplasmosis (RT), can be achieved *in vitro* under specific conditions, enabling researchers to study therapeutic potential of different drugs on both stages of *T. gondii*. The aim of the ToxoReTREAT project is to resolve the problem of RT in HSCT recipients combining medicine, pharmacy, and computer image analysis to provide novel drug candidates. Throughout our project, high-resolution photographs of intracellular formations and extracellular *T. gondii* will be obtained, as well as the images of stage conversion in Vero cells. Experiments will be conducted in two different settings: images will be acquired from infected Vero cells, without administered treatment, and infected Vero cells treated with acridines/acridones (20 acridines already synthesized); both using referent RH strain and human type II strains for infection. Digitalized microscopic images will be analysed by the freely available ImageJ software, utilizing morphological, particle, and fractal analysis, in an attempt to advance automated image scoring for chemotherapy results, and contribute to the optimization of experimental protocols.

This study was supported by the Science Fund of the Republic of Serbia, 7328, Re-invention of the diagnostic algorithm and treatment options for reactivated toxoplasmosis – ToxoReTREAT.

Keywords: image analysis, ImageJ, *Toxoplasma gondii*, acridines, acridones

OS – oral session

Session: [SE15] Update on *Toxoplasma* infection epidemiology and diagnosis

Diagnostic tools for toxoplasmosis: a moving landscape

Florence Robert-Gangneux[1]

1 Univ Rennes, Hospital University of Rennes, France

Abstract

Diagnosis of toxoplasmosis has benefited from many innovative approaches over decades, and many tools have been developed for serological diagnosis, particularly in the frame of congenital toxoplasmosis. In the era of rationalization of laboratory organization and optimization of production costs by manufacturers, the landscape of diagnosis is rapidly evolving. On one hand, some assays have been recently withdrawn from the market (ISAGA, IgA assays), leading to a necessary modification of diagnostic algorithms of congenital diagnosis. How to replace them? On the other hand, new diagnostic tests (WB IgM, ICT) have been commercialized and need large scale evaluations before placing them in a new diagnostic strategy. Besides, the development of pre-analytical and analytical immuno-analysis robotic devices optimizing the traceability of assays, reducing human error and facilitating laboratory accreditation led to a decreased diversity of techniques. These automated assays have various performances, which should be known to interpret results and give an expert advice for counselling during pregnancy. In the same way, molecular diagnosis has moved from conventional PCR to qPCR, first in-house and then commercial techniques

with automated DNA extraction, the quality of which can influence the sensitivity of the PCR. LAMP and now digital PCR are other tools that may be interesting. No infectious disease has benefited from such a comprehensive range of diagnostic techniques, but none is infallible and expert opinion must always be based on several tests.

Keywords: toxoplasmosis, diagnosis, serology, PCR

OS – oral session

Session: [SE15] Update on *Toxoplasma* infection epidemiology and diagnosis

Molecular prevalence of *Toxoplasma gondii* in raw meat products sold in Poland. Assessment of the viability of detected parasites

Jacek Sroka[1], Weronika Piotrowska[1], Angelina Wójcik-Fatla[2], Ewa Bilska-Zając[1], Jacek Karamon[1], Jolanta Małgorzata Zdybel[1], Joanna Dąbrowska[1], Weronika Korpysa-Dzirba[1], Małgorzata Samorek-Pieróg[1], Tomasz Cencek[1]

1 Department of Parasitology and Invasive Diseases, National Veterinary Research Institute, Partyzantów Av. 57, 24-100, Puławy, Poland

2 Department of Health Biohazards and Parasitology, Institute of Rural Health, Jaczewskiego 2, 20-090 Lublin, Poland

Abstract

Raw and undercooked meat products and meat containing *Toxoplasma gondii* tissue cysts are considered the main source of human toxoplasmosis in Europe, however data on this subject in Poland are still insufficient. The aim of the study was to determine the prevalence of DNA and live *T. gondii* parasite in raw meat products commecially available in Poland.

Samples of various types of raw cured bacon, sausage, ham and minced meat (1900 samples) were collected in shops and meat factories (in cooperation with the Veterinary Inspection) in 16 provinces of Poland (2019-2023). Samples (50 g) were digested with pepsin solution, followed by the DNA extraction. Amplifications of 35-fold-repetitive B1 fragment gene were performed by nested polymerase chain reaction (PCR) and Real-time PCR. Multilocus sequence typing (MLST) with 12 additional genetic markers was used to genotype *T. gondii*. To assess the viability of *T. gondii*, isolation assays by cell culture and/or bioassay on mice were performed.

Of the 1900 examined samples, 135 (7.1%) tested positive in PCR. The highest percentage of positive results was found for samples from the Łódzkie voivodeship (14.6%) and Pomorskie voivodeship (12.9), the lowest from the Podlaskie voivodeship (0.7%). The percentage of positive results for particular types of meat products ranged from 1.5% to 9.8%. MLST showed mostly the alleles of clonal type II (47%) and III (31.5%), the combinations of types alleles at different loci were also found (12.7%). The viable *T. gondii* was isolated from 44 samples.

The detection of *T. gondii* DNA and live parasite in 7.1% and 2.3% of tested samples of raw meat products, respectively, indicates a real threat to consumers' health.

Keywords: *Toxoplasma gondii*, raw meat products, PCR, genotyping

OS – oral session

Session: [SE15] Update on *Toxoplasma* infection epidemiology and diagnosis

Isolation, genotyping and phenotype analysis of European *Toxoplasma gondii* strains - what have we discovered?

Aleksandra Uzelac[1], Olgica Djurkovic-Djakovic[1]

1 Institute for Medical Research, University of Belgrade, Serbia

Abstract

Over the last two decades, a considerable effort has been undertaken to isolate, genotype, classify and analyze the phenotype of *Toxoplasma gondii* strains worldwide. Initiated by a desire for scientific discovery, this effort is now fueled primarily through the implementation of the One Health approach. Specifically, the addition of the environment as a novel research frontier provided the rationale, framework and purpose to ask new research questions, develop surveillance strategies and methodologies. In Europe, there has been a tremendous increase in knowledge of the parasite's genetic diversity - both at a low and high level of resolution - as compared to data available a decade ago. Shifting the focus from humans and farmed food animals to free range animals, wildlife and even soil and water as reservoirs of infection, has resulted in the discovery of a higher diversity than anticipated, while experimental analyses of natural isolates revealed that virulent genotypes circulate in the environment. Together, the emerging data is gradually changing conventional epidemiological knowledge of transmission and risk factors and providing a new perspective on *T. gondii* infection and disease management. Exciting future research targets include development of diagnostic tools with source attribution capabilities, strategies for environmental surveillance and novel solutions for transmission prevention.

Keywords: *Toxoplasma gondii*, genotypes, diversity, transmission

OS – oral session

Session: [SE15] Update on *Toxoplasma* infection epidemiology and diagnosis

Development of novel diagnostic assays for Toxoplasmosis

Fernando Cardoso[1], Olga Matos[1]

1 Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Rua da Junqueira 100, 1349-008 Lisboa, Portugal

Abstract

Toxoplasma gondii is an obligate intracellular protozoan parasite that infects a wide range of mammals and birds, causing the disease toxoplasmosis. Diagnosing toxoplasmosis in humans relies on a combination of biological, serological, histological, and molecular methods. This study aimed to develop new diagnostic assays for toxoplasmosis. We employed several methodologies, including the production of monoclonal antibodies via traditional hybridoma technology, and the generation of recombinant antibodies and peptides through phage display. Additionally, recombinant *T. gondii* proteins were produced in *Escherichia coli* for assay development. The monoclonal antibodies produced were effectively utilized in ELISA, dot blot, and immunohistochemistry assays. Furthermore, the cloning and expression of recombinant proteins were successfully achieved. The diagnostic tools developed in this study hold significant potential for enhancing the accuracy and reliability of toxoplasmosis diagnosis, thereby contributing to better clinical outcomes and disease management.

Keywords: phage display, recombinant proteins, *Toxoplasma gondii*, toxoplasmosis, diagnosis, monoclonal antibodies,

PS – poster session

Session: [SE15] Update on *Toxoplasma* infection epidemiology and diagnosis

Toxoplasma gondii recombinant trivalent chimeric proteins as an alternative to *Toxoplasma* lysate antigen (TLA) in Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of IgG in farm animals.

Bartłomiej Ferra[1], Maciej Chyb[2], Justyna Gatkowska[2]

1 Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine in Gdynia, Medical University of Gdańsk, Powstania Styczniowego 9B, 81-519 Gdynia, Poland
2 Department of Molecular Microbiology, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland

Abstract

Toxoplasma gondii is an intracellular, obligatory, protozoan parasite of all warm-blooded animals including humans and livestock. The protozoan causes asymptomatic infection in susceptible animals such as goats, sheep, pigs, or rabbits. Particularly in goats and sheep, the parasite invasion is associated with transplacental infection leading to mortality and morbidity in offspring, which cause significant reproductive losses.

Diagnostics of *T. gondii* infection in farm animals is based mainly on serological methods, however, they require improvement to enable the testing of large numbers of samples in a inexpensive manner.

Our study aimed to construct and evaluate the diagnostic utility of seventeen newly produced recombinant trivalent chimeric proteins (containing three different immunodominant fragments of *T. gondii* antigens) for the detection of *T. gondii* infection in small ruminants. Analyzing the results obtained for two animal species (sheep and goat), it was found that six protein preparations of recombinant trivalent chimeric proteins (SAG1-SAG2-GRA5, SAG1-SAG2-GRA9, SAG1-SAG2-MIC1, SAG1-SAG2-MIC3, SAG1-SAG2-P35, and SAG1-SAG2-ROP1) can be used to develop IgG ELISA tests with 100% sensitivity and specificity, and constitute an alternative to the polyvalent native antigen TLA used in commercial tests.

Considering that more than 80% of the infected human population became infected as a result of eating raw or undercooked meat, this approach to detecting parasite infection seems to be appropriate. It is estimated that 30–63% of *T. gondii* infections in pregnant women are caused by eating improperly prepared meat. Our results give hope for the development of cheaper diagnostic tools; however, we should also remember to raise public awareness of *T. gondii* infection.

This work was supported by the National Centre for Research and Development (grant No. LIDER/34/0188/L-10/18/NCBR/2019) and the National Science Centre (grant No. UMO-2018/31/D/NZ6/02839).

Keywords: *Toxoplasma gondii*, recombinant protein, ELISA, animals, diagnostics

PS – poster session

Session: [SE15] Update on *Toxoplasma* infection epidemiology and diagnosis

What is the added value of anti-*Toxoplasma* IgA for the serological diagnosis of congenital toxoplasmosis in 2024?

Lya Hamet, Florence Robert-Gangneux

Univ Rennes, Irset Inserm U1085, Rennes, France

Abstract

The detection of specific IgA has been included in the panel of biological tools used for the diagnosis of congenital toxoplasmosis since over 25 years, as it had been shown back then, to be complementary to the detection of specific IgM in the newborn serum. However, in the era of the withdrawal of several serological assays for IgA detection and of the wide use of commercial western-blot to compare mother and newborn IgG and IgM, we decided to re-evaluate the benefit of anti-*Toxoplasma* IgA.

We analyzed retrospectively the files of all infants born form mothers infected with *Toxoplasma gondii* during pregnancy, followed in our lab from 2010 to 2023. Patients were included for analysis if they had at least a 6-month serological follow-up.

In total 469 infants (1116 serum samples) were included, of whom 51 were diagnosed with congenital toxoplasmosis.

Fifty-seven babies (89 samples) had at least 1 positive ISAGA A result (index$\geq$6) during follow up, of whom 36 (63.1%) were congenitally-infected. False-positive results were observed in 21 uninfected infants during the first 3 days of life; all babies tested negative 10 days later. The mean time to detect IgA in infected neonates was 7.3 days and 88.9% were positive within 1 month after birth. The positive predictive value at 1 month of life was 72%. Of the 51 infected infants, 23 were diagnosed antenatally, and 31 had a positive PCR on placenta at birth. IgA were simultaneously positive with IgM and WB in 16 cases, with IgM alone in 12 cases and 1 case with WB alone. IgA was the earliest serological parameter in only 1 case (index=7) on cord blood, which decreased at 3 days (index=5), thus was overlooked, and turned negative at 1 month. The infant was finally diagnosed at 3 months on the rise of specific IgG. Overall, no infant was diagnosed on the sole basis of IgA detection.

These results let us think that we can do without IgA assays to manage the diagnosis of congenital toxoplasmosis.

Keywords: congenital toxoplasmosis, anti-*Toxoplasma* IgA, serology

PS – poster session

Session: [SE15] Update on *Toxoplasma* infection epidemiology and diagnosis

Molecular confirmation of the systemic toxoplasmosis in cat

Jacek Sroka[1], Weronika Piotrowska[1], Angelina Wójcik-Fatla[2], Ewa Bilska-Zając[1], Jacek Karamon[1], Jolanta Małgorzata Zdybel[1], Joanna Dąbrowska[1], Małgorzata Samorek-Pieróg[1], Weronika Korpysa-Dzirba[1], Tomasz Cencek[1]

1 Department of Parasitology and Invasive Diseases, National Veterinary Research Institute Partyzantów Avenue 57, 24-100, Puławy, Poland
2 Department of Health Biohazards and Parasitology, Institute of Rural Health, Jaczewskiego 2, 20-090 Lublin, Poland

Abstract

Systemic toxoplasmosis with tissue-spread parasites occurring in intermediate hosts may also occur in immunocompromised cats (e.g. infected with FLV or FIV). The case of the sudden death of 3 out of 4 cats in a cattery, and the detection and molecular characterization of *T. gondii* DNA detected in the tissues of one of the dead cats is presented. According to the literature, no reports on the detection and genotyping of *T. gondii* DNA in cats with extraintestinal toxoplasmosis have been published in Poland yet. Samples of brain, lungs, heart, and liver of the cat that died suddenly were examined for the presence of *T. gondii* DNA (B1 gene) by nested PCR and real-time PCR. DNA positive samples were also genotyped at 12 genetic markers using multiplex multilocus nested PCR-RFLP (Mn-PCR-RFLP) and multilocus sequence typing (MLST).

A total of 9 out of the 20 DNA samples were successfully amplified with nested and/or Real-time PCR. DNA from 3 out of 5 types of tested samples were genotyped (brain, heart and muscle). Mn-PCR-RFLP and MLST results revealed type II (and II/III at SAG1) alleles at almost all loci, except a clonal type I allele at the APICO locus. This profile corresponds to the ToxoDB#3 genotype, commonly identified amongst cats in Central Europe. To the best of our knowledge, this is the first study describing the genetic characteristics of *T. gondii* population determined in a cat in Poland. These data confirm the importance of this host as a reservoir for this pathogen, and demonstrate the genotypic variation of this parasite. Veterinarians should take into account that cats may develop disseminated toxoplasmosis which may lead to the death of the cat.

Keywords: *Toxoplasma gondii*, cat, PCR, genotyping, systemic toxoplasmosis

PS – poster session

Session: [SE15] Update on *Toxoplasma* infection epidemiology and diagnosis

Experimental chemotherapy of reactivated toxoplasmosis: repurposing and *de novo* synthesis of acridine and acridone derivatives

Đorđe Zlatković[1], Jelena Srbljanović[1], Vladimir Dobričić[2], Neda Bauman[1], Olivera Lijeskić[1], Dragana Vujić[3], Branko Bobić[1], Tijana Štajner[1]

1 National Reference Laboratory for Toxoplasmosis, Group for Microbiology and Parasitology, Centre of Excellence for Food- and Vector-borne Zoonoses, Institute for Medical Research, University of Belgrade, Belgrade, Serbia
2 Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia
3 Faculty of Medicine, University of Belgrade, Serbia

Abstract

Toxoplasma gondii is a protozoan parasite infecting nearly one third of the global population. Severe immunosuppression in chronically infected individuals results in reactivated toxoplasmosis (RT), a devastating complication following hematopoietic stem cell transplantation (HSCT), particularly in allogeneic recipients. Treatment options for RT, other than being limited, also prove to be inadequate for HSCT patients due to their side myelotoxicity and ineffective since they act only on circulating parasites. Acridine and acridone derivatives are a group of very potent bioactive compounds with various proven activities such as anticancer, antimalarial, antimicrobial, anti-inflammatory, antiparasitic and fungicidal. *De novo* sinthetized acridine and acridone derivatives (previously investigated for antineoplastic activity) are being tested in *in vitro* experimental model of RT, using *T. gondii* type 2 strain isolated from human patient. *In vitro* testing is based on MTT assay, widely used for measuring cytotoxicity of newly synthetized pharmaceutical compounds. MTT assay is being performed to evaluate cytotoxicity of derivatives on both non-infected and *T. gondii*-infected Vero cell lines. CC50 and IC50 values will be calculated by statistical analysis using GraphPad Prism 10 for all derivatives tested. Results obtained so far suggest that acridines and acridones could display high potency against *T. gondii*, serving as novel drug candidates and perhaps more adequate treatment options for RT. It is expected that the research activities within ToxoReTREAT project's WP2 (Experimental chemotherapy of RT) will provide novel drug candidates for RT with reduced toxicity and increased overall efficacy on *T. gondii* tachyzoites and bradyzoites, in comparison to current treatment options.

This research was supported by the Science Fund of the Republic of Serbia, 7328, Reinvention of the diagnostic algorithm and treatment options for reactivated toxoplasmosis – ToxoReTREAT

Keywords: *Toxoplasma gondii*, experimental chemotherapy, acridines, acridones, cytotoxicity

PS – poster session

[SE16]
Parasitology in multi-disciplinary projects / grants

Session: [SE16] Parasitology in multi-disciplinary projects /grants

Achievements of the "MEmE" multidisciplinary project on E*chinococcus* spp. in Europe and beyond

Adriano Casulli

1 ISTITUTO SUPERIORE DI SANITA / EU Reference Lab Parasites / WHO CC Echinococcosis (Italy)

Abstract

MEmE (Multi-centre study on *Echinococcus multilocularis* and *Echinococcus granulosus s.l.* in Europe: development and harmonization of diagnostic methods in the food chain) is an international collaborative project funded by the European Union under the Framework of One Health European Joint Programme (grant agreement 773830). MEmE aims to fill research gaps highlighted by international agencies for the standardization of detection methods, production of new molecular tools and epidemiological data at large scale on the zoonotic parasites *E. multilocularis* (Em) and *E. granulosus sensu lato* (Eg), causing alveolar echinococcosis (AE) and cystic echinococcosis (CE), respectively.

Major achievements of MEmE were: 1. The validation of the parasitological (SSCT) and molecular diagnostic (multiplex- and MC-RT-PCRs) procedures to detect Em and Eg in different matrices along the food chain; 2. The development, validation and comparison of new molecular tools (comparison of DNA extraction and PCRs assays, novel probe-based qPCRs, PCR-RFLPs and multiplex PCR assays and NGS approach); 3. Multicentre studies for the production of data relevant for epidemiological assessments (contamination of fresh vegetables and fruits for human consumption by Em/Eg DNA; prevalence of Em/Eg in dog faeces); 4. Quantitative assessment on the impact of human CE in Europe by means of systematic review approach; 5. Molecular and clinical epidemiology studies in selected geographical areas. MEmE impacted on animal health, public health and food safety sectors. Beneficiaries of scientific outputs of MEmE are EU National Reference Labs, international organizations and all decision makers. MEmE provided a set of molecular tools, epidemiological risk assessments and quantitative epidemiological models for the detection, surveillance and control of these parasitic infectious diseases in Europe and beyond.

Keywords: cystic and alveolar echinococcosis, *Echinococcus* spp., international public health, multicentre studies

OS – oral session

Session: [SE16] Parasitology in multi-disciplinary projects /grants

Understanding climate and environmental drivers of sand fly borne diseases: the CLIMOS project

Olgica Djurkovic-Djakovic[1], Suzana Blesic[1], Ivana Klun[1], Manos Athanatos[2], Eduardo Berriatua[3], Gioia Bongiorno[4], Remi Charrel[5], Orin Courtenay[6], Juan Jose Saenz de la Torre[7], Jerome Depaquit[8], Vit Dvorak[9], Ozge Erisoz[10], Federica Ferraro[11], Maria Maia[12], Valentina Foglia Manzillo[13], Nenad Gligoric[14], Vladan Gligorijevic[15], Diana Guardado[16], Gordon Hamilton[17], Nils Hempelmann[18], Tally Hatzakis[19], Vladimir Ivovic[20], Edwin Kniha[21], Laor Orshan[22], Yusuf Ozbel[23], Shlomit Paz[24], Florence Robert-Gangneux[25], Jovana Sadlova[9], Luis Samaniego[26], Daniel San Martin[7], Seher Topluoglu[27], Frank Van Langevelde[28], Petr Volf[9], David Wright[29], Carla Maia[30]

1 Institute for Medical Research, University of Belgrade, Serbia; 2 Telecommunications Systems Institute, Chania, Greece; 3 University of Murcia, Murcia, Spain; 4 Istituto Superiore di Sanità, Rome, Italy; 5 Aix-Marseille University, Marseille, France; 6 University of Warwick, Coventry, United Kingdom; 7 Predictia, Santander, Spain; 8 University of Reims Champagne-Ardenne, Reims, France; 9 Charles University, Prague, Czech Republic; 10 Hacettepe University, Ankara, Turkey; 11 Ministry of Health, Italy; 12 Karlsruhe Institute of Technology, Karlsruhe, Germany; 13 University of Naples Federico II, Naples, Italy; 14 Zentrix Lab, Pancevo, Serbia; 15 CubexLab, Amsterdam, Netherlands; 16 F6S Network Ireland Limited, Dublin, Ireland; 17 Lancaster University, Lancaster, United Kingdom; 18 Open Geospatial Consortium, Leuven, Belgium; 19 Trilateral Research Ireland, Marine Port, Ireland; 20 University of Primorska, Koper, Slovenia; 21 Medical University of Vienna, Vienna, Austria; 22 Israeli Ministry of Health, Jerusalem, Israel; 23 Ege University, Izmir, Turkey; 24 University of Haifa, Haifa, Israel; 25 University of Rennes 1, Rennes, France; 26 Helmholtz Centre for Environmental Research, Leipzig, Germany; 27 Turkish Ministry of Health, Ankara, Turkey; 28 Wageningen University, Wageningen, Netherlands; 29 Trilateral Research Ireland, Marine Port, Ireland; 30 University Nova of Lisbon, Lisbon, Portugal

Abstract

Over the last two decades, three successive research consortia (EDEN, EDENext and VectorNet) aimed at improving knowledge, surveillance, and control of vector-borne diseases in Europe and neighboring countries. Among these, sand fly-borne diseases including leishmaniasis and phleboviral infections represent an important public health and veterinary concern. A novel effort to tackle sand fly borne diseases (SFBDs) – the CLIMOS project, has been underway since September 2022. Using sand flies and the diseases they transmit as a model system, CLIMOS - Climate Monitoring and Decision Support Framework for Sand Fly-borne Diseases Detection and Mitigation with Cost-benefit and Climate-policy Measures – aims to complement and build on previous efforts, bringing together researchers, health-care and veterinary practitioners, technology platform designers and at-risk communities, to conduct innovative and applied research seeking to better prepare for current and future impacts of climate and environmental changes on human and animal health. Specifically, CLIMOS will: a) develop a general public health risk assessment method for SFBDs through integration of climate, environmental and One Health disciplines and data sciences; b) utilize big data from Earth-observing satellites and ground-level surveillance records, to map the locations of disease-carrying insects and provide health, climate and environmental services to keep communities safe, and c) integrate economic and social sciences, to enable socio-economic assessments of impacts of the incidence and spread of SFBDs on individuals and societies.

Keywords: sand flies, sand fly borne dieases, climate, environment

OS – oral session

Session: [SE16] Parasitology in multi-disciplinary projects /grants

JPIAMR project: Droplet Digital PCR and PCR-free BIOSensors for the diagnosis of resistance-associated SNPs in *Pneumocystis jirovecii* (DDBIOS)

Marta Kicia[1], Żaneta Zajączkowska[1], Magdalena Szydłowicz[1], Solène Le Gal[2], Gilles Nevez[2], Enrique Calderón[3]

1 Department of Biology and Medical Parasitology Wroclaw Medical University, Wroclaw, Poland

2 Laboratory of Parasitology and Mycology, Brest University Hospital, Brest, France; Fungal Respiratory Infections (FRI) Research Unit, University of Angers, University of Brest, Brest, France

3 Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío/Consejo Superior de Investigaciones Científicas/Universidad de Sevilla, Sevilla, Spain; Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), Madr

Abstract
Pneumocystis jirovecii is a respiratory fungus, whose infection can lead to the development of *Pneumocystis* pneumonia (PcP), especially in immunosuppressed patients. There are no available systems for its *in vitro* culture – currently used diagnostic methods are based mainly on molecular techniques which enable detection and analysis of single nucleotide polymorphisms (SNPs) within genes encoding targets for agents widely used in PcP prevention and treatment. SNPs development may result from the previous use of such agents and be associated with antimicrobial resistance (AMR). However, PCR-based methods still have several limitations and new tools which enable rapid and sensitive pathogen identification and SNPs assessment are required. The aim of this project is the development and evaluation of two methods – droplet digital PCR and PCR-free biosensors – in the diagnosis of *P. jirovecii*, as well as in the quantitative and qualitative analysis of SNPs within four pathogen genes potentially associated with AMR (DHPS, DHFR, IMPDH, CYB), in biological samples collected from patients at high risk of infection. The final objective of the project is to create the database based on the results obtained – the distribution of identified SNPs and their ratio to the wild type, in relation to the examined patients' data, will allow to determine the potential consequences of the use of specific drugs on AMR emergence, as well as the possible impact of such mutations on the effectiveness of treatment in the context of given infection risk factors.

This project [2023/05/Y/NZ6/00166] has been supported by National Science Centre under the framework of the JPIAMR - Joint Programming Initiative on Antimicrobial Resistance.

Keywords: droplet digital PCR, PCR-free biosensors, *Pneumocystis jirovecii*, resistance-associated SNPs, respiratory infections diagnosis

OS – oral session

Session: [SE16] Parasitology in multi-disciplinary projects /grants

SPRINGS: Supporting policy regulations and interventions to negate aggravated global diarrheal disease due to future climate shocks

Vanessa Harris[1], Laura Rinaldi[2], Ludovico Dipineto[2], Josh Colston[3]

1 Academisch Medisch Centrum bij de Universiteit van Amsterdam, The Netherlands
2 University of Napoli Federico II, Italy
3 University of Virginia (US)

Abstract
Climate change and the concomitant destruction of the natural world are threatening human and planetary health now. Global climate projections of increased precipitation, flooding and drought threaten decades of progress achieved in reducing diarrheal disease burden. Key knowledge gaps in these dynamics hinder effective policy responses and prioritisation of mitigation and adaptation strategies to tackle diarrheal threats in Europe and globally. The SPRINGS consortium* brings together scientists from climate, environment, health and the social sciences to collaborate with communities, private sector, public authorities, and policy makers in a transdisciplinary project to address these knowledge gaps and needs. The project builds 4 case studies in Italy, Ghana, Romania, and Tanzania with contrasting vulnerabilities to predict and measure local climate, water quality, and pathogen-specific diarrheal disease burden in order to inform and prioritise effective policy responses. Working with stakeholders and policy makers across sectors, SPRINGS will identify, appraise and prioritize evidence-based interventions, conduct health & value impact assessments, helping policy makers implement sustainable planetary health policies with robust evidence.

**SPRINGS CONSORTIUM Academisch Medisch Centrum bij de Universiteit van Amsterdam (NL), Norwegian Meteorological Institute (NO), University of Virginia (US), Stichting Amsterdam Institute for Global Health and Development (NL), University of Ghana (GH), Three o'Clock (FR), London School of Health and Tropical Medicine (UK), Aarhus University (DK), IHE Delft Institute for Water Education (NL), The Abdus Salam International Centre for Theoretical Physics (ICTP, IT), Vrije Universiteit Amsterdam (NL), University of Naples Federico II (IT), Haydom Lutheran Hospital (TZ), AQUATIM (RO), University of Bucharest (RO), Rijksinstituut voor Volksgezondheid en Milieu (NL)*

Keywords: *Giardia*, *Cryptosporidium*, *Campylobacter*, climate change, One Health

OS – oral session

Session: [SE16] Parasitology in multi-disciplinary projects /grants

LIFE MiCliFEED: tackling climate change through functional feed in small ruminants

Smarada Sotiraki, Life Miclifeed Partners

VRI ELGO DIMITRA

Abstract
Parasitic infections are ubiquitous in grazing small ruminants, and a serious problem, causing reduced productivity, ailing health, and necessitating regular drug use. Parasitic infections affect not only health but also GHG production, besides the impact of gastrointestinal (GI) parasites of small ruminants is of particular importance. GI parasite infections in lambs drive a 33% increase in methane yield (g CH4/kg dry matter intake) revealing the potential benefits of mitigating emissions through controlling parasite burdens.
LIFE MiCliFeed (Full name: Mitigating Climate impact of small ruminants through innovative Feeding approaches), is a co-financed project receiving funding from the EU LIFE Programme and will run for 5 years until early 2026. It will use waste streams from the food industry in Southern Europe (olive, wine, nuts and carob tree production) that remain unused or are used as regular livestock feed to prepare upgraded extracts with bio-active properties that have the potential to reduce methane emissions from sheep and goats. While the use of feed additives to reduce greenhouse gas emission from livestock is not new, the use of a circular approach, upcycling waste streams is novel. Moreover, reduction of methane emissions will be achieved not only via interaction with the ruminal microbiome leading to fermentation, but also through testing the activity of the bio-active substances against common parasitic pathogens. Through this additional effect on the parasite burden, production efficiency can be improved, and the climate impact of livestock production further mitigated. The projects will perform lab and farm experiments in the Mediterranean area to validate the approach and if successful, develop a roadmap how such an approach can be implemented across the industry. The project involves public and private partners from Greece, Italy, France, and Belgium. Project results and news will be announced at the project's website.

Keywords: gastrointestinal parasites, GHG emissions, bioactive feed, LIFE project

OS – oral session

Session: [SE16] Parasitology in multi-disciplinary projects /grants

A cross-disciplinary alliance to PREdict and prePARe for climate sensitive Vector-Borne Diseases in Africa and Europe: the PREPARE4VBD experience

Anna-Sofie Stensgaard

Section for Parasitology and Aquatic Pathobiology, Department for Veterinary and Animal Sciences, Faculty of Health, University of Copenhagen, Denmark

Abstract

Vector-borne diseases (VBDs) constitute a major challenge to both human and animal health in many countries today. However, with on-going climate change and globalization, it is anticipated that new outbreaks of a variety of different zoonotic vector-borne diseases will occur more frequently in the future, along with an increased spread of invasive vectors and risk of VBDs becoming established in new areas. This warrants the need for new and improved tools for early detection and surveillance, as well as strong collaboration among different sectors and research environments, often operating in siloes. In response to this, a multi-disciplinary consortium was formed in 2021, bringing together 10 highly complementary research and policy institutions in both Africa and Europe. The consortium spans a vast geographical gradient of different partners, disciplines, ecosystems and climates in which the target PREPARE4VBD diseases are distributed, to develop new knowledge and surveillance tools transcending disciplines and vector organism to improve preparedness in Africa and Europe for zoonotic VBD emergence. Submitted under the acronym PREPARE4VBD (A Cross-Disciplinary Alliance to Identify, PREdict and prePARE for Emerging Vector-Borne Diseases), the 4 year project was selected for funding by the European Commission (EC) within the European Horizon 2020 RIA framework. As a multidisciplinary consortium, PREPARE4VBD aims to create a broad, conceptual knowledge that reaches beyond specific vector-borne diseases, organisms or disciplines traditionally operating in "siloes", to prepare for a new era of a diverse set of invasive vectors and emerging VBDs under climate change and globalization. With point-of-departure in three different vector groups (mosquitoes, ticks and freshwater snails), we aim to build capacity, develop new detection tools and model-based surveillance systems for a select number of zoonotic VBDs of relevance to both animal and human health.

Keywords: vectors, ticks, mosquitoes, snails, climate change

OS – oral session

[SE17]
Diagnostics of parasites

Session: [SE17] Diagnostics of parasites

Cell-free DNA as a novel diagnostic biomarker for parasite infections in dog

Shirin Akter[1], Ryo Nakao[2], Mohammad Zahangir Alam[1], Ken Katakura[2]

1 Department of Parasitology, Bangladesh Agricultural University, Bangladesh
2 Hokkaido University, Japan

Abstract

Cell-free DNA (cfDNA) comprises fragments of DNA found extracellularly and mainly in theblood circulation. cfDNA is relatively a new analyte and being applied in the current armamentarium of diagnostics. The present study was designed to assess the potential of cfDNA as a novel diagnostic biomarker for parasite infections. We employed 14 serum samples that were collected from stray dogs captured in visceral leishmaniasis-endemic areas of Bangladesh. Our previous studies showed that some of the dogs were infected with several pathogens such as *Leishmania donovani*, *Babesia gibsoni*, and *Anaplasma* sp. using conventional PCR. As negative control, dog sera obtained from 4 laboratory dogs were also included. cfDNA was extracted from 0.6 to 1.7 mL of sera using MagMAX™ Cell-Free DNA Isolation kit and the presence of cfDNA with the size of ~150bpwas confirmed by Agilent Bio analyzer 2100. cfDNA libraries were prepared using the Illumina TruSeq Nano DNA Sample Prep kit and analyzed on Illumina MiSeq platform. After removing low-quality sequences, the resulting reads were subjected to BLAST search analysis. We obtained approximately 46 million reads for 18 dog samples from two independent MiSeq runs. Preliminary analysis showed that most of the reads (>98%) had the highest identities with dog genome sequences. However, some of the reads showed association with parasite sequences (e.g. *L. donovani*, *Dirofilaria* sp.,and *Anaplasma* sp.). In conclusion, the analysis of cfDNA could be a novel diagnostic approach to produce an inventory of parasites carried by dogs in a high throughput sequencing manner.

Keywords: Cell-free DNA, Next-generation sequencer, parasites, dogs

OS – oral session

Session: [SE17] Diagnostics of parasites

Evaluation of the PAPRIKA method for predicting *Cryptosporidium* contamination of karst water resources

Gladys Foissey[1], Philippe Audra[2], Romy Razakandrainibe[1], Loic Favennec[3], Matthieu Fournier[4], Damien Costa[5]

1 Université de Rouen, EA7510 ESCAPE
2 Polytech'Lab - UPR 7498, University Côte d'Azur, France
3 Université de Rouen, EA7510 ESCAPE, CHU de Rouen, CNR Cryptosporidioses, Microsporidies et Autres Protozooses digestives
4 Univ Rouen Normandie, Univ Caen Normandie, CNRS, M2C, UMR 6143, Rouen, France
5 EA 7510 ESCAPE Epidemiosurveillance and Circulation of Parasites in the Environment, University of Rouen Normandie, University Hospital of Rouen, CNR Cryptosporidioses, Microsporidies et Autres Protozooses digestives

Abstract

In 2019-2020, the largest cryptosporidiosis outbreak never reported in France occurred. Several thousands of patients were concerned. An intense rainfall caused an episode of *Cryptosporidium parvum* contamination in the karstic springs. In this context, a study was carried out to evaluate a prediction model for *Cryptosporidium* oocysts dissemination in karst aquifer. The PaPRIKA vulnerability assessment method was evaluated. The PaPRIKa method is based on four criteria: protection of the groundwater, rock type of the reservoir, infiltration and karstification. From January to July 2021, eight surface waters were sampled monthly. Infectivity of isolated *Cryptosporidium* oocysts was evaluated and strains were genotyped. Potential correlation with microbiological potability indicators of tapwater was evaluated. Sampling sites contamination varied according to seasonality and most vulnerable suspected sites using the PaPRIKA vulnerability method were actually the most contaminated. Up to 7/8 sampling sites were contaminated by oocysts in January. No significant correlation was observed with other investigated microbial parameters neither with water features. Regarding the potential correlation between *Cryptosporidium* contamination and precipitation: oocysts were mainly detected when it rained during sampling or when it rained for several days before sampling. Results on subtyping showed that contamination varies over time even for a same sampling site and that a wide diversity of subtypes are circulating. Subtypes IIa were dominating. 86% of detected isolates were still infective. In conclusion, the PaPRIKA vulnerability assessment method appeared effective to predict *Cryptosporidium* oocysts circulation in karst aquifer. Water resources contamination appeared frequent, influenced by rainfall and surrounding livestock areas. The PaPRIKA vulnerability assessment method could be proposed to health authorities for the prediction (or even prevention) of cryptosporidiosis.

Keywords: *Cryptosporidium*, aquifer, model, vulnerability, assessment

OS – oral session

Session: [SE17] Diagnostics of parasites

Detection of *Fasciola hepatica* by LAMP and PCR in intermediate hosts from Portugal

Samira D'Almeida[1], María Martínez-Valladares[2], Isabella Martins[3], Manuela Calado[4], Isabel L. Maurício[4]

1 Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Rua da Junqueira 100, 1349-008 Lisboa, Portugal
2 Instituto de Ganadería de Montaña (CSIC-Universidad de León), León, Spain
3 Veterinary Medicine Department, Center for Agricultural Sciences and Engineering, Universidade Federal do Espírito Santo, Alegre, Espírito Santo, Brazil
4 Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Rua da Junqueira 100, 1349-008 Lisboa, Portugal

Abstract

Fasciolosis is a cosmopolitan disease caused by the trematode species *Fasciola hepatica* and *Fasciola gigantica*, whose intermediate hosts are Lymnaeid freshwater snails, and the reservoir are ruminants. Fasciolosis is considered re-emergent by the WHO and a limiting factor in ruminant pasture production. Drug resistance is an increasing problem and control requires knowledge about intermediate host snail populations for effective pasture control, as well as rapid and accurate diagnostic methods. Molecular diagnostic techniques, such as PCR, are more sensitive than parasitological diagnosis, but isothermal methods, such as Loop-Mediated Isothermal Amplification (LAMP), can have high sensitivity and specificity and be easier to apply in low resource locations.

This study aimed to explore the geographic distribution of intermediate hosts of *F. hepatica* in the region between the Tejo and Sado rivers, districts of Setúbal and Santarém in Portugal, and infection rates by PCR and LAMP using new primers developed from a region of the mitochondrial NAD5 gene. Field work took place from 2017 e 2018, at 29 locations. The collected snails were exposed to light to verify cercariae shedding, and DNA was ex-

tracted. Snail species were confirmed by *Galba truncatula/Galba schirazensis* multiplex microsatellite PCR or by COI amplification and sequencing.

Among the collected Lymnaeid snails, infection by *F. hepatica* was confirmed in nine (4%) by PCR and six (1.8%) by LAMP. Six infected snails were identified by molecular methods as *G. truncatula*, one as the cryptic species *G. schirazensis*, and two as *Ampullaceana balthica*. *Fasciola hepatica* detection by PCR amplification with the new primers for NAD5 was more sensitive than with LAMP but with high specificity for both, and LAMP has the advantage of providing a faster result without the need for electrophoresis. The role of *G. schirazensis* and *A. balthica* in *F. hepatica* transmission in Portugal is discussed.

Keywords: *Fasciola hepatica*, *Galba* spp., *Ampullaceana balthica*, loop mediated isothermal amplification, molecular epidemiology

OS – oral session

Session: [SE17] Diagnostics of parasites

Comparison of indirect ELISA specificity and sensitivity after fast protein liquid chromatography (FPLC) of *Toxocara canis* somatic antigen to diagnose human toxocariasis

Zahra Navi[1], Mohammad Bagher Rokni[1], Reza Falak[2], Mohammad Zibaei[3], Mehdi Mohebali[1], Abbas Rahimi Foroushani[4]

1 *Department of Medical Parasitology & Mycology,School of Public Health, Tehran University of Medical Sciences,Iran.*
2 *Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran,*
3 *Department of Parasitology, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran*
4 *Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

Abstract

We aimed to compare the specificity and sensitivity of indirect Elisa after purification of *Toxocar canis* somatic antigen using fast protein liquid chromatography (FPLC).Antigens for serological analysis were obtained from the adult *Toxocara* worms recovered from naturally infected puppies. The FPLC method, using a DEAE Sepharose-6B, anion-exchange column chromatography technique was set up for antigen purification. Sera from 42 toxocariasis patients and 42 healthy individuals were considered as positive and negative samples, respectively. To evaluate the cross-reactivity of antibodies, we applied 21 heterologous sera with various helminthes and protozoan infections. Indirect Elisa was conducted for checking anti-*Toxocara* antibodies (immunoglobulin G) against *T. canis* somatic antigen before and after the purification using FPLC method.The sensitivity, and specificity for indirect Elisa of *T. canis* crude antigen were detected 100% and 82%, respectively. Cross-reactive sera with crude antigen of *T. canis* contained sera of fascioliasis n= 4, hydatidosis n= 4, strongyloidiasis n=1 and trichostrongylosis n=2. After purification of somatic protein we found 2 protein fractions separated from DEAE Sepharose-6B which showed 100% sensitivity and specificity with no cross reaction in indirect Elisa of the same sera.Purified fractions achieved after FPLC might be considered as suitable candidates for toxocariasis detection kit production. In addition, we found the FPLC method simple, rapid, low cost and reproducible.

We express our sincere thanks to Tehran University of Medical Sciences (TUMS), Tehran, Iran, for supported our study by grant 25088.

Keywords: *Toxocara canis*, FPLC, Elisa

OS – oral session

Session: [SE17] Diagnostics of parasites

In search of *Echinococcus multilocularis* diagnostic markers

Katarzyna Basałaj[1], Mateusz Pękacz[2], Emilia Dvorožňáková[3], Julia Gurtowska[2], Daniel Młocicki[4], Anna Zawistowska-Deniziak[2]

1 *Museum and Institute of Zoology, Polish Academy of Sciences, 00-679 Warsaw, Poland*
2 *Department of Immunology, Institute of Functional Biology and Ecology, Faculty of Biology, University of Warsaw, 02-095 Warsaw, Poland*
3 *Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, 04001 Košice, Slovak Republic*
4 *Department of General Biology and Parasitology, Medical University of Warsaw, Warsaw, Poland*

Abstract

Echinococcosis is one of the most neglected parasitic diseases, with alveolar echinococcosis (AE) being particularly deadly. Caused by the fox tapeworm *Echinococcus multilocularis*, AE is a major zoonotic disease. There is no effective vaccine or control measure for AE in endemic areas. The only curative treatment is invasive surgical resection, which is possible for only about 35 % of patients due to late-stage diagnosis. Owing to the limitations of current diagnostic methods, infections are frequently detected at an advanced stage where metastasis has already occurred to organs like the lungs, brain, or bones, making complete excision unfeasible. There is an urgent need for new therapeutic agents and improved diagnostic methods for early detection and diagnosis.

In our study we analyzed samples from BALB/c mice infected with *E. multilocularis* at various timepoints post-infection. The infections varied in severity depending on the dose (number of protoscolices per mouse). We screened a peptide phage library using plasma from infected mice to identify potential diagnostic markers for *Echinococcus* infection. The prescreening step was carried out using serum samples from uninfected mice, different blocking buffers and polystyrene plate. Ten selected peptides were able to distinguish between healthy and infected mice in an ELISA test with mice sera. Differences were observed in both lightly and heavily infected mice compared to uninfected group. However, we did not observe significant differences based on the infection dosage. Anti-peptide antibody titers in the infected mice were correlated with the number of days post *E. multilocularis* infection. The identified phage clones will undergo further analysis to exclude cross-reactions with sera from mice infected with other parasites. Subsequently, they will be tested as diagnostic markers using human serum samples infected with *E. multilocularis*.

Keywords: *Echinococcus multilocularis*, diagnostic markers, phage display technology

OS – oral session

Session: [SE17] Diagnostics of parasites

Multiplex qPCR a powerful technique for the diagnosis of the intestinal protozoan infections

Claudia Mihaela Istrate[1], Carmen Michaela Cretu[1], Ionica Ceaus-Capota[1], Loredana Gabriela Popa[2], Patricia Elena Mihailescu[1]

1 *Eco-Para-Diagnosis Medical Center, Bucharest, Romania*
2 *Colentina Clinical Hospital, Bucharest, Romania/University of Medicine and Pharmacy „Carol Davila", Bucharest, Romania*

Abstract

Protozoan are parasitic infections frequently meet in human population from children to adults. They are localized in the digestive tract and are very easy to spread from a contaminated person to another via direct contact, or infested food, water and objects. Protozoan organisms, *as Giardia duodenalis (G.d.), Cryptosporidium parvum (C.p.), Blastocystis* spp, *Entamoeba coli/histolytica, Di-*

entamoeba fragilis (D.f.) are a major source of distress for the host no matter the age of the patient. The main symptoms are: nausea, bloating, gas, diarrhea, anorexia, abdominal pain.

Between January 2023 - 15 May 2024, in the laboratory of Eco-Para-Diagnosis Medical Center from Bucharest, Romania, were tested 309 feces samples from patients with suspicion of intestinal parasitic infections. All the samples were analyzed using microscopy (direct) and imunocromatography. 216 out of 309 were tested using molecular biology technique (qPCR), multiplex assays for two different combinations of primers (Parasitic Stool Panel1-R-Biopharm Germany; Viasure Multiplex-Certest, Spain). Using the microscopy 2% (6 /309) were positive G. d.; 1% (3/309) positive *Blastocystis* spp. Molecular methods did show: 8/216 samples were tested for combinations as follow: *Blastocystis* spp., D.f., and the results were: positive 3 D.f. and 5 *Blastocystis* spp. (2 of them were positives for the both pathogens). 208/216 were tested for intestinal protozoans using a different PCR kit -10.4% were positives for G.d. (5) and 6.24% for C.p. (3). All the positive samples identified by microscopy and immunochromatography were confirmed, and in two other negative samples, parasitic DNA was detected.

Molecular biology (qPCR) became more and more necessary to complete the classical methods for parasitological diagnosis. To have a proper diagnosis, we have to combine different techniques (microscopy, immunology and molecular biology) to receive a right therapeutically approach for the patient.

Keywords: qPCR, protozoan, molecular diagnosis

PS – poster session

Session: [SE17] Diagnostics of parasites

Contemporary challenges of medical parasitological diagnostics - using their own observations

Przemysław Kołodziej, Anna Bogucka-Kocka

Chair and Department of Biology and Genetics, Medical University of Lublin, Poland

Abstract

Medical parasitological diagnostics is a very important part and at the same time a challenge of modern laboratory medicine. The changing climate and international migration significantly facilitate the spread of parasitic diseases and mean that in non-endemic countries we increasingly encounter the need to diagnose diseases caused by tropical parasites. In the case of parasitic diseases, the cooperation of various specialists and continuous training and improvement of qualifications of medical staff dealing with medical parasitological diagnostics are very important. Continuous work is also needed to develop new tools and diagnostic tests that will make it easier to detect particular species of parasites and will be more economically available.

Medical parasitological diagnostics requires extensive experience and extensive theoretical and practical knowledge. There is a great need to conduct scientific research that will contribute to the significant development of the field of medical parasitological diagnostics.

Our research fits into this trend because, as part of our multicenter team, we conduct experiments related to the diagnosis of parasitic diseases using methods, among others: microscopic, serological and molecular methods. As part of our team, we also conduct research on the search for new, innovative tools that can be used in broadly understood medical parasitological diagnostics.

Keywords: diagnosis of parasitic diseases, parasitological diagnostics, innovative tools

PS – poster session

Session: [SE17] Diagnostics of parasites

Frequency of parasitic infestation in faecal specimens in patients from Malopolskie region of Poland, examined between 2020 and 2023

Anna Lisowska[1], Zuzanna Radoń[1], Magdalena Zdziechowicz-Wachała[1], Paulina Rapacz- Urbańczyk[2], Danuta Kozłowska[3]

1 Diagnostyka S.A.-Parasitology laboratory department at MDL Kraków - Poland
2 Diagnostyka S.A. IT department BD analysis -Kraków-Poland
3 Diagnostyka S.A. and Andrzej Frycz Modrzewski Krakow University (AFMKU) Faculty of Medicine and Health Sciences department Laboratory Medicine-Kraków-Poland

Abstract

Parasitic infestations continue to be an important health issue. Since 2008, epidemiological surveillance of gastrointestinal parasitoses has been mostly discontinued in Poland. As a result, the current epidemiological situation in this field may not be fully recognized.

The aim of this study was to analyze the volume of ova and parasite stool testing in a private medical laboratory Diagnostyka S.A. in Cracow, Poland over four years (2020 – 2023). Additionally, the distribution of different parasites in age groups was analyzed. Microscopic examinations of samples were performed using Lugol's iodine stained faecal smears, Kato and Miura thick-smear technique (112943 samples), and also Parasep SF fecal parasite concentrator (1804 samples). Separately, Graham's method was used for detection of *Enterobius vermicularis* in perianal swabs (3300 samples), and ELISA was used for detection of *Giardia lamblia* coproantigen (23744 samples).

Altogether, 97 273 patients were analyzed. The number of tests performed increased from 16 703 in 2020 r. to 29 591 in 2023 r. The parasites detected included: *Blastocystis* spp., *Entamoeba histolytica sensu lato, Entamoeba coli, Chilomastix mesnili, Iodameba butschli, Giardia lamblia, Enterobius vermicularis, Taenia saginata, Ascaris lumbricoides*.

The most commonly detected protozoa was *Blastocystis* spp., followed by *Enterobius vermicularis*. In the analyzed period the frequency of *Blastocystis* infestations increased, and this tendency was seen in all age groups. In contrast, no clear trend was observed in the frequency of other protozoan species.

Keywords: parasitic infestation, faecal specimens, Poland

PS – poster session

Session: [SE17] Diagnostics of parasites

External quality control an useful and necessary instrument in daily routine for the parasitologists in clinical laboratories

Patricia Elena Mihailescu, Aurora Cotea, Gabriel Catalin Dinulescu, Ioana Diana Dumitran, Cristina Gabriela Ivan, Alexandra Cîrstea, Adina Elena Stanciu, Georgeta Sorescu, Constanta Popa

CALILAB Association ROMANIA

Abstract

In Romania, specialists from laboratories for medical analysis take part in external quality control schemes (EQC) provided by the CALILAB Association from Bucharest, Romania. From the field of parasitology, the coproparasitological exam is represented by a dedicated scheme. In our country, participation in EQC schemes is, for medical laboratories a mandatory step in the process of accreditation.

Between 2020 - 2024 (including two rounds from 2024), during the activity carried out together with the CALILAB team, we analyzed the results reported by the participants in EQC schemes for the coproparasitological examination. During this period of time, were carried out, 26 rounds of EQC, with a total number of 10923 results reported by to medical laboratories with a distribution between 166 and 690 participants per round/participation. The object of the proficiency test provided by the CALILAB Association to medical laboratories, in Romania, was represented by digital images transmitted to participants in EQC schemes in each round in order to identify parasites present in the object of the proficiency test or to specify the absence of parasitic elements. During the analyzed period of time, the participants reported a total number of 10923 results to the EQC scheme „Coproparasitological examination"; The percentage of „satisfactory" results obtained at the external evaluation of the quality of the results provided by the participants in EQC scheme varied during the 26 rounds of 2020-2024 (including April 2024) from 86.31% to 100%.

From the analysis of the above results we did notice as follow:

- a difficulty in identifying negatives samples (without parasitic elements), due to the presence of a multitude of non-specific forms that can generate a false positive response;
- a good performance of the medical laboratories participants in the EQC scheme during the analyzed period of time.

Keywords: External quality control, EQC, Coproparasitology exam

PS – poster session

Session: [SE17] Diagnostics of parasites

Comparison of the performance of the AllPlex GI Parasite® assay (Seegene) and microscopy for the diagnosis of intestinal parasites in routine

Florence Robert-Gangneux[1], Brice Autier[1], Clement Cazala[2], Sorya Belaz[3], Anne Dupuis[3], Helene Guegan[1], Jean-pierre Gangneux[1]

1 Univ Rennes, Irset Inserm U1085, Rennes, France
2 Univ Rennes, France
3 Centre Hospitalier Universitaire de Rennes, France

Abstract

Commercial multiplex qPCR assays are now widely used for the diagnosis of intestinal parasitoses, particularly for protozoan infections, but no prospective studies on large patients cohorts evaluated their performances.

We extracted data from our information system from 01/01/2021 to 15/03/2024. All stool samples analysed in routine during the study period were included. Parasites were searched in all samples using the AllPlex GI Parasite® assay (Seegene), direct examination and 2 concentration methods. *Cryptosporidium* spp were searched by microscopy using acid fast staining only when specifically precribed.

During the study period, 3495 stools were analysed from 2127 patients (1.5±0.8 sample/clinical episode). Only one sample was available for 1571 patients, while ≥2 samples were analyzed for 737 patients.

Overall *Giardia intestinalis* (Gi), *Cryptosporidium* spp (Cr), *Entamoeba histolytica* (Eh), *Dientamoeba fragilis* (Df) and *Blastocystis hominis* (Bh) were found by multiplex qPCR in 45, 30, 9, 310, and 673 samples, respectively, alone or in combination (n=909 samples). Microscopy was positive for Gi, Cr, Entamoeba histolytica/dispar (Eh/Ed), Df and Bh in 25, 8, 24, 22 and 229, samples, respectively, alone or in combination (n=286 samples). There was no samples with qPCR-/Microscopy+ results for Gi, Cr, Eh, but Df and Bh were detected only with microscopy in 6 and 20 samples, respectively. Microscopic examination allowed the detection of parasites not targeted by the multiplex panel (5 *Cystoisospora belli*, 331 samples with a non-pathogenic protozoa and 68 samples with a helminth). Interestingly, those parasites were associated with a positive qPCR (any protozoa) in 75% of cases.

The multiplex qPCR proved very efficient to detect protozoan parasite, but a microscopic technique should be performed when *C. belli* is suspected. In the vast majority of cases, a protozoa was detected on the first stool sample. A concentration method must be still combined to detect helminths.

Keywords: intestinal parasites, multiplex PCR, diagnosis, protozoan parasites

PS – poster session

[SE18]
Snails, trematodes, diseases / MOTT

Session: [SE18] Snails, trematodes, diseases / MOTT

Molecular diagnosis of intestinal schistosomiasis: An overview of current protocols and what is needed, highlighted using data from a *S. mansoni* and *S. haematobium* co-endemic area

John Archer[1], Silvia Mesquita[2], Russell J. Stothard[3], Bonnie Webster[4]

1 Liverpool School of Tropical Medicine & Natural History Museum, UK
2 Natural History Museum, UK & Fundação Oswaldo Cruz, Brazil
3 Liverpool School of Tropical Medicine, UK
4 Natural History Museum, UK

Abstract

Intestinal schistosomiasis is typically diagnosed using Kato-Katz faecal microscopy or point-of-care circulating cathodic antigen (POC-CCA) rapid diagnostic tests. Whilst these assays are valuable tools in certain settings, highly sensitive assays, such as PCR-based methods, are also needed for impactful disease diagnosis and surveillance. However, PCR is unsuited for use in most schistosomiasis-endemic settings. Both PCR itself, as well as the preliminary steps needed to isolate DNA from faecal material, require sophisticated equipment, specialised personnel, and reliable laboratory infrastructure seldom available in endemic areas. In addition, there is currently no standardised diagnostic PCR assay that is routinely used to differentiate between the various human-infecting *Schistosoma* species in multispecies co-endemic areas.

Isothermal DNA amplification methods offer an alternative to PCR-based amplification and are better suited for use in resource-poor settings as they can be highly portable and user-friendly. The most widely used isothermal DNA amplification method is loop-mediated isothermal amplification (LAMP). Again, however, no standardised *S. mansoni*-specific LAMP assay capable of reliably diagnosing intestinal schistosomiasis is currently available. In addition, an *S. mansoni*-specific Recombinase Polymerase Amplification (RPA) assay has also been recently developed, however, this has not yet been fully validated using human faecal material.

We present an overview of current protocols available for the molecular diagnosis of intestinal schistosomiasis, inclusive of methods needed to isolate DNA from faecal material, PCR, and isothermal DNA amplification approaches. We also outline what is needed to develop, standardise, and deploy highly sensitive and specific molecular diagnostic tools that can be used at the point-of-care in low-endemicity areas, highlighted using data recently generated from a *S. mansoni* and *S. haematobium* co-endemic area.

Keywords: Intestinal schistosomiasis, diagnosis

OS – oral session

Session: [SE18] Snails, trematodes, diseases / MOTT

The Schistosome and Snail Resource (SSR) – Maximising snail and cercariae production by investigating snail-schistosome compatibility

Adam Cieplinski[1], Fernanda Sales Coelho[2], Guillaume Delhaye[3], Carla Gustave[1], Vanessa Yardley[4], Amaya Bustinduy[2], Aidan Emery[2], Bonnie Webster[1]

1 Natural History Museum, United Kingdom
2 London School of Hygiene and Tropical Medicine, United Kingdom
3 Royal Botanic Gardens Kew, United Kingsdom
4 London School of Hygiene and Tropical Medicine,United Kingdom

Abstract

The Schistosome Snail Resource (SSR) is a Wellcome Trust-funded schistosomiasis resource, run through a partnership between the Natural History Museum (NHM) and the London School of Hygiene and Tropical Medicine (LSHTM) in London. Schistosomiasis is a parasitic disease caused by schistosomes and transmitted by freshwater snails. Although, it is common, due to the complexity of its lifecycle few laboratories maintain it. Moreover, strains of both snails and parasites maintained in laboratories lack genetic heterogeneity present in nature. The aim of the SSR resource is to provide research material (snails and schistosome life-cycle stages), live and preserved, for applied and clinical research.

The NHM snail lab has been maintaining different strains of *Biomphalaria glabrata* snails for many years. Although, all these strains have been selected for their compatibility to infection with *Schistosoma mansoni*, we have observed differences in infection efficiency. To streamline our culturing efforts and to limit potential bias in future experiments we compared infection rates, cercarial production, shell growth rate and mortality for four strains of *Biomphalaria glabrata* to identify the best host.

We found significant differences in infection rate and in numbers of cercariae between different strains. Infected snails also grew much faster. Notably, the highly inbred melanistic strain of *B. glabrata* that was recently derived from one individual snail showed the highest level of infection rate as well as the highest cercarial output.

We decided to focus our infections on the highly inbred snail strain which resulted in higher infection rates and lowered workload necessary to maintain snail cultures. Our experiment shows the necessity of constant assessment of snail/schistosomes compatibility as the infection rate can easily increase or decrease by random selection of alleles impacting transmission.

Keywords: schistosomiasis, *Biomphalaria*, Schistosomes, cercariae

OS – oral session

Session: [SE18] Snails, trematodes, diseases / MOTT

Passive environmental DNA samplers for *Schistosoma* spp. and freshwater snail eDNA capture and detection in water

Sónia Cunha[1], Fernando Cardoso[2], Isabel L Maurício[2]

1 Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Rua da Junqueira 100, 1349-008 Lisboa, Portugal
2 Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Rua da Junqueira 100, 1349-008 Lisboa, Portugal

Abstract

Environmental DNA (eDNA) has increasingly been used as proxy for the presence, in water, of trematodes and of their intermediate hosts, freshwater snails, including *Schistosoma* spp. as an alternative to traditional snail surveys and cercariae collection. Most studies rely on water collection and filtration, which requires transportation of equipment to the field, or conservation of water to the laboratory, and filter clogging can be an issue. Passive environmental samplers (PEDS) have the advantage of sampling eDNA for a longer period and of being less sensitive to varying amounts of circulating eDNA. PEDS have been evaluated for eDNA detection in water, but not for trematodes.

We aimed to evaluate the efficacy of PEDS for capture of parasite and intermediate hosts eDNA. Various resins (10) and filter materials (19) were evaluated with exposure to free DNA and to infected snails in tanks, for various time periods, followed by CTAB extraction method. The best results from tank trials, overall, were achieved for Whatman filter paper 5 and the resin Amberlite XAD-4, which were deployed in the field in Mozambique in a urinary schistosomiasis endemic location. PEDS remained in situ from one to nine days and a good amount of DNA was ex-

tracted. *Schistosoma mansoni*, *Schistosoma haematobium*, *Schistosoma bovis* and intermediate host snail DNA was successfully amplified from field samples, and no cross-contamination was detected with negative controls. Better detection results were obtained from resin samples.

Low cost PEDS showed to be a good alternative to water filtering for eDNA capture and were not disturbed by the population or animals for several days in field trials. *Schistosoma* spp. and snail monitoring strategies that include PEDS should reduce costs and effort associated with intermediate snail host detection and population surveys.

Keywords: Passive environmental DNA samplers, *Schistosoma* spp., freshwater snails, environmental DNA, monitoring

OS – oral session

Session: [SE18] Snails, trematodes, diseases / MOTT

Long non-coding RNAs putatively involved with *Schistosoma mansoni* resistance to Praziquantel

Pedro Jardim Poli[1], Agatha Fischer-carvalho Fischer-Carvalho[1], Ana C. Tahira[1], Winka Le Clec'h[2], Frédéric D. Chevalier[2], Timothy J.C. Anderson[2], Sergio Verjovski-Almeida[1], Murilo Sena Amaral[1]

1 Laboratório Ciclo Celular, Instituto Butantan, São Paulo, Brazil
2 Texas Biomedical Research Institute, San Antonio, TX, USA

Abstract

Schistosomiasis is a very debilitating disease that affects over 250 million people globally. Treatment relies on Praziquantel (PZQ) monotherapy, which may contribute to emergence of parasite resistance. Recent studies have revealed that the primary target of PZQ, a transient receptor potential melastatin ion channel (Sm. TRPMPZQ), underlies variation in PZQ responses in *Schistosoma mansoni*. However, genetic variants associated with PZQ resistance were only found in non-coding regions, suggesting that regulatory changes may influence this trait. This study aims at identifying long non-coding RNAs (lncRNAs) possibly involved in the development of PZQ resistance by *S. mansoni*. We have previously shown that *S. mansoni* lncRNAs are vital for pairing-dependent adult worm homeostasis and fertility, and lncRNAs have a well-established role in drug resistance in human cancers. Here, differentially expressed (DE) lncRNAs were identified through re-analysis of two public RNA-Seq datasets from *S. mansoni* laboratory resistant subpopulations, which show 368-fold higher EC50 than sensitive ones. The RNA-Seq re-analyses revealed hundreds of DE lncRNAs, which were narrowed to 6 after filtering only DE genes located within the major quantitative trait *locus* linked to PZQ response. These lncRNAs raise interest as they are DE in multiple conditions and are located ~450kb downstream from the *Sm.TRPMPZQ* gene, representing putative *Sm.TRPMPZQ* regulators. These results were used to select DE lncRNAs that will be assessed by RT-qPCR using the same 368-fold resistant subpopulations used in the RNA-Seq assays. This is the first characterization of lncRNAs putatively involved in drug resistance in a parasite and may contribute to a better understanding of PZQ resistance in *S. mansoni*.

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Keywords: *Schistosoma mansoni*, schistosomiasis, drug resistance, praziquantel, lncRNAs

OS – oral session

Session: [SE18] Snails, trematodes, diseases / MOTT

The Hidden Parasitome: Digenean Diversity in African National Parks

Anya Tober[1], Joanne Cable[1], Isa-rita Russo[1], Bonnie Webster[2], Danny Govender[3]

1 Cardiff University, Wales
2 Natural History Museum, London
3 South African National (SAN)Parks, Kruger

Abstract

Freshwater snails are intermediate hosts for digenean parasites of both veterinary and medical importance (e.g. fasciolids and schistosomes). These snails have been reported across southern Africa; however, little is known about their parasite diversity and distribution. Traditional methods of detecting digenean diversity in snail hosts have focused on light induced cercarial shedding or crushing and dissecting snails, followed by identification of individual cercariae using morphological keys. However, these methods are laborious and time consuming with a high likelihood of human error. This study used a metabarcoding approach to amplify the nuclear 28S gene region of digenean parasites within whole freshwater snails, assessing within host diversity in two southern African National Parks (Kruger National Park, South Africa and Bwabwata National Park, Namibia). The metabarcoding approach highlighted large digenean diversity including species of medical and veterinary importance, across different snail genera in both parks. The Invasive *Tarebia granifera* snail in KNP showed a significantly higher digenean alpha diversity compared to other snail genera. Infection experiments are necessary to determine whether *Tarebia* snails can shed these parasites or whether they may cause a dilution effect on parasite transmission. Metabarcoding is a promising tool for assessing the diversity of digenean parasites and within host communities. This study also highlights the need for improved molecular databases and the value of combining traditional and modern approaches.

Keywords: metabarcoding, digeneans, diversity, freshwater snails, wildlife

OS – oral session

Session: [SE18] Snails, trematodes, diseases / MOTT

Phylogeography of *Schistosoma bovis*, *S. curassoni* and associated *S. haematobium* group hybrids across sub-Saharan Africa; revealed by mitochondrial cox1 analyses

Shannan Summers[1], Tom Pennance[2], John Archer[3], Nathan Tian-Bi[4], Ame Shaali[5], Senghor Bruno[6], Aidan Emery[7], Bonnie Webster[7]

1 London School of Hygiene and Tropical Medicine, UK
2 Western University of Health Sciences, USA
3 Liverpool School of Tropical Medicine, UK
4 Université Félix Houphouët-Boigny, Cote D'Ivoire
5 Zanzibar Ministry of Health, Tanzania
6 Vectors-Tropical and Mediterranean Infections (VITROME) Laboratory, Senegal
7 Natural History Museum, UK

Abstract

Schistosomiasis is a chronic disease of profound medical and veterinary importance. In Africa, there are five species infecting livestock of which *Schistosoma bovis* has the broadest distribution. *Schistosoma bovis* has become a research focus due to its ability to hybridize with the human-infecting species, *Schistosoma haematobium*. This study analysed mitochondrial *cox*1 data to explore geographical and potential host structuring of *S. bovis, Schistosoma curassoni* and *S. haematobium* group hybrids. The *cox*1 data consisted of both published (n=258) and new (n=169) datasets from adult worms, miracidia and cercariae from *S. bovis* and *S. haematobium-bovis* hybrids from across sub-Saharan Africa, Spain and Corsica. *S. curassoni* data were more limited to samples from Senegal, Niger and Mali. All data were aligned, trimmed and subjected to phylogenetic and haplotype analyses.

The analyses revealed a complex population structure, with a notable divide between East and West African *S. bovis* popula-

tions. Considerable reduced genetic diversity was found in the *S. haematobium-bovis* hybrid populations compared to *S. bovis* populations with two distinct hybrid clusters observed. These *S. haematobium-bovis* hybrids, which are primarily associated with human infections, showed limited, if any, current genetic mixing with *S. bovis* populations. *S. curassoni* also showed high levels of genetic diversity compared to *S. haematobium* and the hybrid populations but no population structuring was observed.

Our findings illustrate genetic diversity and population structuring of different *Schistosoma* species whilst highlighting the need to analyse samples from both humans and animals to identify population structuring by geography and host. Further analyses of such samples at the genome wide level is certainly warranted to shed light on inter species hybridisation and the level of actual ancient and contemporary mixing between human and animal *Schistosoma* species.

Keywords: *Schistosoma*, hybridisation, bovine, molecular, diversity

OS – oral session

[YSA]
Young Scientist Award Session

Session: [YSA] Young Scientist Award Session

Impact of *Anisakis* extracellular vesicles on human intestinal organoids: insights into immunomodulation and potential tumorigenic linkages

Ilaria Bellini[1], Daniela Scribano[1], Cecilia Ambrosi[2], Claudia Chiovoloni[1], Silvia Rondòn[1], Agostina Pietrantoni[3], Anna Kashkanova[4], Annamaria Pronio[5], Stefano D'Amelio[1], Serena Cavallero[6]

1 Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy
2 Department of Human Sciences and Promotion of the Quality of Life, San Raffaele Open University, IRCCS, Rome, Italy
3 Core facilities, Istituto Superiore di Sanità, Rome, Italy
4 Max Planck Institute for the Science of Light, Erlangen, Germany
5 Digestive Endoscopy Unit, Department of General Surgery and Surgical Specialties "Paride Stefanini", Sapienza University of Rome, Azienda Policlinico Umberto I, Rome, Italy
6 Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy; Department of Public Health and Infectious Diseases, Sapienza University of Rome, Laboratory affiliated to Pasteur Institute - Fondazione Cenci Bolognetti, Rome

Abstract

Anisakiasis is an accidental zoonosis caused by consuming raw fish parasitized with infective *Anisakis* spp third stage larvae (L3). Severe symptoms may include mucosal ulcers, granulomas and chronic inflammation in the gastro-intestinal tract, features potentially linked to tumorigenesis. A novel aspect of *Anisakis* biology involves the release of extracellular vesicles (EVs), potential players in host-parasite dynamics and disease progression. This study explores, for the first time, immunomodulatory and potential tumorigenic effects of *Anisakis* EVs on 2D human intestinal organoids (HIO), an innovative model mirroring gut epithelial during human infection.

470 *Anisakis* spp. L3 were collected from fish visceral cavities and cultured in RPMI 1640 in 1x P/S at 37°C with 5% CO2 for 24h. EVs were isolated using Exoquick kit and characterized through Nanoparticle Tracking Analyses (NTA), Interferometric Nanoparticle Analysis (iNTA), an innovative technique never used on helminths EVs, and electron microscopy (SEM, TEM). HIO were exposed to *Anisakis* EVs for 48h, followed by analyses of gene expression and cytokines amounts using comparative transcriptomics, qRT-PCR and Luminex assay.

Treating HIO with 2.92 x 109 particles/cells altered several transcripts with a potential involvement in tumorigenesis. Significant downregulation of EPHB2 and LEFTY1 and upregulation of NUPR1, genes associated with colorectal cancer, was observed. Additionally, a reduction in specific mediators of inflammation and cell cycle regulation like IL-33R, CD40, CEACAM-1, IL-1b, GM-CSF, IL-15 and IL-23 was detected, supporting the suggested parasite's immunomodulatory effect on the accidental human host, aiding *Anisakis* to establish and persist in its niche.

Keywords: *Anisakis*, extracellular vesicles, human intestinal organoids, immunomodulation, tumorigenic potential

OS – oral session

Session: [YSA] Young Scientist Award Session

The range of *Clinostomum complanatum* within French freshwater habitats hosting European perch (*Perca fluviatilis*)

Maureen Duflot[1], Françoise Pozet[2], Sophie Le Bouquin-Leneveu[3], Céline Richomme[4], Odile Bourgau[1], Isabel Blasco-Costa[5], Mélanie Gay[1]

1 Anses, Laboratory for Food Safety, Boulogne-sur-Mer, France
2 Jura Departemental Analysis Laboratory, Poligny, France
3 Anses, Ploufragan-Plouzané-Niort Laboratory, Epidemiology Health and Welfare Unit, Ploufragan, France
4 Anses, Nancy Laboratory for Rabies and Wildlife, Malzéville, France
5 Department of Invertebrates, Natural History Museum of Geneva, Geneva, Switzerland

Abstract

In the recent years, the consumption of fish products has surged in European countries, being an essential part of a healthy diet. Despite representing a small part of European production, freshwater fisheries hold considerable significance for non-professional anglers and restaurant owners. The spread of new eating habits lead to a raising risk for consumers due to food-borne parasitic zoonoses. *Clinostomum complanatum* (Platyhelminthes: Digenea) is present, at the larval stage, in numerous freshwater fish. This species is zoonotic and induce pharyngitis or laryngitis in humans following consumption of raw infected fish. Until now, the occurrence of the zoonotic trematode *C. complanatum* in Europe was restricted to the Danube basin and the North-East of Italy. The present study reports the distribution of these parasites from wild perch (*Perca fluviatilis*) in French freshwaters.

An epidemiological study was performed on batches of perch from different departments. The samples were collected from December 2020 to September 2023. Fish were dissected, metacercariae were isolated and characterized from a molecular perspective. Infection levels were calculated.

Comparison of generated sequences with available data of literature confirmed the presence of *C. complanatum* in France. Phylogeny and BLAST analysis of the new specimens showed high homology with individuals from Italy and Australia. Prevalences were very variable from 4 to 80% of infected fish per batch.

This survey constitutes the first mapping of *C. complanatum* in French freshwaters ecosystems. As these parasites are zoonotic, these findings emphasize the importance of monitoring and controlling the presence of *Clinostomum* in aquatic environments to minimise the risk of infections and ensure food safety. Moreover, further studies are needed to understand the distribution and expansion of these parasites, analysing their biological and epidemiological aspects, and the factors that influence their proliferation.

Keywords: Zoonosis, Clinostomum, freshwater fish, trematode

OS – oral session

Session: [YSA] Young Scientist Award Session

Nematodes of the genus *Thelazia* as one of the potential cause of infectious keratoconjunctivitis in European bison in Poland.

Katarzyna Filip-Hutsch[1], Anna Pyziel[1], Tomasz Hutsch[2], Karolina Duk[3], Daniel Klich[4], Wanda Olech[4], Aleksander Demiaszkiewicz[5], Krzysztof Anusz[1]

1 Department of Food Hygiene and Public Health Protection, Institute of Veterinary Medicine, Warsaw University of Life Sciences–WULS, Nowoursynowska 159, 02-776, Warsaw, Poland.
2 Department of Pathology and Veterinary Diagnostics, Institute of Veterinary Medicine, Warsaw University of Life Sciences-WULS, Nowoursynowska 159, 02-776, Warsaw, Poland.
3 Veterinary Diagnostics Laboratory ALAB plus – ALAB bioscience, Stępińska 22/30 00-739 Warsaw, Poland.
4 Department of Animal Genetics and Conservation, Institute of Animal Sciences, Warsaw University of Life Sciences - WULS, Ciszewskiego 8, 02-787, Warsaw, Poland.
5 Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Twarda 51/55, 00-818, Warsaw, Poland.

Abstract

Numerous clinical cases of infectious keratoconjunctivitis (IKC) has been observed in European bison in Poland during last few years. The aim of the present study was to describe infection with nematodes of the genus *Thelazia*, isolated from eyeballs of different European bison populations in Poland in relation to severity of ocular changes.

The eyeballs of 198 European bison, both showing ocular signs and clinically healthy, were collected in Poland in years 2021 - 2024. Conjunctival sac, tear ducts, corneal surface and nicitating membrane were rinsed with saline solution. Nematodes of the genus *Thelazia* were isolated from the decantated sediment and identified on the basis of morphological and molecular analyses. Eyeballs were subjected to histopathological examination. Two species of *Thelazia* nematodes has been identified – *T. skrjabini* and *T. gulosa*. Prevalence of infection reached over 62% and the infection intensity ranged from 1 to 28 nematodes per individual. Histopathology showed varying degrees of lesions, including: corneal erosions or ulcers, diffuse purulent infiltrates to lymphocytic infiltration in the cornea, and lymphocytic or mixed conjunctivitis with CALT stimulation. Occurrence of nematodes in eyeballs not always corresponded with the severity of ocular changes. Results of our studies revealed that nematodes of the genus *Thelazia* are common parasites of European bison in Poland, however their role in an outbreak of IKC demands further examination.

This study was supported by National Fund for Environmental Protection and Water Management (priority program No. 5.1.1), in frame of the project "Evaluation of the epidemic situation of Thelazia spp. infection (thelaziosis) in European bison in Poland". Part of the study was financed by the project "Complex project of European bison conservation by State Forests", financed by the Forest Found (Poland), contract no OR.271.3.10.2017.

Keywords: *Bison bonasus*, eyeworms, infectious keratoconjunctivitis, histopathology

OS – oral session

Session: [YSA] Young Scientist Award Session

When one become three – species diversity of *Cotylurus* spp. parasitizing in swans in the light of molecular data and integrative approach

Julia Gabrysiak[1], Grzegorz Zaleśny[2], Gerard Kanarek[3], Sandra Wydra[2], Joanna Hildebrand[1]

1 Department of Parasitology, Faculty of Biological Sciences, University of Wrocław, Poland
2 Department of Systematic and Ecology of Invertebrates, Wrocław University of Environmental and Life Sciences, Poland
3 Ornithological Station, Museum and Institute of Zoology, Polish Academy of Sciences, Gdańsk, Poland

Abstract

The genus *Cotylurus* Szidat 1928 is a relatively small group of widespread, highly specialized flukes, specific gastrointestinal tract parasites and bursa of Fabricius of waterfowl, coots and waders.. The genus *Cotylurus* represents the family Strigeidae with a long and complicated taxonomic history. The independent status of *Cotylurus* has been confirmed in recent molecular studies, but the structure, validity of individual species, and actual diversity within the genus are still far from definitive.

In recent years, studies have been combining morphological and molecular analyses of representatives of Strigeidae of Central Europe from definitive hosts aimed at taxonomic verification of this group of digeneans. Surprisingly, these studies revealed a high level of molecular diversity within some species, for example, *C. syrius* (a specific species parasitic in swans), pointing to their polyphyletic nature. In our research, we focused on this species, and based on extensive material, we performed analyses using an integrative approach.

Our analysis allowed us to distinguish three genetic lines of *Cotylurus* in swans. One is the species *C. brandivitellatus*, which was somewhat „forgotten" until this point. Still, due to its undoubted molecular and morphological distinctiveness, we want to restore its taxonomical status. The second line is presumably represented by a new species, also morphologically and molecularly distinct, and additionally using leeches as the second intermediate host and the third line, currently the most enigmatic, represents the *Cotylurus* species, probably using the snails as the second intermediate host.

The results of our study indicate that the polyphyletic character of *C. syrius* is the result of misinterpretation of morphological features and essentially excessive allegiance of earlier authors to the phenomenon of host specificity. Our findings presumably may also apply to other representatives of the family Strigeidae.

Keywords: Digenea, Strigeidae, Cotylurus,

OS – oral session

Session: [YSA] Young Scientist Award Session

Do *Acanthamoeba* sp. have an impact on host kidneys? Immunological interactions between *Acanthamoeba* sp. and host

Karolina Kot[1], Patrycja Kupnicka[2], Marta Grabowska[3], Maciej Tarnowski[4], Danuta Kosik-Bogacka[5], Natalia Łanocha-Arendarczyk[1]

1 Department of Biology and Medical Parasitology, Pomeranian Medical University in Szczecin, Szczecin, Poland
2 Department of Biochemistry, Pomeranian Medical University in Szczecin, Szczecin, Poland
3 Department of Histology and Developmental Biology, Pomeranian Medical University in Szczecin, Szczecin
4 Department of Physiology in Health Sciences, Pomeranian Medical University in Szczecin, Szczecin, Poland
5 Independent Laboratory of Pharmaceutical Botany, Pomeranian Medical University in Szczecin, Szczecin, Poland

Abstract

Even though *Acanthamoeba* spp. was reisolated for the first time from primary monkey kidney tissue culture, they attack mostly the brain, cornea and lungs of the hosts. Animal studies, on the other hand, have revealed that amoebae can penetrate the kidneys where they can cause dysfunction. Exact kidney injury mechanisms in parasitic infections are poorly known in many cases, bringing major difficulties to specific therapeutic interventions. Additionally, kidney involvement in parasitic diseases is almost always late, being an important cause of medical complications. Hence, the aim of the study was to analyze immunological response in the kidneys of host infected with *Acanthamoeba* spp. Immunocompetent and immunosuppressed mice were intranasally inoculated with *Acanthamoeba* sp. (T16 genotype) and then sacrificed at 8, 16, and 24 days post infection (dpi). Based on the results of our studies, it is known that *Acanthamoeba* sp. activated Toll-like receptor 2 and NOD-like receptor protein 3 (NLRP3) that play a key role in the innate immune. Activation of these proteins significantly increased the synthesis of various cytokines and chemokines, including interleukin 17α (IL-17α). IL-17α lead to greater activity of extracellular matrix metalloproteinases (MMPs) responsible for e.g. extracellular matrix destruction and tissue damage. Moreover, IL-17α probably acted with interferon γ to increase the production of monocyte chemoattractant protein-1. *IL-17*α promoted proliferation and inflammation in the kidneys but also it induced *apoptosis.* Additionally, hypoxia played a key role in pathophysiology of acanthamoebiasis in the host kidney. We noted that higher levels of MMPs and increased level of MCP-1 could be caused by hypoxia inducible factor 1α and 2α. This study provides important new information for understanding the mechanisms of the disseminated acanthamoebiasis.

Karolina Kot was supported by the Foundation for Polish Science (FNP).

Keywords: *Acanthamoeba* sp., immunological status, kidney

OS – oral session

Session: [YSA] Young Scientist Award Session

Molecular analysis of *Hydatigera parva*: parasite identification in autochthonous Iberian small mammals, Spain

Milan Miljević[1], Marija Rajičić[1], Javier Marco[2], Jelena Blagojević[1], Ruth Rodríguez-Pastor[2], Branka Bajić[1], Javier Millán[3]

1 Department of Genetic Research, Institute for Biological Research "Siniša Stanković"—National Institute of the Republic of Serbia, University of Belgrade, Bulevar Despota Stefana 142, 11000 Belgrade, Serbia
2 Departamento de Patología Animal, Facultad de Veterinaria, Universidad de Zaragoza, Miguel Servet 177, 50013-Zaragoza, Spain
3 Instituto Agroalimentario de Aragón-IA2 (Universidad de Zaragoza-CITA) 50013-Zaragoza, Spain; Fundación ARAID, Avda. Ranillas 1, 50018-Zaragoza, Spain

Abstract

The origin of *Hydatigera parva* is thought to be on the African continent, and it was introduced to the Iberian Peninsula together with its definitive host (*Genetta genetta*), where it subsequently adapted to specific intermediate hosts. This study aimed to identify and characterize *H. parva* and other taeniid metacestodes in autochthonous small mammals in the Iberian Peninsula. Three different small mammal species (410 individuals: *Crocidura russula* 69, *Mus spretus* 257 and *Apodemus sylvaticus* 84) were field-captured in riparian habitats by the Ebro River in Aragón (northwestern Spain) in 2022 in two types of habitats: natural riparian habitats and agricultural fields close to the natural areas. Captured animals were necropsied and visually examined for cysts and visible lesions. The mitochondrial (mt) *cox1* and 12S rDNA genes were used for amplification and sequencing in all extracted taeniid samples. Cysts containing cestode larvae were found in 27 *A. sylvaticus* (32.14%) and a single *M. spretus* (0.39%). All *C. russula* were negative. Sequence analysis revealed that only *H. parva* was present. Genetic analysis of 25 cox1 sequences (344 bp) revealed two haplotypes with a haplotype diversity (Hd) of 0.380. Statistical analysis for *A. sylvaticus* revealed no significant differences in infection prevalence between sexes (χ^2=0.136, df=1, p=0.712), habitats (nature and agriculture) (χ^2=0.904, df=1, p=0.342), and seasons (winter, spring, summer, autumn) (χ^2=1.357, df=3, p=0.716). Apparently, *A. sylvaticus* serves as the primarily suitable intermediate host for *H. parva* and shows a relatively high infection prevalence in the studied area. The observed low genetic diversity could support the hypothesis regarding the introduction of this parasite to the Iberian Peninsula.

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, No. 451-03-66/2024-03/ 200007 and by Gobierno de Aragón (grant LMP90_21).

Keywords: *Apodemus*, rodents, *Hydatigera*, PCR, genetic

OS – oral session

[YSA] Young Scientist Award Session

The great escape: unveiling the immunomodulatory properties of *Dirofilaria* antigens using an *in vitro* moDC model

Mateusz Pękacz[1], Katarzyna Basałaj[2], Alicja Kalinowska[2], Agnieszka Wesołowska[2], Alicja Laskowska[3], Rodrigo Morchón[4], Elena Carretón[5], Anna Zawistowska-Deniziak[3]

1Division of Parasitology and Invasive Diseases, Department of Preclinical Sciences, Institute of Veterinary Medicine, Warsaw University of Life Sciences (SGGW), Poland
2Museum and Institute of Zoology, Polish Academy of Sciences, Poland
3Department of Immunology, Institute of Functional Biology and Ecology, Faculty of Biology, University of Warsaw, Poland
4Zoonotic Diseases and One Health Group, Faculty of Pharmacy, University of Salamanca, Spain
5Internal Medicine, Faculty of Veterinary Medicine, University of Las Palmas de Gran Canaria, Spain

Abstract

Dirofilariosis is one of the fastest-spreading vector-borne zoonoses in Europe. The most medically significant species are *Dirofilaria repens* and *Dirofilaria immitis*, which cause subcutaneous and cardiopulmonary dirofilariosis, respectively. The spread of the diseases is driven by climate change, which introduces new vectors, and human activities, such as traveling to endemic areas with unprotected pets. Over years of co-evolution, helminths have developed various mechanisms to modulate immune responses and establish chronic infections. Antigen-presenting cells (APCs), which shape the host's immune response, are key targets for parasites. Unfrotunately, our understanding of molecular interactions between *Dirofilaria* and humans remains limited due to historical neglect. The immune responses elicited by the various developmental stages of the parasites, as well as the specific molecules that modulate the immune response, remain poorly understood. In this study, we examined the effects of *Dirofilaria* antigens on monocyte-derived dendritic cells (moDCs). Cytokine secretion analysis revealed a reduced inflammatory response, characterized by lower TNF-α and IL-6 levels and a modest increase in IL-10 following stimulation with somatic antigens. Additionally, decreased expression of co-stimulatory markers was observed, indicating a potential shift towards a Th2 response. Proteome Profiler analysis identified key cytokines, including Dkk-1, which was upregulated during various stages of *D. repens* infection, possibly implicating role of Wnt signaling pathway in the subcutaneous immune response. Moreover, *Dirofilaria*-stimulated DCs showed reduced CD4+ T cell proliferation, potentially due to increased Dkk-1 secretion.

Although further research is needed to elucidate the exact mechanisms, our findings provide general insights into the immune response induced by *Dirofilaria*, indicating potential avenues for vaccine development and therapeutic strategies for autoimmune diseases.

OS – oral session

Session: [YSA] Young Scientist Award Session

In vitro efficacy of red lapacho (*Tabebuia avellanedae*) against *Giardia duodenalis*

Giulia Rigamonti[1], Marco Lalle[2], Christian Klotz[3], Stefano De Angelis[4], Alessia Tognoloni[1], Leonardo Brustenga[1], Riccardo De Santo[5], Elisabetta Chiaradia[1], Fabrizia Veronesi[1]

1 University of Perugia, Department of Veterinary Medicine, Italy
2 Istituto Superiore di Sanità, Department of Infectious Diseases, Unit of Foodborne and Neglected Parasitic Diseases, Italy
3 Robert Koch-Institute, Department of Infectious Diseases, Unit 16 Mycotic and Parasitic Agents and Mycobacteria, Germany
4 Deakos SRL, Italy
5 Sapienza University, Department of Chemistry, Italy

Abstract

Giardia duodenalis is an intestinal protozoan that affects mammals, including humans, causing symptoms such as abdominal pain and diarrhea. Treatment involves synthetic drugs like metronidazole (MTZ), but drug resistance in giardiasis treatment is increasing. Thus, therapeutic alternatives are needed. Here we have examined the efficacy of *Tabebuia avellanedae* dry extract (TD) and hydroalcoholic extract (TH), as well as one of its active compounds, β-lapachone (β-lap), as potential treatment against *G. duodenalis* infection. *In vitro* activity (IC50 after 48h) was quantified by viability assay on isolates of *G. duodenalis* Assemblage A and B, using MTZ as reference drug. *In vitro* cytotoxicity was evaluated on Caco-2 and MDCK cell lines

and selectivity index (SI=IC50/CC50) evaluated at 48h post treatment (p.t). Furthermore, to better mimic intestinal situation, cytotoxicity was assessed on intestinal Organoid Derived Monolayers (ODMs) in a trans well set-up by measuring the transepithelial electrical resistance (TEER) and cell viability. We observed good anti-*Giardia* activity of all the compounds; β-lap demonstrated IC50 values lower than MTZ. The viability assays showed that TD exhibited toxicity at the highest concentration (2 mg/ml) after 12, 24, and 48 hours in both cell lines, whereas no cytotoxicity was observed for TH. Notably, the SI for TH approaches infinity as no cellular toxicity was observed. Despite very effective against *G. duodenalis*, β-lap proved to be toxic against both cell lines, likely due to its known anticancer activity. A remarkable low toxicity was observed for TD and β-lap on ODMs, while no toxicity was detected for TH. TEER values didn't show a decrease at 48h p.t. Our *in vitro* results pointed out a potential therapeutic applicability of *T. avellanedae*. This is the first time that ODMs were used for testing anti-*Giardia* compounds.

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Keywords: *Giardia duodenalis*, *Tabebuia avellanedae*, *in vitro* activity, safety

OS – oral session

Session: [YSA] Young Scientist Award Session

Point-of-care molecular diagnostics for schistosomiasis surveillance using environmental DNA

Cecilia Wangari Wambui[1], Mila Viaene[1], David Were Oguttu[2], Benjamin André[1], Hannah Njiriku Mwangi[3], Bart Hellemans[1], Tine Huyse[4], Hugo F. Gante[1]

1 Unit of Ecology, Evolution and Biodiversity Conservation, Department of Biology, KU Leuven, Belgium
2 Department of Biology, Mbarara University of Science and Technology, Uganda
3 Unit of Geography and Tourism, Department of Earth and Environmental Sciences, KU Leuven
4 Department of Biology, Royal Museum for Central Africa, Belgium

Abstract

Schistosomiasis is only second to malaria in terms of morbidity and prevalence in developing nations. Monitoring of schistosomiasis has often relied mainly on epidemiological data rather than direct parasite detection in the environment. There is a critical need for ASSURED (Affordable, Sensitive, Specific, User-friendly, Robust, Equipment-free, and Deliverable) diagnostic tools for effective large-scale monitoring. While LAMP is increasingly being used in schistosomiasis diagnostics, its application in environmental DNA (eDNA) monitoring remains untapped. Coupling eDNA with LAMP could enable real-time identification of transmission sites. We hypothesise that species-specific LAMP assays will rapidly detect *Schistosoma* parasites eDNA, facilitating targeted control. Moreover, implementing this technique promises to significantly reduce the time and cost of schistosomiasis surveillance compared to classical methods. Three methods including open membrane (OM), Sylphium capsule (SC) and Waterra capsule (WC) were tested to optimise eDNA filtration. The optimisation process includes evaluation of filtration efficiency, ease of field operation and time efficiency. Preliminary results suggest that SC has a higher average DNA yield (38.49 ng/µl) and operational efficiency compared to WC (16.31 ng/µl) and OM (2.5 ng/µl). In the next step, we will conduct species-specific qPCR to determine the efficiency and sensitivity of the different filtration methods. The ongoing optimisation of eDNA filtration techniques shows promising results in terms of DNA captured and operational efficiency. Once optimised, the most efficient and user-friendly filtration method will be coupled with *Schistosoma* LAMP assays for real-time identification of transmission sites in schistosomiasis-endemic regions. This integrated approach has the potential to improve schistosomiasis surveillance, thereby contributing to targeted control and eradication efforts in resource-limited settings.

Keywords: Schistosomiasis, environment DNA, LAMP, Africa

OS – oral session

[SY1]

Artificial intelligence and digital health: multidisciplinary examination of potential applications in geospatial analysis

Session: Symposium [SY1] Artificial intelligence and digital health: multidisciplinary examination of potential applications in geospatial analysis

Geospatial Health: development of a maturing scientific journal 2006 – 2023

Sherif Amer, Ellen-Wien Augustijn

Faculty of Geoinformation Science and earth Observation, University of Twente, The Netherlands

Abstract

Geospatial Health is an international, peer-reviewed scientific journal produced by the International Society of Geospatial Health. The journal covers all aspects of geographical information system (GIS) applications, remote sensing and other spatial analytic tools focusing on human and veterinary health.

Our study illustrates how the interdisciplinary domain of Geospatial Health has evolved over time by analysing the published content of the journal for the period 2006 – 2023.

A systematic bibliometric analysis was conducted based upon all (review) articles and editorials published in Geospatial Health. To capture key research topics, methods & techniques, geographic areas studied, and temporal development, our analysis proceeds through three stages: (1) high-frequency keywords identification, (2) co-occurrence analysis that identifies how high-frequency keywords are related, and (3) cluster analysis that identifies groups of interlinked keywords. Impact analysis based on citation counts is included.

Original research papers represent the largest publication category, followed by editorials, and review papers. Three quarters of the publications focus on human health, the rest is on veterinary applications. Geographic focus was initially largely on Africa and Asia. This remained up to around 2020 after which a strong decline in African studies, and a major increase of Asian studies is observed. Initially, focus was on schistosomiasis and malaria, but the scope widened to 35+ different diseases, injuries and risk factors. Over time there is growing attention for non-communicable disease such as cancer and obesity. Spatial and statistical analytical techniques used are diverse and exhibit increasing sophistication over time.

Scaling up the analysis to include related journals is straightforward. This would enable a solid understanding of the temporal development of the scientific discipline Geospatial Health as a whole.

Keywords: geospatial health, bibliometric analysis

OS – oral session

Session: Symposium [SY1] Artificial intelligence and digital health: multidisciplinary examination of potential applications in geospatial analysis

Development of an automated system to recognize and count parasitic elements of veterinary and medical relevance

Paola Pepe[1], Maria Paola Maurelli[1], Giuseppe Martone[2], Salvatore Capuozzo[3], Mirella Santaniello[1], Antonio Bosco[1], Paola Vitiello[1], Stefano Marrone[3], Michela Gravina[3], Biase Celano[4], Carlo Sansone[3], Laura Rinaldi[1], Giuseppe Cringoli[1]

1 *Department of Veterinary Medicine and Animal Production, University of Naples Federico II, CREMOPAR, Naples, Italy*
2 *Universitas Mercatorum, Faculty of Economics, Rome, Italy*
3 *Department of Electrical Engineering and Information Technology (DIETI), University of Naples Federico II, Naples, Italy*
4 *Officina Elettronica SPA S.r.l., Naples, Italy*

Abstract

The development of new (semi-)automated system for the diagnosis in parasitology offers potential solutions to overcome gaps and limitations (i.e. human errors and time for analysis) of Faecal Egg Count (FEC) techniques in veterinary and human fields.

For this reason, from a collaboration among Dept. of Veterinary Medicine and Animal Production, Dept. of Electrical Engineering and Information Technology and a company of Mechanical Engineering a new automated system for diagnosis of helminth eggs in animals and humans was developed the Kubic FLOTAC Microscope (KFM). The KFM is a compact, low-cost digital microscope that combines the high sensitivity, accuracy and precision of the Mini-FLOTAC/FLOTAC techniques with a reliable system based on Artificial Intelligence (AI) predictive model, that after capturing high resolution pictures, is able to recognize and count the parasitic elements in the analysed samples. This system can be remotely controlled via software by smartphone, tablet or PC, and via internet it is possible to transfer the captured pictures to other laboratories, that could be very useful to create a network or to support operators directly in the field. A dataset with more than 5,000 objects for each analysed parasite was used. The acquired images are first segmented into background and objects, which can be either impurities or pseudo-parasites with the main goal of being able to characterize the parasite species and to automatically count eggs. The AI system is able to recognize the 90.0% of the parasitic elements analysed. Therefore, the KFM is a promising automated system for a rapid and accurate assessment of FEC to improve the diagnosis of parasitic infections in veterinary and human fields.

This study was supported by EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Future Artificial Intelligence Research (Project no. PE00000013, FAIR)

Keywords: Artificial Intelligence, Digital microscope, Kubic FLOTAC microscope, Faecal Egg Count

OS – oral session

Session: Symposium [SY1] Artificial intelligence and digital health: multidisciplinary examination of potential applications in geospatial analysis

Geospatial tools and digital health for sustainable strategies to control cystic echinococcosis in endemic areas

Martina Nocerino, Paola Pepe, Antonio Bosco, Elena Ciccone, Nicola Lattero, Laura Rinaldi

University of Napoli Federico II, Italy

Abstract

Cystic echinococcosis (CE), caused by the larval stage of the cestode *Echinococcus granulosus*, is one of the most widespread zoonoses in Mediterranean countries. In this area, the traditional actions taken to control CE are still inefficient, because surveillance and treatment strategies fail to reach inaccessible grazing areas (accessible to stray canids) and are usually designed for wide geographical areas without considering that the prevalence of CE can differ widely in different locations of the same region.

The aim of this study is to show the potential of geographical information systems (GIS)-based innovative tools to support control strategies against cystic echinococcosis in highly endemic areas. The use of GPS devices makes it possible to track the movements of animals (sheep and dogs) and identify the most frequented locations within the grazing areas. Furthermore, the application of anthelmintic baits (laced with praziquantel) using unmanned aerial vehicles (UAVs) allows the development of treatment strategies specifically designed for capillary and automatic distribution of anthelmintics in the study areas, minimizing waste of time and resources. Other innovative devices to implement the control of CE include camera traps to continuously monitor the stray canids for praziquantel-laced baits.

These innovative tools and technologies have been successfully used to control CE in southern Italy as part of the International Echino-Safe-Med project.

This research was funded as part of the project "New sustainable tools *and innovative actions to control cystic ECHINOcoccosis in sheep farms in the MEDiterranean area: improvement of diagnosis and SAFEty in response to climatic changes - ECHINO-SAFE-MED", supported by PRIMA (Partnership for research and innovation in the Mediterranean area).*

Keywords: *Echinococcus granulosus*, geospatial tools, maps, drones, GPS

OS – oral session

Session: Symposium [SY1] Artificial intelligence and digital health: multidisciplinary examination of potential applications in geospatial analysis

The importance of the urban environment for the sentiment during the COVID-19 pandemic

Iuria Betco[1], Cláudia M.[2], Ana Isabel Ribeiro[3], David Sousa Vale[4], Jorge Rocha[1]

1 Institute of Geography and Spatial Planning, University of Lisbon, Portugal
2 Viana
3 Institute of Public Health, University of Porto, Portugal
4 Faculty of Architecture, University of Lisbon, Portugal

Abstract

COVID-19 was one of the biggest public health problems in the world. It also had an impact on people's mental health, both as a direct result of the viral infection and because of the social and economic transformations largely caused by the decisions made to contain the global spread of the virus. In this context, it is essential to understand how COVID-19 measures affected sentiment in Portugal. For this, we used sentiment analysis utilizing a lexicon of sentiments and emotions, and data from the social network Twitter (actual X). Several studies have extracted significant information from Twitter databases over the years. Although individual and family circumstances, including age, income, marital status, economic situation, genetics, and subjective indicators of each person, have a significant impact on the subjective feelings of residents, environmental factors are also determining. Rapid urbanization has led to an increasing number of challenges, such as unhappiness, air pollution, irrational land use, traffic, and environmental degradation. However, the lockdown proved beneficial for the environment. Positive impacts were evident in large cities, where pollution levels decreased. Nevertheless, there were also adverse effects, including an increase in biomedical and plastic waste. Some of the measures adopted by Portugal during the lockdown were the mandatory adoption of remote work and the prohibition of movement between municipalities. These measures were responsible for increasing the time people spent indoors or in their neighborhood. Due to the potential of social media data, it sought to determine which aspects of the urban environment experience affected sentiment. For this, a machine-learning model was used along with an agnostic model, to increase the understanding of the factors in the urban environment that affected sentiment during the COVID-19 pandemic.

Keywords: sentiment analysis, twitter data, machine learning, Covid-19, agnostic model

OS – oral session

Session: Symposium [SY1] Artificial intelligence and digital health: multidisciplinary examination of potential applications in geospatial analysis

Analysis of spatial determinants of community-acquired pneumonia incidence in Portugal mainland

Iuria Betco, Cláudia Morais Viana, Jorge Rocha

Institute of Geography and Spatial Planning, University of Lisbon, Portugal

Abstract

Community-acquired pneumonia (CAP) is a common and serious infectious disease with various causative agents. Understanding the risk factors associated with different pathogens is crucial for effective treatment and management strategies. The incidence of CAP increases with age, with the highest rates seen in individuals over 65 years old. Environmental factors play a role in the development of CAP. Exposure to tobacco smoke, air pollution, and overcrowded living conditions increase the risk of acquiring pneumonia. Machine learning (ML) models, particularly the XGBoost algorithm, have shown effectiveness in predicting adverse outcomes in CAP patients, such as hospital admission and mortality. These models outperformed traditional clinical stratification tools, like the Pneumonia Severity Index (PSI), in terms of prediction accuracy. However, limitations of using ML for CAP diagnosis include the need for reliable and balanced datasets, potential biases in training data, and the black-box nature of artificial intelligence models. As such, we analyzed a set of more than 30 spatial determinants at a municipality level for Portugal mainland (chosen through bibliographic review) and after eliminating multicollinearity problems using Variance Inflation Factor (VIF) we tested Geographical Random Forest (GRF) which is a spatial extension of the random forest algorithm to address spatial heterogeneity in population modelling, Traditional RF, Support vector machines (SVM) and eXtreme Gradient Boosting (XGBoost). In the end, GRF performed better and we used explainable Artificial Intelligence (xAI), i.e., an agnostic model, for evaluating the importance of each variable, namely Triplot, that consider the hierarchical correlation structure of variables. Overall, a comprehensive understanding of the driving factors of CAP is essential for improving public health.

Keywords: Community-acquired pneumonia, Machine learning, explainable Artificial Intelligence, agnostic models

OS – oral session

Session: Symposium [SY1] Artificial intelligence and digital health: multidisciplinary examination of potential applications in geospatial analysis

Machine learning and eXplainable Artificial Intelligence for the prevention of waterborne cryptosporidiosis and giardiosis

Panagiota Ligda[1], Nikolaos Mittas[2], George Kyzas[2], Edwin Claerebout[3], Smaragda Sotiraki[1]

1 VRI, ELGO DIMITRA, GREECE
2 Democritus University of Thrace, GREECE
3 Faculty of Veterinary Medicine, Ghent University Belgium

Abstract

Cryptosporidium and *Giardia* are important parasitic protozoa due to their zoonotic potential and impact on human health, often causing waterborne outbreaks of disease. Several attempts have been made to investigate the association between the presence of those protozoa in waters with other biotic or abiotic factors, with inconclusive findings. The aim of this study was to develop an holistic approach leveraging *Machine Learning* (ML) and *eXplainable Artificial Intelligence* (AI) techniques, to provide evidence related to the presence and prediction of *Cryptosporidium* and *Giardia* (oo) cysts in water samples. For this, we, initially, model the complex relationship between *Cryptosporidium* and *Giardia* (oo)cysts and a set of parasitological, microbiological, physicochemical, and meteorological parameters via a model-agnostic meta-learner algorithm. Based on this approach, a set of four well-known ML candidates were, evaluated in terms of their predictive capabilities. The best-performed algorithms were further examined through XAI techniques. Findings reveal that the Random Forest achieves the highest prediction performance when the objective is the prediction of both contamination and contamination intensity with *Cryptosporidium* oocysts in water samples, with meteorological/ physicochemical and microbiological markers being informative, respectively. For the prediction of contamination with *Giardia*, the eXtreme Gradient Boosting with physicochemical parameters was the most efficient algorithm, while the Support Vector Regression that takes into consideration both microbiological and meteorological markers was more efficient for evaluating the contamination intensity with cysts. Results show that the adoption of ML and XAI approaches can be a valuable tool for unveiling the complicated correlation of the presence and contamination intensity with these parasites constituting a basis for the development of early warning systems to prevent of waterborne disease outbreaks.

Keywords: Machine learning, explainable artificial intelligence, Cryptosporium, Giardia, monitoring system, waterborne outbreak

OS – oral session

[SY2]

Parasites and Vectors in a Warming World: predicting and preparing for future scenarios

Session: Symposium [SY2] Parasites and Vectors in a Warming World: predicting and preparing for future scenarios

Fasciola hepatica and Lymnaeidae host snails in southern Italy

Maria Paola Maurelli[1], Lavinia Ciuca[1], Ines Hammami[2], Paola Vitiello[1], Antonio Bosco[1], Laura Rinaldi[1]

1 Unit of Parasitology and Parasitic Diseases, Department of Veterinary Medicine and Animal Production, University of Naples Federico II, CREMOPAR, Via Delpino, 1, 80137 Napoli, Italy
2 Laboratoire de Parasitologie, École Nationale de Médecine Vétérinaire de Sidi Thabet, Univ Manouba, 2020 Sidi Thabet, Tunisia

Abstract
Fasciolosis is a worldwide parasitic disease caused by flatworms, belonging to the genus *Fasciola*, also known as liver flukes. In Italy the information about the adult liver flukes is scarce and on their intermediate hosts is missing. For these reasons, this study aimed to assess: (i) the species of adults of *Fasciola* spp. in cattle in southern Italy; ii) the species of snails on pastures; iii) the presence of *Fasciola* spp. as well as of the rumen fluke *Calicophoron daubneyi* (presenting the same intermediate hosts) in host snails. The study was carried out in cattle farms and slaughterhouses located in Campania, Basilicata and Molise region (southern Italy). A total of 84 adult liver flukes were collected at slaughterhouses from eight animals, while 190 snails were collected from water-sources close to 11 cattle farms resulted positive for liver/rumen flukes. Ten snail samples from each farm (N=110) were selected for molecular studies. For identification of adult liver flukes and snails by morphological analysis reading keys were used. The molecular analyses were performed from adult liver flukes and snail specimens using ITS2+ region. The presence of *Fasciola* spp. and *C. daubneyi* was tested using respectively ITS2+ region and COX1 gene. All the amplicons obtained were sequenced. All the adult liver flukes were identified as *F. hepatica*. The snails were identified as *Galba truncatula* (56.4%) and *Physella acuta* (43.6%). *F. hepatica* was found only in *G. truncatula* in one pool from the Basilicata region, while *C. daubneyi* was found in both snail species in six pools: four from the Campania region and two from the Basilicata region. Our findings highlight the need to perform malacological surveys in the different Italian regions to develop integrated strategies for snail control, preventing the spread of liver and rumen flukes.

This research was funded under the European Union's Horizon 2020 research and innovation program under Grant Agreement No. 101000365.

Keywords: fasciolosis, snails, cattle, liver flukes, rumen flukes

OS – oral session

Session: Symposium [SY2] Parasites and Vectors in a Warming World: predicting and preparing for future scenarios

Application of environmental DNA-based monitoring of vector snails and liver fluke transmission across different field settings along a latitudinal gradient from Denmark to South Africa

Mita Eva Sengupta[1], M.P. Malatji[2], P.I. Ngcamphalala[2], S. Mukaratirwa[2], L. Ciuca[3], M.P. Maurelli[3], A. Bosco[3], L. Rinaldi[3], G. Materu[4], S. Kinunghi[4], M. Adriko[5], L. Mugisha[6], A-s. Stensgaard[1]

1 University of Copenhagen, Denmark
2 University of KwaZulu-Natal, South Africa
3 University of Naples, Italy
4 National Institute for Medical Research (NIMR-Mwanza Centre), Tanzania
5 Ministry of Health-VCD, Uganda
6 Makerere University, Uganda

Abstract
Fasciolosis is an emerging snail-borne zoonotic infection of humans and animals in both Europe and Africa, caused by the two liver flukes, *Fasciola hepatica* and *F. gigantica*. The distribution of these liver fluke species is closely linked to the occurrence of their vector snails, therefore identifying areas where these snail species are present is essential to evaluate trematode epidemiology and inform control strategies. The traditional monitoring method of snail collections and subsequent detection of parasite infections in the snails remain labour intensive and costly. Environmental DNA (eDNA)-based detection is therefore increasingly being considered as tool to accurately detect, identify and monitor the presence of snail borne parasites in aquatic environments. However, it remains under-explored for use in African settings. In the PREPARE4VBD-project, we therefore set out to develop and evaluate the use of eDNA-based monitoring of liver flukes and vector snails across a variety of field-settings ranging from Denmark to South Africa. In 2023, we conducted parallel eDNA surveys, including water sampling and filtering, following the same protocol in the countries Denmark, Italy, Uganda, Tanzania, and South Africa respectively, alongside traditional snail surveys. The eDNA samples were stored in two different ways for comparison, i.e. RNAlater and dry, and kept until DNA extraction (ongoing). Next, species-specific PCR will be applied to detect eDNA traces of liver flukes and vector snails in the water bodies in areas with known transmission. We expect to clarify which environmental and climatic factors of different field-settings are associated with the detection of snail and liver fluke eDNA, and in turn evaluate the applicability of eDNA-based monitoring of snail borne parasites in diverse environmental settings along a larger latitudinal gradient.

Keywords: eDNA, vector snail, fasciola, monitoring

OS – oral session

Session: Symposium [SY2] Parasites and Vectors in a Warming World: predicting and preparing for future scenarios

Investigation of *Ixodes ricinus* tick infections and associated microbiome composition across a variety of human-impacted landscapes in two macro-climatic zones

Mita Eva Sengupta[1], J.T.P. Verhoeven[1], M.P. Maurelli[2], L. Ciuca[2], A. Bosco[2], L. Rinaldi[2], A.J. Hansen[1], A-S. Stensgaard[1]

1 University of Copenhagen, Denmark
2 University of Naples, Italy

Abstract
Investigations of the tick microbiome and how it affects important biological traits associated with tick fitness and infection is an emerging field of research, fueled by a need to understand the ongoing expanding distribution of ticks and tick-borne diseases. The composition and diversity of the tick microbial communities is highly variable and driven by numerous external factors, i.e. host species, host meals, habitats and seasons. The aim of our study is to characterize and compare tick microbiome composition and diversity of field-collected *Ixodes ricinus* ticks from populations across a diversity human-impacted landscape types in two macro-climatic zones such as Denmark and Italy. Ticks were collected using traditional 'flagging-method' in the environment to capture questing tick larvae, nymphs and adults. In Denmark, questing ticks were collected from four main habitat types (forest, grazing pastures, sub-urban residential areas and urban areas), at

three time points in 2023 (spring, summer and autumn). A total of 1406 *I. ricinus* specimens were collected in DK and in Italy the counting of ticks is still ongoing. All Danish ticks have been morphologically identified to species, and DNA is being extracted from tick bulk samples using an optimized protocol. Next, pools of 10 ticks will be screened for parasites and other pathogens using species-specific qPCR-based methods (fluidigm), and the bacterial microbiota diversity and composition will be characterized by targeting the V3-V4 region of the bacterial 16S rRNA region. We expect to clarify if certain microbiota profiles are associated with specific landscape types, and in turn elucidate if these pose higher risk of tick-borne disease transmission.

Keywords: Ixodes, tick, microbiome, tick-borne disease, climate change

OS – oral session

Session: Symposium [SY2] Parasites and Vectors in a Warming World: predicting and preparing for future scenarios

Dynamics of *Fasciola hepatica* and vector snail populations after an extreme summer drought event in Denmark

Mita Eva Sengupta[1], Leah Lourenço[1], Nao Takeuchi-Storm[2], Stig Milan Thamsborg[1], Anna-sofie Stensgaard[1]

1 University of Copenhagen, Denmark
2 National Food Institute, Technical University of Denmark

Abstract

Fasciolosis, caused by *Fasciola hepatica*, is a devastating snail-borne, parasitic trematodiasis affecting ruminants. Recent studies have suggested altered or even increased transmission patterns of *F. hepatica* in some parts of Europe, possibly due to changing temperature and rainfall patterns because of climate change. Here, we investigated the *F. hepatica*-host snail dynamics in one hotspot of naturally occurring transmission sites in Denmark. The study was initiated in 2018, coinciding with the most severe summer drought and heat wave ever recorded in Denmark, which presented a unique opportunity to study the impact of an extreme weather event on snail-parasite transmission dynamics. We report the results from investigations of *i*) the timing of exposure in the sheep and *ii*) the population dynamics and infection levels of the vector snail *Galba truncatula* sampled at regular intervals from April 2018 to May 2020. Furthermore, water samples for environmental DNA (eDNA) detection of both snail and parasite DNA, were collected at the same sites in 2018–2023. We observed a peak in snail abundance in spring 2018, just before the onset of the extremely hot and dry summer of 2018, after which only a few snails were observed again until late autumn 2019. Accordingly, only 1/22 lambs became infected with *F. hepatica* in the grazing season in 2018 (and only 1/24 lambs as detected by ELISA in 2020), despite that > 90% of ewes were patently infected throughout the seasons. ELISA results indicate one case was a likely case of "winter infection". The extreme weather in the summer of 2018 dried out a substantial part of snail habitats, leading to declines in the snail populations and low parasite transmission. These effects were also noticeable in the following grazing seasons. In conclusion, our study provides insight into the impacts on *Fasciola*-snail transmission under adverse weather events, which are expected to occur at higher frequencies in Europe with future climate change.

Keywords: Fasciola, vector snail, population dynamics, climate change

OS – oral session

Session: Symposium [SY2] Parasites and Vectors in a Warming World: predicting and preparing for future scenarios

A citizen-science-based approach to mosquito monitoring - experiences from a car-netting project in Denmark

Mita Eva Sengupta[1], Cecilie Skræp Svenningsen[2], Anders Tøttrup[2], Erika Thorhauge-Thejll[2], Anna-Sofie Stensgaard[1]

1 Department for Veterinary and Animal Science, University of Copenhagen, Denmark
2 The Natural History Museum of Denmark, University of Copenhagen, Denmark

Abstract

The emergence and growing importance of invasive mosquito species and mosquito□borne diseases in Europe has fueled the need to scale-up mosquito surveillance efforts in many countries. The conventional approach to mosquito monitoring (systematic trapping and morphological species ID by trained personal), can however be very time- and resource demanding. In recent years, many countries have therefore sought to combine mosquito monitoring with community participation in citizen science programs. As part of a larger citizen-based project to map insect diversity in Denmark, 'The Insectmobile', we set out to evaluate the usability of car-netting as a supplement to national surveillance efforts, where citizens drive their own cars with roof-top mounted insect nets along designated routes and landscapes across the country. Using the nationwide samples from the InsectMobile for the years 2018 and 2019, we could map and identify 12 biting mosquito species (*Culicidae*), and 32 *Culicoides* species across 533 localities in Denmark, using a DNA metabarcoding approach. We furthermore performed a second study with the purpose *i*) to compare the efficiency of traditional mosquito traps with the car-netting, across different types of habitats in Denmark and *ii*) to optimize the DNA metabarcoding protocol by specifically targeting important endemic and invasive vector mosquitoes & mosquito-borne parasites. We found that both methods where able catch all five genera of mosquitoes known to occur in Denmark. The traditional traps caught significantly more female *Culex* mosquitoes than car-netting, while car-netting caught higher numbers of female *Aedes* mosquitoes, in particular in urban areas. In conclusion, car-netting has potential as a supplementary tool to up-scale the national surveillance of problematic vector mosquitoes, in particular for in relation to *Aedes* female mosquitoes in urban/sub-urban areas.

Keywords: citizen science, mosquitoes, surveillance, metabarcoding

OS – oral session

Session: Symposium [SY2] Parasites and Vectors in a Warming World: predicting and preparing for future scenarios

Breaking down the siloes to prevent and prepare for climate-sensitive vector-borne disease in Africa and Europe: the PREPARE4VBD approach

Anna-Sofie Stensgaard[1], Mita Eva Sengupta[1], Birgitte Vennervald[1]

1 Department for Veterinary and Animal Sciences, University of Copenhagen, Denmark

Abstract

Vector-borne diseases (VBDs) constitute a major challenge to both human and animal health in many countries today. However, with on-going climate change and globalization, it is anticipated that new outbreaks of vector-borne diseases will occur more frequently in the future, along with an increased spread of invasive vectors and risk of VBDs becoming established in new areas. Yet, our understanding of why some vectors and VBDs are able to successfully spread and establish in new, previously disease-free areas, whereas other do not, is limited. This warrants the need for improved surveillance, prediction and research capacity for early outbreak detection. This can only be achieved through collaboration among different sectors and research environments, often operating in siloes. In response to this, a multi-disciplinary consortium of 10 partners from three European and five African countries was formed to develop new knowledge and surveillance tools transcending disciplines and vector species, to improve preparedness in Africa and Europe for VBD emergence. Submitted under the acronym PREPARE4VBD (A Cross-Disciplinary Alliance to Identify, PREdict and prePARE for Emerging Vector-Borne Diseases), the project was selected for funding by the European Commission (EC) within the European Horizon 2020 RIA framework. PREPARE4VBD was launched in September 2021 and will run for 4 years. The Consortium are working to improve vector surveillance and control by: *i*) build knowledge of neglected tick-, mosquito- and snailborne VBDs of importance for animal and human health in endemic African countries; *ii*) assess their capacity to adapt and spread to new areas using a holo-genomics approach and novel climate change impact modelling, *iii*) develop novel diagnostic tools for rapid vector/VBD discoveries and model-based surveillance for early warning, and finally *iiii*) strengthen the research and surveillance capacity within vectors and vector-borne diseases in both Africa and Europe.

Keywords: Vectors, ticks, mosquitoes, snails, climate change

OS – oral session

Session: Symposium [SY2] Parasites and Vectors in a Warming World: predicting and preparing for future scenarios

Investigating the impact of heat stress on *Fasciola* intermediate host snail immune defenses and life history traits across a latitudinal gradient (Denmark, Italy, Uganda, Tanzania and South Africa)

Jennifer Upfold[1], Mita Sengupta[1], Godlisten Materu[2], Safari Kinunghi[2], Chester Kalinda[3], Agrippa Dube[3], Tawanda Manyangadze[3], Moses Chimbari[3], Samson Mukaratirwa[3], Moses Adriko[4], Lawrence Mugisha[5], Lavinia Ciuca[6], Laura Rinaldi[6], Anna- Sofie Stensgaard[1]

1 University of Copenhagen, Institute for Veterinary Medicine and Animal Science, Dyrlægevej 100, Frederiksberg C
2 National Institute for Medical Research (NIMR), Mwanza Centre, Tanzania
3 University of KwaZulu-Natal (UKZN), South Africa
4 Vector Control Division, Ministry of Health, Uganda
5 Makerere University, Uganda
6 University of Naples, Italy

Abstract

Fascioliasis, an emerging global disease caused by *Fasciola* trematodes, poses significant challenges to public health and agriculture worldwide. Within the framework of the PREPARE4VBD project, this study investigates the impact of heat stress on the immune defenses and life history traits of two key intermediate host snail species, *Radix balthica* (*F. hepatica*) and *R. natalensis* (*F. gigantica*), across a latitudinal gradient spanning from Denmark and Italy (northern range margin) through Uganda and Tanzania (middle) to South Africa (southern range margin). Given the estimated global rise in temperatures due to climate change, evaluating how heat stress may alter fascioliasis disease dynamics by potentially reducing or increasing the intermediate host snail fitness, and consequently its ability to resist infections is vital. Furthermore, *Radix* snails have a global distribution therefore it is important to investigate how the intermediate host snail species react to heat stress along a wide-ranging macroclimatic spectrum. Between April and June 2024, field-collected snails along the latitudinal gradient were subjected to increased temperatures for ten days to induce heat stress. We assessed the constitutive levels of immune defense, including total hemocyte counts, phenoloxidase (PO)-like activity, and antibacterial activity of the hemolymph, as well as life history traits such as growth and oviposition rates. These measurements were compared with responses from snails maintained at ambient temperatures. Our study aims to elucidate how future climate change-induced temperature increases could modify fascioliasis disease dynamics by influencing snail fitness and infection resistance. We anticipate variations in these responses among snail populations along the latitudinal gradient. Ultimately, our findings will inform the development of targeted disease control and mitigation strategies in diverse environmental contexts, contributing to global health efforts.

Keywords: Fascioliasis, Heat stress, Immune defenses, Climate change

OS – oral session

Session: Symposium [SY2] Parasites and Vectors in a Warming World: predicting and preparing for future scenarios

Combining vector snail habitat suitability and parasite infection models to map the risk of *Fasciola gigantica* in Africa under current and future climates

Tiem Van der Deure, Anna-Sofie Stensgaard

1 Section for Parasitology and Aquatic Pathobiology, Department for Veterinary and Animal Sciences, Faculty of Health, University of Copenhagen, Denmark

Abstract

Fascioliasis is an infection of liver flukes caused by *Fasciola gigantica* or *Fasciola hepatica* and infects mammals including cattle, wildlife, and humans. *Fasciola* species have a complex life cycle that includes a lymnaeid snail intermediate host. Warmer and wetter conditions generally favour transmission of *Fasciola*, a connection that has been used to forecast *F. hepatica* risk in Europe since the 1950s. However, the disease has been much less studied in Africa, where both *F. gigantica*, and *F. hepatica* circulate and occasionally hybridize.

Here, we aim to model and map the risk of *F. gigantica* infection and the prevalence of its main intermediate host snail species, *Radix natalensis,* across Africa.

We first extracted occurrence records for *Radix natalensis* from two main sources: 1) GBIF (381 unique locations) and 2) 564 newly georeferenced occurrence records from 370 unique locations from the Mandahl-Barth/The Danish Bilharziasis Laboratory mollusc collection maintained at the Natural History Museum, University of Copenhagen, Denmark. In total, we collected records covering 39 African countries.

We then developed two distinct models: 1) a point process model that uses environmental covariates to estimate the density of *R. natalensis* throughout Africa, and 2) a mechanistic model that utilises the temperature constraints for the extrinsic development of *F. gigantica* to predict where transmission is possible if an intermediate host is present. These models are then combined to estimate continental-scale infection risk.

The next step will be to validate the model's performance using an independent dataset of reported *Fasciola* prevalence extracted from the literature.

Using this approach, we aim to identify important large-scale drivers of current snail-parasite occurrence in Africa, anticipate changes in risk due to climate change, facilitate targeted interventions, and highlight areas where parasite hybridization is possible.

Keywords: fascioliasis, climate change, trematode, spatial models

OS – oral session

Session: Symposium [SY2] Parasites and Vectors in a Warming World: predicting and preparing for future scenarios

Evidence of autochthonous transmission of *Dirofilaria repens* in Slovenia

Barbara Šoba[1], Urška Glinšek Biškup[1], Tatjana Avšič Županc[1], Miša Korva[1], Nataša Knap[1], Katja Adam[2], Tea Knapič[3], Patricija Pozvek[1], Tina Kotnik[4]

1 *Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia*
2 *Faculty of Mathematics, Natural Sciences and Information Technologies, University of Primorska, Koper, Slovenia*
3 *Slovenian Museum of Natural History, Ljubljana, Slovenia*
4 *Small Animal Clinic, Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia*

Abstract

The infection with *Dirofilaria repens* has been historically endemic in Mediterranean countries. In the last two decades, it has spread to the rest of Europe, where it is considered an emerging infection. Canids act as a reservoir for the parasite, while mosquitoes serve as vectors. Humans can also become infected, but are considered a dead-end host. Several cases of *D. repens* infection in humans have been reported in Slovenia. Due to the proximity to neighbouring Croatia and Italy, both endemic for dirofilariasis, it is difficult to determine whether the cases are autochthonous or not.
A collaborative approach was taken by microbiologists, veterinarians and zoologists to clarify the possibility of autochthonous transmission of *D. repens* dirofilariasis in Slovenia.
A statistically representative number of 465 dogs older than 1 year and born in Slovenia were recruited between April and October 2018. Epidemiological data were collected and blood samples were taken. qPCR was performed to detect *D. repens* DNA. Mosquitoes were collected and identified between 2021 and 2022 as part of a vector and vector-borne diseases monitoring project. All adult mosquitoes were grouped into primary pools (up to 40 per vial) according to species and sex. In secondary pools, 10 DNA extracts from the primary pools were grouped by capture date and location. For molecular detection and identification of filarioid species, a qPCR approach was used, followed by conventional PCR and sequencing.
Three out of 465 (0.64 %) dogs tested positive for *D. repens*. Two of them had never travelled outside the country suggesting autochthonous infection. *D. repens* was detected in 4 *Aedes*, 1 *Coquillettidia*, and 1 *Anopheles* pool. The detection of *D. repens* in mosquito vectors indicates that the causative agent of subcutaneous dirofilariasis is present in Slovenia.

The study was supported by the Slovenian Research and Innovation Agency the Ministry of Health and Ministry of the Environment and Spatial Planning.

Keywords: *Dirofilaria repens*, collaborative approach, humans, dogs, mosquitoes

OS – oral session

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Bio-Rad - your partner in parasitological research; application possibilities of ddPCR

Katarzyna Kowalczyk

Field Application Specialist Genomics, CEE
BIO-RAD Sp. z o. o.
E-mail: katarzyna_kowalczyk@bio-rad.com; mateusz_pajak@bio-rad.com

Droplet Digital PCR (ddPCR) provides ultrasensitive nucleic acid detection and direct absolute quantification without the need for standard curves. Not only does this simplify experiment set-up but it also increases reproducibility. In ddPCR, the PCR reaction is partitioned by droplet generator into 20,000 uniform sized droplets, which has been externally validated and shown to be highly reproducible.

In summary, this technology stands out by simplified quantification - no calibration standards or reference (ΔΔCq method) are required for absolute quantification. We could reduce consumable costs - reaction volumes range from pico to nanolitres, reducing reagent consumption and the amount of sample required for each data point. The emulsion-based reaction system means that PCR reactions can be performed in a standard thermocycler without complex chips or microfluidics. ddPCR technology allows 20,000 droplets per 20 µl sample, or almost two million partitioned PCR reactions in a 96-well plate which increases precision and sensitivity compared to qPCR or even dPCR.

This direct method of quantification can be applied to any application that uses primers or primers/ probes to detect nucleic acid sequences and we have tools to help you switch any qPCR assay over to ddPCR. Some but not all applications include environmental monitoring of pathogens, species determination, residual host cell contamination assessment, viral load analysis, mutation detection, copy number variation (CNV), minimal residual disease (MRD), microbial quantification, NGS library quantification, genome editing assessment (HDR and NHEJ), small-fold change gene expression analysis, miRNA quantification and methylation sensitive restriction enzyme (MSRE) ddPCR without the need for bisulfite conversion. Probe based analysis using a one-step kit for RT-ddPCR provides minimal hands-on time for reliable 4-plex (QX200) gene expression analysis.

A novel application of ddPCR is linkage assessment. Which can be used to identify intact proviral DNA, the integrity of viral vectors, inversion assessment, plasmid impurities, cis/trans mutations, and whole cell DNA analysis. Linkage works by using two or more assays (FAM and HEX) at either end of your target that are expected to be co-amplified (e.g. vectors, viral pathogen, suspected cis-mutations, whole cells isolated in droplets). The software can then determine the amount of linkage above that of random distribution to determine the concentration of linked loci and the %linkage, this technique is solely unique to digital PCR. The Bio-Rad system is a well-established platform (>8300 publications across a wide range of applications), with easy-to-use software, high resolution data, experienced technical support, and continued product development to broaden the scope and applications of ddPCR. You can also search Bio-Rad publication list to look for applications of interest.

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In memoriam: prof. Wacław Szybalski

Wacław Szybalski, born in 1921 in Lviv to a noble family, had a remarkable journey. He completed his early education, graduating in 1933 from the St. Joseph Elementary School and in 1939 from Casimir III the Great 8th Gymnasium in Lviv. His passion for chemistry led him to study at the Lviv Polytechnic, where he worked under the guidance of Prof. Adolf Joszt, an expert in fermentation processes. During his studies, he developed a method for separating chemical compounds using paper chromatography and worked at the Institute for Research on Typhus and Viruses, led by the renowned Polish microbiologist Prof. Rudolf Weigl.

After World War II, he had his chemical engineering diploma recognized at the Silesian University of Technology in Gliwice. In the same year, he moved to Sopot and joined the Gdańsk University of Technology, where he became the head of the Department of Fermentation Industry and Biotechnology. It was during this time that he began delving into biotechnology and genetics and successfully defended his doctorate in 1949. In 1950, he emigrated to Denmark and then to the United States, where he made significant scientific contributions. In Denmark, he worked at the Carlsberg Laboratory and also conducted research at the Academy of Technical Sciences and the Niels Bohr Institute for Theoretical Physics. His move to the USA in 1951 marked the beginning of his groundbreaking work at Cold Spring Harbor, where he was part of a team that laid the foundations for molecular biology and genetic engineering. He had the opportunity to work alongside Prof. James Watson, the Nobel Prize-winning co-discoverer of the DNA structure. Subsequently, he continued his research at the Institute of Microbiology at the University of New Brunswick (working with the Nobel Prize winner, prof. Selman Waksman) and later became a professor at the McArdle Laboratory for Cancer Research at the University of Wisconsin-Madison. His pioneering work included developing methods for studying DNA and identifying chemical compounds that induce mutations in bacterial cells. Together with his wife Elisabeth Szybalski, he also played a key role in developing technology for gene therapy. His influential work extended to the coining of the concept and foundations of synthetic biology. His notable scientific achievements were published in prestigious journals such as "Science," "Nature," "Proceedings of the National Academy of Sciences of the United States of America (PNAS)," and "Virology." He also founded and served as the editor-in-chief of the international journal "GENE" and held positions on the editorial boards of numerous scientific journals.

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