

PROCEEDINGS

8th ESDA Days

and the

2025 Annual EVPC Scientific Meeting

Thessaloniki, Greece

May 22-24, 2025



ESDA official website:

<http://www.esda.vet/>

email: info@esda.vet, esdadays@gmail.com

EVPC official website:

<https://www.eurovetpar.org/>

8th ESDA DAYS AND EVPC 2025 official website:

<https://esda2025.gr/>

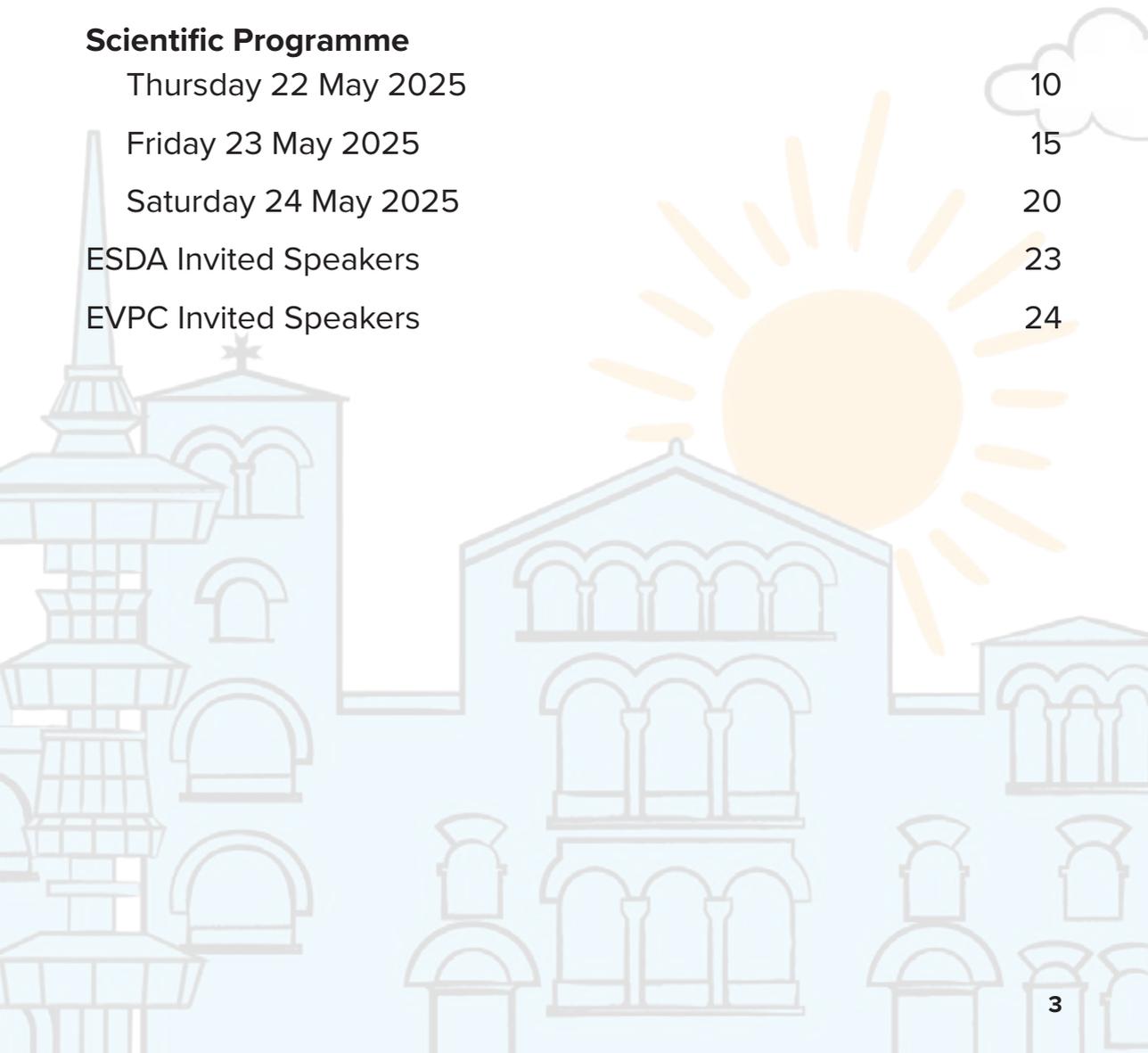
email: esda2025@symvoli.gr

The 8th ESDA Days and the 2025 Annual EVPC Scientific Meeting are organized under the patronage of:



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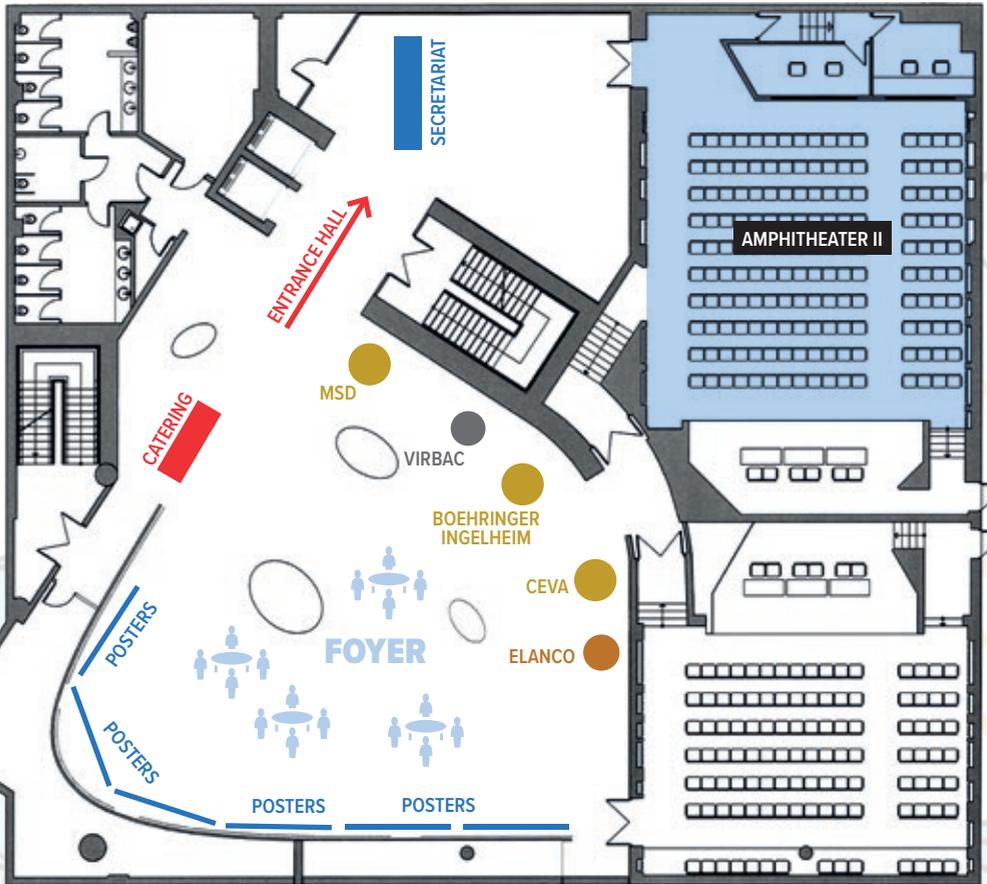


VENUE

Aristotle University's Research Dissemination Center (KEDEA) was founded in 2006 and opened its doors for the first time in April 2011. The building is located downtown at Aristotle University's campus between the Student Club and the University Gymnasium. The conference will take place at the AMPHITHEATER II which is located on the -1 floor. Exhibition spaces, posters and catering services will operate at the Foyer right outside the conference hall.



FLOOR PLAN



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CONFERENCE MANAGEMENT



COMMITTEES

Organising Committee

Presidents

Anastasia Diakou
Smaragda Sotiraki

Members

Emilie Bouhsira
Lavinia Ciuca
Ljubomir Curcin
Mariana Ionita
Aranzazu Meana Manes
Isaia Symeonidou
Constantina Tsokana
Luigi Venco

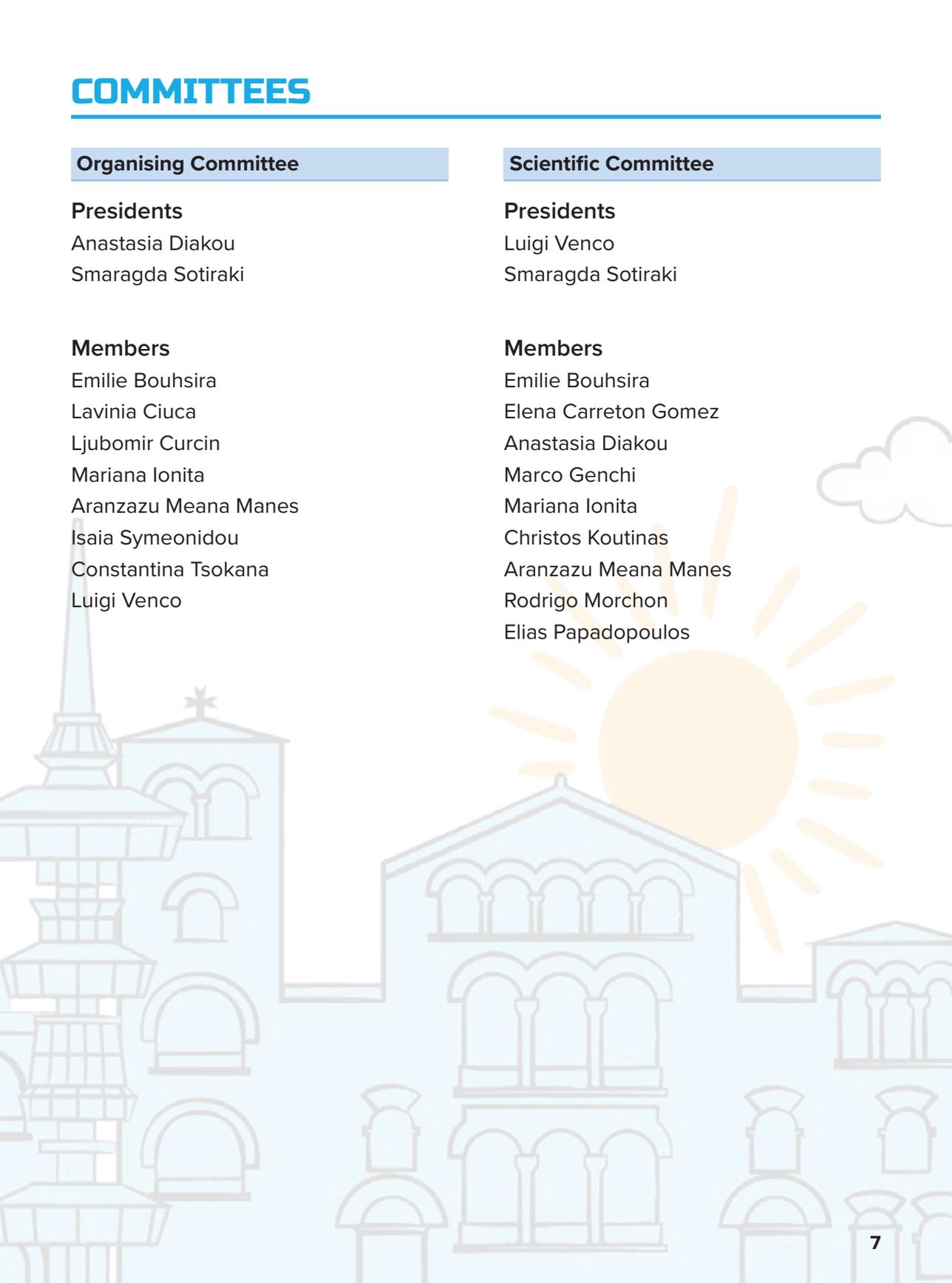
Scientific Committee

Presidents

Luigi Venco
Smaragda Sotiraki

Members

Emilie Bouhsira
Elena Carreton Gomez
Anastasia Diakou
Marco Genchi
Mariana Ionita
Christos Koutinas
Aranzazu Meana Manes
Rodrigo Morchon
Elias Papadopoulos



GREETINGS FROM THE ORGANIZING COMMITTEE

Dear friends,

We are pleased to announce the joint event organised by **ESDA** and **EVPC** in Thessaloniki, for the **8th ESDA Days** and the **2025 Annual EVPC Scientific Meeting**.

The European Society of Dirofilariosis and Angiostrongylosis (ESDA) is dedicated to educating the scientific community on the prevention, diagnosis, treatment, and public health risks associated with *Dirofilaria immitis*, *Dirofilaria repens* and *Angiostrongylus vasorum* in both animals and humans. ESDA holds a tri-annual scientific meeting, the “ESDA Days”, where the latest advancements in the field are shared. The objectives of ESDA days are closely aligned with the **European Veterinary Parasitology College (EVPC)** mission which is to advance the field of Veterinary Parasitology and to promote a high standard of education.

For 2025, we are pleased to announce that **ESDA** will join forces with the **EVPC**, organising a common event namely the **8th ESDA Days-2025 Annual EVPC Scientific Meeting**, 22-24 of May, in Thessaloniki, Greece.

This joint event represents a unique opportunity for students, veterinary practitioners, researchers, and academics to share state-of-the-art advancements in epidemiology, diagnosis, treatment, and prevention of parasites relevant to the veterinary and medical communities.

Our aim is that this year’s event will become a meeting point for the scientific community, including young scientists, who will be the future members of ESDA and EVPC.

The venue for this meeting will be in Thessaloniki at the Aristotle University’s Research Dissemination Center, strategically located close to the city centre. Thessaloniki is the second biggest city in Greece, build around Thermaikos Gulf with high touristic activity. In close proximity to the meeting venue, there are various possibilities for accommodation, restaurants, bars and other facilities.

For those whose circumstances prevent them from meeting with us in Thessaloniki, there will be the opportunity to attend online, allowing this important event to open a communication window to the world.

We are confident that this meeting, organised with great enthusiasm, will serve as an engaging forum, fostering improvement and advancement in the health of both pets and humans.

Best Regards,

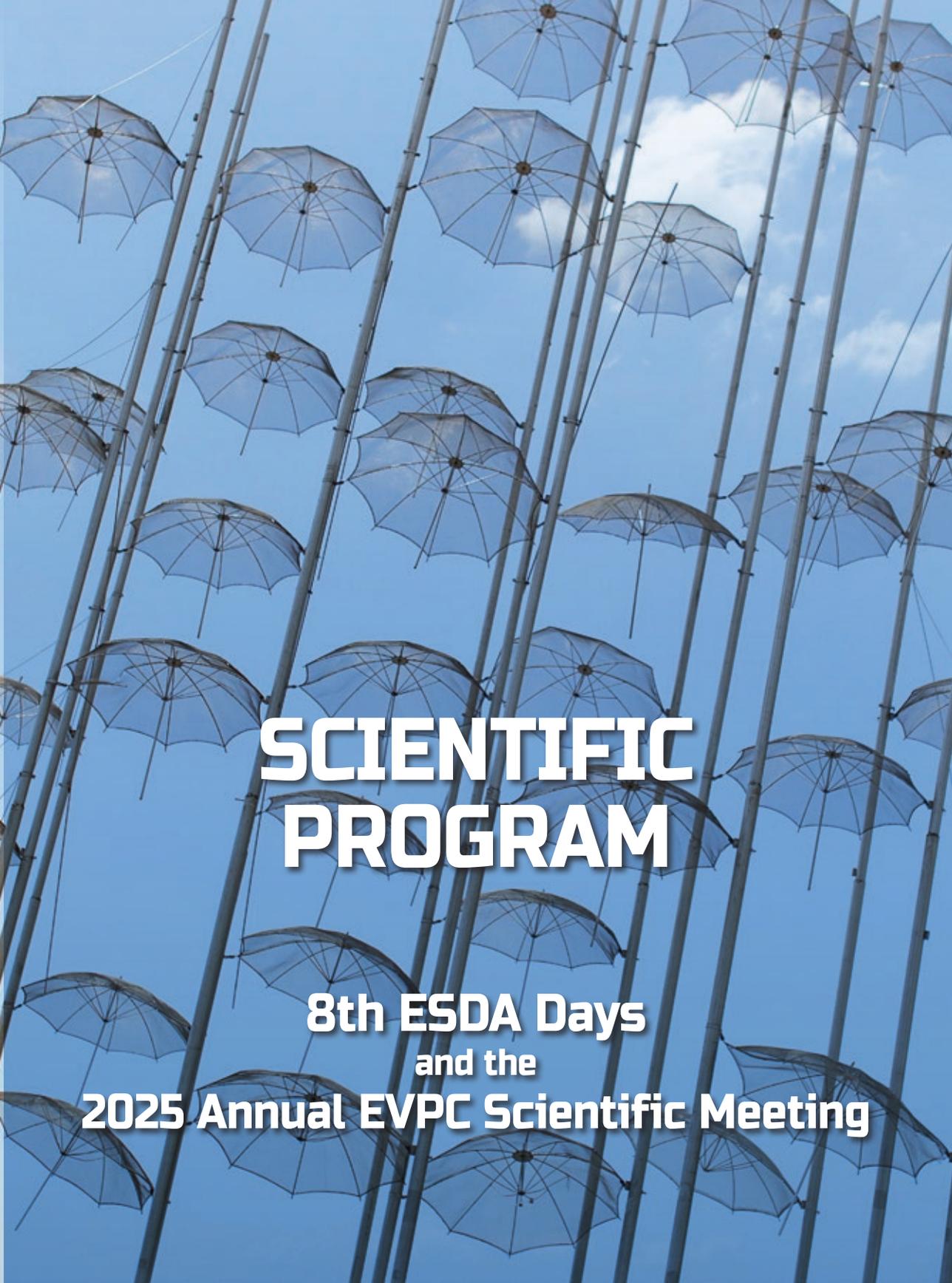
On behalf of the Organizing Committees

Anastasia Diakou

ESDA

Smaragda Sotiraki

EVPC



SCIENTIFIC PROGRAM

**8th ESDA Days
and the
2025 Annual EVPC Scientific Meeting**

Thursday 22 May 2025

Annual EVPC Scientific Meeting

08:00-09:00 Registration

09:00-11:00 Chair: Smaragda Sotiraki

Invited Speakers

Three + decades of EBVS: an overview of the strategy for the 4th decade

Zoe Polizopoulou *Professor School of Veterinary Medicine, Faculty of Health Sciences, AUTH, EBVS® European Specialist in Veterinary Clinical Pathology, Senior Vice President of the EBVS (Greece)*

Best practices in education

Dr. Mark Bowen *EBVS® European Specialist in Veterinary Sports Medicine and Rehabilitation, Director of Education EBVS*

Experimental and in-field poultry coccidiosis: A clinical perspective of diagnosis and control

Vasileios Tsiouris *Associate Professor, School of Veterinary Medicine, Faculty of Health Sciences, AUTH, EBVS® European Specialist in Poultry Veterinary Science (Greece)*

Mite-related skin diseases in dogs

Rania Farmaki *Assistant Professor, School of Veterinary Medicine, Faculty of Health Sciences, AUTH, EBVS® European Specialist in Veterinary Dermatology (Greece)*

11:00-11:30 COFFEE BREAK AND EVPC POSTER SESSION (PART I)

P01 Assessment of the speed of transmission of *Ehrlichia canis*, *Anaplasma phagocytophilum*, and *Borrelia burgdorferi* sensu stricto by infected ticks through an in vitro experimental system

Frederic Beugnet *(France)*

P02 Occurrence of Hookworms and Other Important Intestinal Helminths in Dog Populations from Romania: Risk Factors and Zoonotic Implications

Mariana Ionita *(Romania)*

P03 The Prevalence of *Crassicauda anthonyi* in Cuvier's Beaked Whales (*Ziphius cavirostris*) Stranded in Greece and Its Molecular Characterization

Elias Papadopoulos *(Greece)*

P04 *Stomoxys calcitrans*: a potential mechanical vector of *Anaplasma phagocytophilum*?

Emilie Bouhsira *(France)*

P05 Prevalence of *Hepatozoon canis* in Iberian Wolves (*Canis lupus signatus*) from Spain

Susana Remesar *(Spain)*

P06 Aberrant migration of *Spirocerca lupi* in the urinary bladder of a dog presented with hematuria

Alexandra Daravigka *(Greece)*

P07 Severe co-infection of *Toxoplasma gondii* and *Ehrlichia canis* in a dog: a compelling clinical case

Georgiana Deak (*Romania*)

P08 Dairy goats helminthosis and its potential predictors in Greece: findings from an extensive countrywide study

George C. Fthenakis (*Greece*)

P09 *Cystoisospora suis* in the region of Murcia: study of prevalence in swine farms

Fuensanta Hernández Ruipérez (*Spain*)

P10 Exploring the Iberian wolf's diet and the epidemiological risks linked to cestodes

María del Rocío Ruiz de Ybáñez Carnero (*Spain*)

11:30-13:30 Chair: Emily Boushira

11:30-12:00 Keynote Speech

Toxoplasma gondii - what is a parasite of cats doing in the Arctic?

Emily Jenkins Professor, Head, Zoonotic Parasite Research Unit, Department of Veterinary Microbiology, University of Saskatchewan, (Canada)

12:00-13:30 Presentations

Evaluating the use of salivary ANTI-CARLA IgA testing to reduce gastrointestinal parasitism in Canadian pastured sheep

Andrew Peregrine (*Canada*)

Species diversity and zoonotic subtypes of *Cryptosporidium* in calves, Denmark

Rosalina Rotovnik (*Denmark*)

BESNOBIT-Updates on bovine besnoitiosis in Italy: novel insights, perspectives, and future directions of a neglected disease

Alessia Libera Gazzonis (*Italy*)

Spreading of *Parafilaria bovicola* cases in Switzerland

Gastón Moré (*Switzerland*)

Nonpruritic clinical manifestation of *Sarcoptes scabiei* on distal limbs of a horse

Petra Bandelj (*Slovenia*)

Comparative efficacy of topical pyrethroids and benzoyl peroxide for treating chorioptic mange in Spanish-Breton horses

Juan David Carbonell (*Colombia/Spain*)

A survey of Diptera involved in transmitting diseases to horses in Galicia (NS Spain)

Cristiana Filipa Cazapal-Monteiro C. (*Spain*)

Increased presence of *Hyalomma marginatum* in the NW Iberian Peninsula: One Health inference

Inés Isabel Abreu Ramos (*Spain*)

13.30-14.30 LUNCH BREAK AND EVPC POSTER SESSION (PART II)

- P11** PARASSESS, a digital owner-facing tool based on algorithm to assess parasite risk for dogs and cats
Frederic Beugnet (*France*)
- P12** Severe Filariasis and Trichinellosis in a free-ranging brown bear in Alaska - parasites know no boundaries
Emily Jenkins (*Canada*)
- P13** *Thelazia callipaeda* infection in Iberian wolves from northwestern Guadalupe Miró (*Spain*)
- P14** Establishment and validation of red fox (*Vulpes vulpes*) and domestic dog (*Canis lupus familiaris*) airway epithelial cell cultures at the air-liquid interface as a platform to investigate host-parasite interactions
Andreas Oehm (*Switzerland*)
- P15** Seroprevalence of *Cephenemyia stimulator* in roe deer from the Iberian Peninsula
Rosario Panadero (*Spain*)
- P16** Cases of Lungworm infections in hedgehogs (*Erinaceus europaeus*) in Greece and their therapeutic approach
Elias Papadopoulos (*Greece*)
- P17** Prevalence and molecular identification of *Eimeria* spp. infection in peri-weaned dairy calves
Elias Papadopoulos (*Greece*)
- P18** In vitro effect of plant extracts on different development stages of *Trichostrongylus colubriformis* and *Haemonchus contortus* of sheep
Antonio Bosco (*Italy*)
- P19** Echocardiographic strain imaging does not reveal myocardial deformation in dogs with Leishmaniasis
Paola Paradies (*Italy*)
- P20** Variations in the hematology of asymptomatic horses exposed to bloodborne pathogens
Adolfo Paz-Silva (*Spain*)
- P21** Ticks and tick-borne pathogens in the Galician stages of the French St. James way
Susana Remesar (*Spain*)
- P22** A preliminary study to determine the risk of culicids in the transmission of vector-borne diseases in wetlands from NW
Cristiana Filipa Cazapal Monteiro (*Spain*)
- P23** Analysis of current risk of *Leishmania infantum* transmission in Greece and its projection
Ivan Rodríguez Escolar (*Spain*)
- P24** Testing the effect of plant compounds on *Ascaridia galli* with an in vitro larval motility assay
Rosalina Rotovnik (*Denmark*)

P25 Relationship between environmental factors and occurrence of cardiopulmonary nematodes in the Eurasian badger (*Meles meles*) in semi-arid Mediterranean areas of Spain

María del Rocío Ruiz de Ybáñez Carnero (*Spain*)

P26 Vector-borne diseases in pet dogs from Bucharest, Romania: insights from a private veterinary clinic

Angela Monica Ionica (*Romania*)

P27 Repeated reproductive failure of a mare due to *Halicephalobus* sp. infection

Andrea Springer (*Germany*)

P28 Phylogeny of the subgenus *Phortica* sensu stricto (diptera: Drosophilidae) from Europe

Maria Sophia Unterköfler (*Austria*)

P29 Treatment of *Sarcoptes mange* in llamas and alpacas with Moxidectin

Beck Wieland (*Germany*)

P30 Circulation and diversity of *Trichinella* spp. in Romanian wildlife: Molecular identification and new epidemiological data

Ana-Maria Marin (*Romania*)

14:30-16:20 Chair: Georg von Samson-Himmelstjerna

Presentations

PARASSESS, A digital tool based on algorithm to assess parasite risk for dogs and cats

Frederic Beugnet (*France*)

Feline dirofilariosis diagnosis in two cats from Italy: expecting the unexpected.

Mariaelisa Carbonara (*Italy*)

Gastrointestinal parasites of shelter dogs in Israel

Salant Harold (*Israel*)

Enteric helminth infection impairs the antigen-specific CD8+ t response to vaccination

Ophélie Piedfort (*Belgium*)

Gastrointestinal helminth infections in dogs in sheep and goat farms in Greece: Prevalence, involvement of wild canid predators and use of anthelmintics

George C. Fthenakis (*Greece*)

Occurrence of antibodies against *Encephalitozoon cuniculi* in horses in Austria

Jutta Pikalo (*Austria*)

Wild hosts, hidden threats: Tick-host associations and tick-borne pathogens in wild carnivores from Northwestern Italy

Barbara Moroni (*Italy*)

Cystoisospora spp. infections and other intestinal parasitoses in young dogs and cats with diarrhea

Simone Morelli (*Italy*)

Three veterinary cases of aberrant parasite locations

Andreas Oehm (*Switzerland*)

16.20-16.30 COFFEE BREAK

16:30-18:00 [EVPC Annual General Meeting](#)

18.00-18.30 Opening ceremony and Welcome to the 8th ESDA Days and the 2025 Annual EVPC Scientific Meeting

18.30 Welcome reception



Friday 23 May 2025

8th ESDA Days

08:00-09:00 Registration

09:00-11:00 Chair: Rodrigo Morchon

09:00-09:40 Keynote Speech

The history of heartworm prevention - Macrocyclic lactones

Jorge Guerrero *Adjunct Full Professor of Parasitology, University of Pennsylvania, USA*

09:40-9:50 Presentation

BIRAGO project - what has happened forty years after the introduction of macrocyclic lactones in a historically endemic area for *Dirofilaria immitis*?

Marco Genchi (*Italy*)

09:50-10:10 Invited presentation

The “slow-kill” protocol in clinical practice: experience from its selective application

Christos Koutinas *Professor of companion animal medicine and cardiology, Companion Animal Clinic, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece*

10:10-10:20 Gold Sponsor Presentation (CEVA)

STRONGER TOGETHER by CEVA: Initiatives to fights against VBDs

10:20-11:00 Presentations

Dirofilaria immitis and *Angiostrongylus vasorum* in wild carnivores from Romania: overview and new data

Georgiana Deak (*Romania*)

Long-term surveillance of *Dirofilaria immitis* in wild carnivores from Serbia (2013–2025)

Aleksandra Penezić (*Serbia*)

Dirofilaria immitis in a red fox (*Vulpes vulpes*) in Austria - Is *D. immitis* establishing in Eastern Austria?

Hans-Peter Fuehrer (*Austria*)

11.00-11.30 COFFEE BREAK

11.30-13.30 Chair: Marco Genchi

11.30-11.50 Invited presentation

The history of *Wolbachia* and *Dirofilaria* spp.: a symbiotic saga of tiny guests and their wormy hosts

Laura Kramer *Full Professor of Veterinary Parasitology and Parasitic Diseases, University of Parma Veterinary School, Italy*

11:50-12:10 Invited presentation

Ecological niche modeling and vector-*Dirofilaria* sharing as tools to assess the risk of *Dirofilaria* infection

Rodrigo Morchon *Full Professor and director of the Zoonotic Diseases and One Health research group, University of Salamanca, Spain*

12.10-12.20 Presentation

Detection rates of *Dirofilaria immitis* and other canine filarial infections in Germany (2019-2023)

Viktor Dyachenko *(Germany)*

12.20-12.40 Invited presentation

From dogs to humans: The growing challenge of Dirofilariosis

Simona Gabrielli *Associate Professor of Parasitology and Parasitic Diseases, University of Rome Sapienza, Italy*

12.40-13.30 Presentations

Emerging human *Dirofilaria repens* infections: new cases in southern Italy

Lavinia Ciuca *(Italy)*

Persistence of *Dirofilaria repens* microfilaremia in a patient from Croatia

Relja Beck *(Germany)*

Human cases of dirofilariasis in Northeastern Europe

Anna Bajer *(Poland)*

Human exposure to *Dirofilaria immitis* following a canine heartworm disease elimination program in Linosa island

Simona Gabrielli *(Italy)*

13:30-14.30 LUNCH BREAK

14:30-16:00 Chair: Donato Traversa

14:30-14:50 Invited presentation

ESDA guidelines for Canine Angiostrongylosis

Anastasia Diakou *Full Professor, Laboratory of Parasitology and Parasitic Diseases, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki (Greece)*

14:50-15:00 Gold Sponsor Presentation (MSD)

MSD Animal Health Parasiticide Innovation Update

Despoina Athanasiou *(Greece)*

15:00-16:00 Presentations

Prognostic factors in canine angiostrongylosis complemented with a quantitative synthesis of the existing data on clinical signs

Manuela Schnyder *(Switzerland)*

Angiostrongylus vasorum compromises endothelial integrity and vascular health

Manuela Schnyder *(Switzerland)*

Successful treatment of *Angiostrongylus vasorum* infections in dogs after a single administration of a fluralaner, moxidectin, and pyrantel chewable tablet

Lea Heinau (Germany)

Successful prevention of angiostrongylosis in dogs after a single administration of a fluralaner, moxidectin, and pyrantel chewable tablet

Lea Heinau (Germany)

Rainfall and temperature driven emergence of neural angiostrongylosis in eastern Australia, 2020-2024

Jan Šlapeta (Australia)

Microscopic lesions in rats naturally infected with *Angiostrongylus cantonensis* mirror that of *Dirofilaria immitis* in dogs

Heather Walden (USA)

Cotton-top tamarins (*Saguinus oedipus*) may develop-fatal infections and are definitive hosts of the metastrongyloid rodent parasite *Angiostrongylus dujardini*

Walter Basso (Switzerland)

16:00-17:00 COFFEE BREAK AND ESDA POSTER SESSION

P01 Preliminary Insights into the genetic diversity of human's *Dirofilaria repens*:

A molecular analysis on cox1 gene

Mohammed Alsarraf (Poland)

P02 Human Dirofilariasis in Slovakia - Epidemiology and clinical manifestations

Daniela Antolová (Slovakia)

P03 Transmission risk maps of canine Angiostrongylosis considering multiple potential intermediate hosts in the Iberian Peninsula and Balearic Islands.

Alfonso Balmori-de la Puente (Spain)

P04 Serological investigation of vector-borne infections in Turkish dogs with special reference to *Dirofilaria immitis*

Veli Yilgor Cirak (Turkey)

P05 Evaluation of the interaction of *Angiostrongylus vasorum* with the host using an in vitro model of endothelial cells

Manuel Collado Cuadrado (Spain)

P06 Relationship of *Dirofilaria repens* with the host through the study of the effect of the Dr20/22 protein on the expression of angiogenesis-related proteins

Manuel Collado Cuadrado (Spain)

P07 The role of 4 recombinant proteins of *Dirofilaria immitis* in the angiogenic process as a mechanism of survival in the host

Manuel Collado Cuadrado (Spain)

P08 Cyprus: A Heartworm-free country under the threat of infection invasion

Anastasia Diakou (Greece)

P09 Co-infection of mosquitoes with *Dirofilaria* spp. and the West Nile virus

Marta Fozzer (Italy)

P10 DIROGEN: Deciphering the genetic diversity of *Dirofilaria repens* and *D. immitis*: A global and European perspective

Hans-Peter Fuehrer (*Austria*)

P11 *Angiostrongylus vasorum* in Slovakia - what we know, what we don't, and what's ahead

Zuzana Hurníková (*Slovakia*)

P12 Mapping the current risk of transmission of dirofilariosis in Colombia month by month through ecological niche modeling

Elena Infante González-Mohino (*Spain*)

P13 New insights into the epidemiology of canine Dirofilariosis and other selected vector-borne diseases in Romania

Mariana Ionita (*Romania*)

P14 Comparative palatability assessment of Milbemycin Oxime and Praziquantel Oral chewable formulations and tablets

Sofia Loukeri (*Greece*)

P15 Canine Heartworm disease - an emerging veterinary challenge in Slovakia

Martina Miterpáková (*Slovakia*)

P16 *Dirofilaria* spp. in mosquitoes from a Canine Dirofilariosis-endemic area

Maria Ortensia Montella (*Italy*)

P17 Prevalence and etiology of lower respiratory tract diseases in cats in Spain

Rodrigo Morchón García (*Spain*)

P18 Preliminary results of risk of atopy in owners of dogs with Heartworm disease

Rodrigo Morchón García (*Spain*)

P19 Influence of *Dirofilaria immitis* on atopic development in a hyperendemic population

Rodrigo Morchón García (*Spain*)

P20 Distribution pattern of *Dirofilaria immitis* in the province of Aveiro (Portugal)

Rodrigo Morchón García (*Spain*)

P21 Prevalence of *Dirofilaria* spp. and a novel *Wolbachia* supergroup B strain in *Aedes vexans* from Ukraine

Olena Nahimova (*Poland*)

P22 Vascular endothelial cell involvement in the pathogenesis and host-parasite interaction of canid angiostrongylosis: a comparative in-vitro study in red foxes (*Vulpes vulpes*) and domestic dogs

Andreas Oehm (*Switzerland*)

P23 Characterisation of *Angiostrongylus vasorum* excretory-secretory products identifies key components for host-parasite interaction and prospective therapeutic targets conserved across a wide range of helminths of veterinary and zoonotic relevance

Andreas Oehm (*Switzerland*)

P24 Current risk of transmission of *Dirofilaria* in Europe and its projection to 2100
Ivan Rodríguez Escolar (*Spain*)

20.00

Conference Diner

Avenue Restaurant

Address: Leof. Meg. Alexandrou 6, 54641-Thessaloniki

(Near Makedonia Palace hotel-Entrance is available both from the street or the waterfront).



Saturday 24 May 2025

8th ESDA Days

9:00-11:00 Chair: Laura Kramer

9:00-9:40 Keynote

Macrocyclic lactone resistance in *Dirofilaria immitis*: Risk for Europe?

Roger Prichard *Distinguished James McGill Professor Emeritus, McGill University, Canada*

9:40-10:00 Invited presentation

ML resistance in Europe - first imported case

Donato Traversa *Full Professor of Veterinary Parasitology and Parasitic Diseases of Animals, Department of Veterinary Medicine, University of Teramo, Italy*

10:00 -10:10 Gold Sponsor Presentation (Boehringer Ingelheim)

Evaluation of the efficacy of permethrin and fipronil (FRONTLINE TRI-ACT®) spot-on in reducing the transmission of *Dirofilaria immitis* in dogs under natural parasitic exposure

Loic Antoine *(France)*

10:10-11:00 Presentations

Population genomics reveals an ancient origin of heartworms in canids

Jan Šlapeta *(Australia)*

New insights into the phylogeographic history of *Dirofilaria immitis* in the Canary Islands

Alfonso Balmori-de la Puente *(Spain)*

An analysis of the current risk of transmission of *Dirofilaria* in United States of America and its future projection under climate change scenarios

Ivan Rodríguez Escolar *(Spain)*

Effect of *Dirofilaria immitis* excretory/secretory and somatic antigen on the expression of angiogenesis-related proteins as a survival mechanism

Manuel Collado-Cuadrado *(Spain)*

11:00-11:30 COFFEE BREAK

11.30-13.30 Chair: Christos Koutinas

11:30-11:50 Invited presentation

ESDA Canine Heartworm guidelines

Laura Kramer *Full Professor of Veterinary Parasitology and Parasitic Diseases, University of Parma Veterinary School, (Italy)*

11:50-12:10 Invited presentation

Proposal for using TTE (Transthoracic Ecocardiography) and VU (Vascular Ultrasound) as an ultrasound guiding technique in CSS (Caval Syndrome Surgery) for GP (General Practitioner)

Florin Leca *DMV, PhD, Laboratory of Veterinary Interventional Cardiology, "Doctor's Vet Universe" Clinic, Romania*

12:10-12:30 Invited presentation

(AHS) Management of symptomatic heartworm disease in dogs

Marisa Koyo Ames *Associate Professor of Cardiology, University of California, Davis School of Veterinary Medicine, USA*

12:30-13:30 Presentations

Reevaluating doxycycline dosage in canine heartworm disease: is less more?

Daniel J. Vera-Rodríguez *(Spain)*

Use of corticosteroids during adulticide treatment in dogs with heartworm: is it a risk or a benefit?

Noelia Costa-Rodríguez *(Spain)*

Application of the slow kill protocol against canine heartworm disease in Greece

Elias Papadopoulos *(Greece)*

Dirofilaria spp. extracellular vesicles and MiRNAs as game-changers in diagnosis and host-parasite dynamics

Serena Cavallero *(Italy)*

Identification of *Dirofilaria immitis*, *Dirofilaria repens* and *Acanthocheilonema reconditum* microfilariae through matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)

Ettore Napoli *(Italy)*

13.30-14.30 LUNCH BREAK

14:30-17:00 Chair: Anastasia Diakou

14:30-14:50 Presentations

Radiographic diagnostics of the canine heartworm disease: old and new methods

Ljubica Spasojević Kosić *(Serbia)*

From incidental discovery to clinical relevance: the journey of *Dirofilaria immitis*

Emanuela Seu *(Italy)*

14:50-15:35 Round Table

Diagnostic tools to stage the disease and choose the best therapy. Radiology and POCUS. Does 1+1 always make 2?

Luigi Venco *(Chair)*

Marisa Koyo Ames, Angelo Francesco Basile, Florin Leca, Christos Koutinas, Emanuela Seu

15:35-15:55 Invited presentation (online)

American Heartworm Guideline updates: what we kept and what we changed and why

Andy Moorhead *Small animal Parasitologist, Associate Professor, North Carolina State University, College of Veterinary Medicine, USA*

15:55-17:00 Presentations

Can dogs be infected with different lineages of *Dirofilaria immitis*? Whole genome sequencing has the answer

Mustafa Alsarraf *(Poland)*

Trend in the circulation of *Dirofilaria immitis* DNA in mosquitos of Emilia-Romagna region (Italy) in years 2022-2023-2024 captures

Alice Vismarra *(Italy)*

Beware of *Dirofilaria* risk when travelling with your dogs!

Francesca Nonnis *(Italy)*

An analysis of the current risk of transmission of animal and human dirofilariosis in Portugal and Spain month by month through ecological niche modeling

Elena Infante González-Mohino *(Spain)*

17:00-17:30 COFFEE BREAK

17:30-18:30 ESDA General Assembly



ESDA

European Society
of Dirofilariosis and
Angiostrongylosis



18:30-19:00 Closing Session

ESDA Invited Speakers



Roger Prichard

Distinguished James McGill Professor Emeritus, McGill University, Canada



Jorge Guerrero

Adjunct Full Professor of Parasitology, University of Pennsylvania (1983-2018), USA



Laura Kramer

Full Professor of Veterinary Parasitology and Parasitic Diseases, University of Parma Veterinary School, Italy



Andy Moorhead

Small animal Parasitologist, Associate Professor, North Carolina State University, College of Veterinary Medicine, USA



Marisa Ames

Associate Professor of Cardiology, University of California, Davis School of Veterinary Medicine, USA



Donato Traversa

Full Professor of Veterinary Parasitology and Parasitic Diseases of Animals, Department of Veterinary Medicine, University of Teramo, Italy



Simona Gabrielli

Associate Professor of Parasitology and Parasitic Diseases, University of Rome Sapienza, Italy



Rodrigo Morchón García

Full Professor and director of the Zoonotic Diseases and One Health research group, University of Salamanca, Spain



Anastasia Diakou

Full Professor, Laboratory of Parasitology and Parasitic Diseases, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece



Christos Koutinas

Professor of companion animal medicine and cardiology, Companion Animal Clinic, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece



Leca Florin

DMV, PhD, Laboratory of Veterinary Interventional Cardiology, "Doctor's Vet Univer's" Clinic, Romania

EVPC Invited Speakers



Emily Jenkins

Professor and Graduate Chair of the Department of Veterinary Microbiology, Head of the Zoonotic Parasite Research Unit, Western College of Veterinary Medicine at the University of Saskatchewan, Saskatoon, Canada



Zoe Polizopoulou

DVM, PhD, DipECVCP Diagnostic Laboratory School of Veterinary Medicine Aristotle University of Thessaloniki, Greece



Vasilios Tsiouris

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PLENARY PRESENTATIONS

8th ESDA Days
and the
2025 Annual EVPC Scientific Meeting
Thessaloniki, Greece
May 22-24, 2025



I-1. THREE + DECADES OF EBVS: AN OVERVIEW OF THE STRATEGY FOR THE 4TH DECADE.

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The European Board of Veterinary Specialisation (EBVS) has started its fourth decade with the same passion and a new ambitious strategy. With the efforts of the Executive Committee, EBVS staff, Committee members, and individual passionate Diplomates from various Colleges, significant progress has been achieved in multiple domains. The recognition of EBVS standards continues to grow.

With a refined strategy and a dedicated team, milestones on social media have been reached, significantly increasing engagement and visibility. The strategic meeting in Vienna in January 2025 provided a pivotal moment for assessing and refining communication strategies, ensuring continued expansion of EBVS' outreach and influence.

The development of the "EBVS Strategy 2025-2030" will guide EBVS in the coming years, reinforcing its objectives and vision. Simultaneously, collaboration with key European partners, including the Federation of Veterinarians of Europe (FVE) and the Union of European Veterinary Practitioners (UEVP) and International Veterinary Student Association (IVSA), has been further strengthened, ensuring that EBVS remains at the forefront of shaping veterinary specialisation policies across Europe.

The ongoing guidance for Colleges regarding Resident credentialing and examination processes ensures that training pathways remain robust and accessible. Furthermore, the EBVS team continues to engage with colleagues from Eastern European countries, offering guidance on integrating into the College system and exploring modular national specialisation frameworks. With a committed network of specialists, staff, and executive members, EBVS continues to drive progress, ensuring a sustainable and impactful future for veterinary specialisation in Europe.

I-2. EXPERIMENTAL AND IN-FIELD POULTRY COCCIDIOSIS: A CLINICAL PERSPECTIVE OF DIAGNOSIS AND CONTROL.

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Poultry coccidiosis, a protozoan disease caused by *Eimeria* spp. poses a significant threat to the global poultry industry due to its impact on the performance, health and welfare of poultry as well as on the profitability. Clinical diagnosis remains a critical component in the effective management and control of the disease. Affected birds often present with symptoms such as diarrhea (often bloody in severe cases), reduced feed intake, weight loss, and lethargy. Traditional diagnostic approaches include lesion scoring during necropsy, fecal examination for oocyst detection using flotation techniques and microscopic evaluation. Rapid and accurate clinical diagnosis facilitates timely intervention, improving treatment outcomes and supporting the implementation of preventative strategies such as vaccination and anticoccidial drug programs. However, advances in molecular diagnostics and antigen detection assays are offering enhanced sensitivity and specificity. Therefore, we must combine clinical and pathological observations with laboratory diagnostics to ensure comprehensive disease management.

I-3. MITE-RELATED SKIN DISEASES IN DOGS.

R. Farmaki

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Mites such as *Sarcoptes scabiei*, *Otodectes cynotis*, *Cheyletiella* spp, *Demodex canis*, *Neotrombicula autumnalis*, and *Straelensia cynotis* are responsible for several skin diseases, some of which have zoonotic potential. Sarcoptic mange is an extremely pruritic, highly contagious disease, characterized by papulocrustous lesions that commonly affect face and ventral areas, specifically the ear pinnae, elbows, and hocks. Additional lesions are seen as a result of self-trauma and chronicity of skin inflammation. Scabies incognito is a term used to describe cases with minimal lesions that are difficult to diagnose. Norwegian or crusted scabies is a rare skin disease characterized by diffuse crusted, non-pruritic dermatitis with high numbers of mites in skin scrapings that is associated with immunosuppression due to systemic disease or malnutrition. Multiple skin scrapings may be required to reveal the presence of the parasite, and ideal sites for skin examination are new lesional areas and marginal areas of the pinnae, especially where pruritus is noted during

scraping. Serological tests are also available for *Sarcoptes scabiei*. Otodectic mange is a common cause of otitis in dogs, accompanied by excessive dark brown ear wax, the characteristic appearance of “coffee grounds”, and itching. Mites may be seen less commonly seen outside of the ear canal on the body. Cheylletiellosis, also known as walking dandruff, is characterized by scaling of the skin, and pruritus is variable. It can be easily diagnosed by examining skin and hair shafts. Demodicosis may be juvenile or adult onset, localized, with limited or multiple affected areas, or generalized with variable pruritus. Skin lesions are mainly characterized by alopecia-hypotrichosis, erythema, crusting and comedones. Juvenile onset demodicosis is the result of an inherited defect in the immune system and may be self-limiting. On the other hand, adult-onset demodicosis is associated with underlying diseases and immunosuppression. Trombiculosis is a seasonal dermatitis, with variable pruritus caused by the larval stage that is seen clustering on protected body areas and skin folds on the head, pinnae, feet and tail. *Straelensia cynotis* is a papular to nodular dermatitis usually non-pruritic that affects the dorsal body and mild to moderate forms are asymptomatic in contrast to a more severe infestation that can be painful.

I-4. TOXOPLASMA GONDII – WHAT IS A PARASITE OF CATS DOING IN THE ARCTIC?

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Toxoplasma gondii is arguably the world’s most successful parasite, with felids as the ultimate source. For over a decade, we have sought to unravel the ecology and transmission of this parasite which is enigmatically prevalent in wildlife and people in the Canadian Arctic, where felids are rare. We take a One Health approach that indicates that foxes are excellent sentinels for transmission of and human exposure to *T. gondii*, that *T. gondii* travels as a “parasite pollutant” through multiple routes into the Arctic, and that exposure to *T. gondii* is increasing in polar bears as sensitive indicators of climate change in one of the most rapidly warming locations on Earth.

I-5. THE HISTORY OF HEARTWORM PREVENTION - MACROCYCLIC LACTONES.

The development of macrocyclic lactones for heartworm prevention in dogs and cats represents a remarkable achievement in veterinary medicine. From the early research and discoveries to the introduction of a variety of groundbreaking compounds like ivermectin, milbemycin oxime, selamectin and moxidectin, the journey has been marked by innovation, collaboration, and a steadfast commitment to animal health. As we move forward, continued advancements in this field will undoubtedly contribute to the ongoing efforts to protect our beloved pets from the threat of heartworm disease.

I-6. THE “SLOW-KILL” PROTOCOL IN CLINICAL PRACTICE: EXPERIENCE FROM ITS SELECTIVE APPLICATION.

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Heartworm disease is one of the most common parasitic diseases in the dog, caused by the parasite *Dirofilaria immitis*. Due to the global presence of its intermediate host, mosquitos belonging mainly to the *Culex* and *Aedes* species, it also has a worldwide distribution and is endemic in many temperate and equatorial countries. *D. immitis* is a parasite of the cardiovascular system, reaching maturity in the pulmonary arteries and causing vascular and lung lesions that lead to pulmonary hypertension, right heart failure and occasionally death, especially when the worms transport back into the right ventricle and atrium. Treatment with arsenic compounds, mainly melarsomine, has been the mainstay of adulticide treatment for many years. Melarsomine, at the dose of 2.5 mg/kg, has a relatively rapid onset of action, reaching therapeutic levels within a few days after deep intramuscular injection. Both the American Heartworm Society (AHS) and the European Society of Dirofilariosis and Angiostrongylosis (ESDA) recommend three doses of melarsomine, within a period of one month, in order to achieve complete cure. Prior to the injection, the guidelines suggest a preparatory period of 60 days, during which doxycycline is prescribed at the dose of 5-10 mg/kg, every 12 hours, for 30 days, to eliminate *Wolbachia pipientis*, a bacterial symbiont of the parasite. This results in reducing the initial worm burden and minimizing the severe inflammatory reaction of the host after the death of the parasites. At the same time, both Societies advocate the use of macrocyclic lactones (e.g. moxidectin, milbemycin), at the beginning

of the 60-day preparatory period, and monthly after that, to further reduce adult worm numbers and eliminate mosquito-infecting microfilariae from the circulation. It has been documented that ivermectin and moxidectin also have adulticidal properties (Kramer et al 2018) and, at the same time, clearance of microfilaraemia reduces the specific lung pathology caused by microfilariae. After the initial melarsomine injection, the most common and lethal complication is pulmonary thromboembolism caused by the dead and disintegrating adults. This risk can be significantly reduced by concurrently administering glucocorticoids at anti-inflammatory doses and enforcing cage-rest of the dog. The entire treatment regiment can take up to 4 months in total, after which all adult *D. immitis* worms in the canine host are largely eliminated. In general, the clinical condition markedly or gradually improves after treatment and pulmonary vascular and parenchymal lesions that accompany heartworm infection are gradually resolved. It should be noted that in advanced stages of the disease (stage III and IV, i.e. right heart failure and vena cava syndrome), the 60-day preparatory period could and probably should be significantly shortened, while accommodating heart failure treatment and surgical removal of the worms.

During the past few years, it has been suggested that a month of doxycycline, along with monthly moxidectin administration, can result in the eventual death of *D. immitis* adults and clinical as well as parasitological cure, even at late stages of the disease (Kramer et al 2018, Genchi et al 2019, Patterson et al 2020). As a result of limited availability of melarsomine in certain countries, along with its high cost (70 – 100 euros per 50 mg injectable bottle in Greece), this “slow-kill” protocol has gained popularity and has been a secondary low-cost alternative to the treatment plan with melarsomine that is traditionally suggested by both AHS and ESDA. Experimental studies have assessed the efficacy and safety of this protocol, with good results in parasite cure rates (Savadelis et al 2017) and there have been a few clinical studies evaluating the long-term effects of continuing worm presence in the pulmonary vasculature (Kramer et al 2018, Genchi et al 2019). However, the so called “moxi-doxy” approach is not currently endorsed by either Society as alternative treatment to melarsomine injections. Moreover, it has not been proven definitively that all worms are eventually eliminated in due time, and there have been reports of parasite survival for up to 3 years (Alberigi et al 2020, Ames et al 2020).

The clinical experience in Greece and our currently running retrospective analysis of 138 dogs that received only the combination of moxidectin, as a topical solution, and doxycycline (several different formulations), suggests that this type of treatment is primarily relatively safe in the long-term. There have been very few cases (<5%) of appearance or progression of symptoms in the dogs receiving moxidectin and doxycycline, even if the majority of dogs for which we have repeat serology did not become negative for *D. immitis* antigen within one year of starting treatment. At the same time, the monthly administration of moxidectin reduces the possibility of mosquito infection by microfilariae, which is a significant parameter in a long-term treatment plan that involves the continuing presence of the adult worms in the final host.

It should be noted that all of our cases were selected based on two main criteria; primarily, dogs receiving the combination of moxidectin and doxycycline were free of advanced symptoms (e.g. systemic signs, right heart failure or vena cava syndrome) or evidence of moderate to severe pulmonary hypertension in echocardiography, which was necessarily performed in all dogs prior to starting treatment; and secondarily, a heavy worm burden, as subjectively assessed based on presence of worms in the right pulmonary artery and its occlusion by the parasites, necessitated the use of melarsomine and precluded a “slow-kill” approach, regardless of other changes. In addition, this treatment’s follow-up period included echocardiographic re-examination, 3 – 4 months after administration of doxycycline, as well as continued administration of monthly moxidectin until serology for *D. immitis* became negative. Even if eventually some dogs did not undergo a second echocardiographic evaluation, in those that did there was very little evidence of disease progression. At this point, it should be stressed that a large number (>70%) of the animals that received this protocol were shelter dogs, some of which were eventually relocated or adopted and the carers were unable to bring them in for a second echocardiographic examination. As a result, there is also limited information regarding the serological antigen status of these dogs after the proposed treatment. However, in recent direct communication with the shelter carers or the owners themselves (75-80% of cases), the dogs were either clinically healthy and alive or had died of extracardiac causes not directly attributed to heartworm disease.

Since severely affected dogs will require further treatment for heart failure or mechanical removal, with the addition of sildenafil as a prerequisite, forgoing melarsomine administration for financial reasons becomes a moot point. In our experience, prolonged therapy with sildenafil, which is required in most cases with moderate to severe pulmonary hypertension, significantly reduces the advantage of a low-cost but long-term parasitocidal treatment. This is the main reason why we don’t advocate the use of a delayed adulticidal protocol in stage III and IV dogs. At the same time, our experience with shelters suggests that carers are much more likely to concentrate their financial efforts on severely affected or high-worm-burden dogs requiring melarsomine, resulting in a much more nuanced approach, with the caveat that echocardiography, using a budget-friendly and focused approach, should always precede any treatment

decision. A robust follow-up protocol is additionally required to evaluate the long-term parasitic and clinical profile of these dogs.

Parallel to the concern of progression of pulmonary vasculature and parenchymal lesions, as well as systemic involvement (e.g. glomerulonephritis), a significant deterrent for “slow-kill” treatment remains the possibility of creating resistant strains of *D. immitis* by exposing the living adult worms to repeated doses of macrocyclic lactones. This may itself endanger the efficacy of prophylaxis in endemic countries. Until the effects of a sustained exposure to macrocyclic lactones and long-term effects of allowing worm presence inside the pulmonary arteries are better understood, the education of practitioners on the dangers of each treatment plan, along with favoring a nuanced, per-case approach to adulticidal therapy, remains the best option.

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I-7. THE HISTORY OF WOLBACHIA AND DIROFILARIA SPP.: A SYMBIOTIC SAGA OF TINY GUESTS AND THEIR WORMY HOSTS.

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Keywords: Wolbachia, *Dirofilaria immitis*, history

Introduction. Wolbachia is the most widespread endosymbiont in animals. It was discovered in arthropods nearly 100 years ago. Since then, it has been the object of over 4,000 scientific publications, including studies on host tropism, co-evolution, genomics and implications for human and animal health. Wolbachia is indeed an amazing microorganism that has changed the way we understand the world of heartworm disease, in particular pathogenesis, immunology and treatment. Here, we will go back in time and unravel the fascinating story of Wolbachia and *Dirofilaria immitis*.

How it all began. If you ask any researcher who studies endosymbiosis in filarial nematodes (and there are many!), they will likely say that it all began in 1995, when colleagues from the universities of Milan and Pavia (Italy) published the study entitled “Molecular evidence for a close relative of the arthropod endosymbiont Wolbachia in a filarial worm” (Sironi et al., 1995). Even though intracellular bacteria had been seen in filarial nematodes with electron microscopy in the ‘70s (McClaren et al., 1975), nobody knew what they were, who they were or why they were there. There had also been a couple of published studies on the anti-filarial effects of tetracycline, but no one thought about those tiny

bacteria, thinking rather that the antibiotic had some sort of direct effect on the worms. Many years later, Sironi and colleagues gave these bacteria a name, and by doing so, opened the door to a whole new filarial world. This fundamental discovery was thanks to their curiosity, intuition and experience in the field of endosymbiosis.

What we know. As soon as Sironi and colleagues described Wolbachia in heartworms, a flurry of excitement went through the scientific community. Subsequent research was aimed at looking for other filarial nematodes that harbored the bacteria and at defining the nature of the Wolbachia-filarial nematode relationship. Wolbachia was described in a further nine species of animal and human filarial nematodes by 1999. Results of further studies were highly suggestive that Wolbachia are obligatory mutualist symbionts because: i) every individual of every filarial species known to harbor Wolbachia, harbors Wolbachia (100% prevalence); ii) the two organisms have evolved together for millions of years (matching evolution); iii) Wolbachia is transmitted vertically from female worms to microfilariae; iv) elimination of Wolbachia is detrimental for the worm. Indeed, by the year 2000, several authors had reported that the anti-filarial effects of tetracycline observed 25 years before were due to the depletion/elimination of Wolbachia, and the focus of subsequent research became the targeting of Wolbachia for the treatment of human and animal filarial diseases. Studies on the effects of antibiotic treatment on *Dirofilaria immitis*-infected dogs (with doxycycline) reported that bacterial depletion from adult worms, elimination of microfilariae and adult worm death were all more efficient when antibiotics were combined with macrocyclic lactones (MLs; Bazzocchi et al., 2008) and that this combination also reduced the potentially severe post-adulticide effects of melarsomine (Kramer et al., 2008). We also learned that *D. immitis*-infected dogs mount an immune response to Wolbachia, likely through exposure to dead or dying parasites, and that Wolbachia contributes to the immunopathology of heartworm disease (Kramer et al., 2005; Diosdado et al., 2017). Some insights on what exactly Wolbachia and *Dirofilaria* spp. do for each other were also gained when, in 2012, genome sequencing of *D. immitis* and its Wolbachia was completed (Godel et al., 2012). Wolbachia are unable to synthesize several vitamins and cofactors (coenzyme A, biotin, folate), making them metabolically dependent on the *D. immitis* host. On the other hand, the known effects of elimination of Wolbachia from *D. immitis* (loss of fertility and vitality of adult filarial worms) are likely due to a lack of pathways for the synthesis of heme, purine and pyrimidine in *Dirofilaria*, which are however present in Wolbachia.

What we do not know. Even though Wolbachia has been identified in *Dirofilaria repens* (Grandi et al., 2008), we do not know what role it plays in the pathogenesis of subcutaneous dirofilariasis nor if doxycycline/ML combinations are adulticidal. We also do not know if these combinations are effective for treating feline heartworm disease. Finally, there is some recent evidence that Wolbachia from ML-resistant *D. immitis* are slightly different than Wolbachia from susceptible worms (Shin et al., 2020), suggesting that the Wolbachia genome may be able to evolve in response to selection pressures, including the use of antibiotics.

Conclusions. All of the ground-breaking studies cited here confirm that targeting Wolbachia is a winning strategy against heartworm disease, and this has led inevitably to the revision of international guidelines for the treatment of infection. There is still much to do, and it must be remembered that curiosity and intuition are and always will be the most important keys to research success!

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I-8. ECOLOGICAL NICHE MODELING AND VECTOR-DIROFILARIA SHARING AS TOOLS TO ASSESS THE RISK OF DIROFILARIA INFECTION.

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Keywords: Heartworm disease, *Culex pipiens*, *Aedes albopictus*, Ecological Niche Model, Europe.

In Europe, *Dirofilaria* infection is of significant veterinary and economic importance due to its impact on the health and welfare of both domestic and wild animals, primarily canids and felids. The presence of *Dirofilaria immitis* and *D. repens* is influenced by bioclimatic and environmental factors that determine the survival of its vectors—culicid mosquitoes. To analyse the risk of infection of vector-borne parasitic diseases as a control measure, ecological niche models, which take into account the behaviour of parasites in vectors, are good tools for this purpose, and colour maps can be made to report the areas at risk of infection with a precision of 1 km². We used ArcMap 10.8 to process both bioclimatic variables (related to temperature and precipitation) and environmental variables (such as water bodies, human footprint, and herbaceous and shrub density). After identifying the key predictor variables, we developed ecological niche models for *Culex pipiens* and *Aedes albopictus* using the MaxEnt algorithm, automated via the Kuenm R package. Additionally, we calculated the number of *Dirofilaria* spp. generations both annually and on a monthly basis as a function of temperature, using a custom R script. In most parts of the continent, the risk of infection is minimal during the winter months, except in some Mediterranean coastal regions, where some residual risk persists. In contrast, spring and summer bring an increased risk of infection in a significant part of the European continent, with the exception of the higher latitude areas together with the United Kingdom, the Scandinavian Peninsula (Norway, Sweden and Finland) and Russia, as well as high mountainous areas. Southern Europe, characterised by its warmer climate, experiences a high risk of infection, while in central Europe the risk becomes moderate to high. More risk assessments using the same methodology have also been carried out in Spain, and Portugal, Greece, Italy and Serbia with very interesting results. The forecasts do not bode well and a significant expansion of the risk of infection towards the north-eastern regions of the continent is foreseen. The creation of this type of maps where the risk of infection is analysed is a good control tool that should be taken into account by veterinary and public health staff, from their One Health point of view.

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I-9. FROM DOGS TO HUMANS: THE GROWING CHALLENGE OF DIROFILARIOSIS.

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Keywords: Human dirofilariosis; Diagnosis; Clinical presentation.

Dirofilariosis, an infectious disease caused by filarial nematodes of the genus *Dirofilaria*, is transmitted to humans through the bite of infected mosquitoes from the genera *Aedes/Ochlerotatus*, *Anopheles*, and *Culex*. Conventionally, dirofilariosis has been regarded as a disease affecting domestic and wild canines and felines, as well as various other carnivores. However, recent evidence suggests that it is now emerging as a zoonotic infection, with an increasing incidence in humans.

Among the various species, *Dirofilaria immitis* and *Dirofilaria repens* are of particular concern in terms of their clinical impact on humans and animals. Recent epidemiological data indicate their spreading beyond its traditional endemic zones in southern Europe, parts of Asia, and the Americas. Factors contributing to this trend include climate change—extending the active seasons and habitats of mosquito vectors—along with increased international pet travel and relocation. Surveillance studies indicate a steady increase in canine prevalence in northern and central Europe, correlating with the emergence of human cases in previously unaffected regions (1).

From a clinical perspective, *D. immitis* primarily causes cardiopulmonary disease in canines, while *D. repens* is typically responsible for subcutaneous and ocular infections. In humans, dirofilariosis frequently manifests as benign subcutaneous nodules, ocular infections (particularly in the periorbital region), or pulmonary “coin lesions” that may resemble neoplasms, occasionally resulting in unwarranted invasive procedures. Furthermore, there is a paucity of literature concerning unusual localisations in the brain, testis, breast or other uncommon sites. This complicates diagnosis due to the absence of specific symptoms and the unfamiliarity of such cases among clinicians (2).

The definitive diagnosis typically relies on histopathological examination following surgical removal of the lesion or worm. Imaging techniques like CT and MRI can help identify nodular lesions, but diagnosis is often delayed due to nonspecific symptoms and the rarity of human cases. Molecular and immunological tests are available to supplement or replace traditional morphology-based diagnostics, and experimental ELISAs have been developed for human serology, though they are not yet widely used in clinical practice.

Despite humans have long been considered accidental, “dead-end” hosts, since parasites do not generally reach reproductive maturity in human tissue, there have now been almost 27 documented cases of human infections involving gravid *D. repens* females. This finding suggests the possibility of male and female worms maturing in the human host, thereby challenging the long-standing assumption that humans are incapable of supporting the full life cycle of such worms. In contrast, only one case of human *D. immitis* infection with a female worm has been documented, and the patient was found to have acute lymphoblastic leukaemia and an immune system compromised by the disease. It is important to note that there are several other non-canine species that have the potential to infect humans. These include *D. tenuis* (transmitted from raccoons), *D. ursi* (transmitted from American black bears), *D. subdermata* (transmitted from North American porcupines), *D. spectans* (transmitted from Brazilian otters), and *D. striata* (transmitted from bobcats) which should be considered in differential diagnosis with other possible human filarial infections (3).

The growing zoonotic potential of dirofilariosis calls for enhanced awareness among clinicians, veterinarians, and public health authorities. Strengthening vector surveillance, improving diagnostic protocols, and promoting preventive measures such as canine prophylaxis and mosquito control are crucial. This presentation will advocate for the adoption of a One Health approach to address the evolving landscape of dirofilariosis, emphasizing interdisciplinary collaboration to mitigate its impact on both animal and human health.

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I-10. MACROCYCLIC LACTONE RESISTANCE IN DIROFILARIA IMMITIS: RISK FOR EUROPE?

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Keywords: *Dirofilaria immitis*, heartworm preventives, macrocyclic lactone resistance, resistance monitoring, resistance spread

Heartworm disease, caused by *Dirofilaria immitis*, can result in severe morbidity and death, particularly in dogs, due to cardiovascular pathology. It can also cause disease in cats and can be zoonotic. The infective stage L3 larvae are transmitted by many species of mosquitoes. The parasite has an almost global prevalence, wherever there are mosquitoes and enough warm days for the parasite to develop in the vector. Transmission is often seasonal. Global warming, with resultant wider distribution and intensity of mosquito transmission, and larval development (warm) days, may increase the risk of heartworm infection and disease.

Once infected and adult worms develop in dogs with resultant pathology and potential for transmission. Parasites can persist for years. Adult worms may be removed with arsenicals. However, there is some risk associated with clinical treatment, and it is expensive. Because of the expense, some infected dogs are not treated, and they can be the source of infection to other hosts via the mosquito vector. Other dogs are treated with a 'slow-kill' approach involving prolonged administration of macrocyclic lactones (MLs) and doxycycline. For 'slow-kill', treatment for about a year is required to remove all worms and it has not been recommended because of the concern for selection of drug resistance. The main control is prevention with macrocyclic lactone preventives which kill the L3/L4 stages prior to the pathogenic adults establishing. However, resistance to MLs has developed in the USA. Despite intensive campaigns for year-round prevention in the USA, we see that the incidence of heartworm infection has increased dramatically over the past 25 years, according to the American Heartworm Society. The question arises as to whether this increased incidence in the USA is due to better monitoring, or to other factors such as lower compliance with prevention despite a relentless campaign to use preventives, a dramatic increase in vector activity, or a loss in efficacy of MLs due to selection for drug resistance? Loss of efficacy of ML preventives was first noted in 2005 (Hampshire, 2005), and a first confirmed case of ML-resistance was reported in 2011 in a dog imported into Canada from Louisiana, USA (Bourguinat et al., 2011).

Genetic (SNP – single nucleotide polymorphic) markers have been developed to monitor for genotypic evidence of ML-resistance in *D. immitis* (Bourguinat et al., 2015; Ballesteros et al., 2018; Kumar et al., 2023) and have been used in several small and large surveys for resistance to ML heartworm preventives in the USA. In addition, the microfilariae suppression test (MFST), as well as the use of experimental infections while animals have been under heartworm preventive regimes, have been used to confirm phenotypic resistance. In addition, information on compliance with heartworm prevention has been gathered. These numerous studies in the USA now show that ML-resistance is widespread and can reach alarming levels in some locations (Fisher, Keller, Prichard, 2024; Curry et al., submitted). This indicates a serious breakdown in containing heartworm disease in the USA, and the potential for resistance to spread to new regions/countries with the possible transport of infected dogs, not to mention vector movement and spread aided by climate change.

What is the situation outside the USA? A study in eastern Australia (Queensland and NSW) showed no evidence of genotypic or phenotypic resistance to ML-preventives in 45 heartworm infected dogs (Power, Slapeta, 2022). We conducted a limited survey, in 2015-2016, in heartworm infected dogs from southern Ontario, Canada and found genotypic markers for ML-resistance in 3 of 39 *D. immitis* samples (Engell et al., in preparation).

Heartworm infections appear to be spreading in Europe. *Culex pipiens*, *C. theileri*, *Aedes albopictus*, *Ae. caspius*, *Ae. vexans*, *Anopheles maculipennis*, *Coquilletidia richiardii* are found in Europe and can transmit *D. immitis* if there are sufficient days (8 to 24 days, depending on temperature) with suitable parasite development conditions (temperatures above 14°C) (Morchón et al., 2012). There is evidence for the spread of mosquitoes of the genus *Aedes* in Europe. Furthermore, both published data, and data collected for myVBDmap™, from clinics indicate a spread of heartworm infections to regions not previously considered endemic for *D. immitis*, such as Germany, and possible increase in incidence of heartworm infections in Europe. We have conducted two limited surveys, based on the SNP markers for ML-resistance, in heartworm infected dogs in 5 countries in Europe. These surveys suggested that ML-resistance either was not yet present in Europe or only occurred infrequently. However, it must be considered that very little monitoring for ML-resistance is occurring, so far in Europe and other countries outside of the USA. Nevertheless, my colleagues have recently reported a case of both genotypic and phenotypic evidence of ML-resistance in a dog treated in Italy (Traversa et al., 2024). Some months before the resistant infection was detected, the dog had been

transported from the USA to Italy and although the dog had been checked for heartworm (antigen test) before being transported, it is possible that the ML-resistant heartworms were imported with the dog. Nevertheless, this shows the risk of ML-resistance being introduced when animals are transported and possibly establishing resistant foci in Europe and other regions.

It is time to discuss how we can reduce the risk of spreading ML-resistance in *D. immitis* and what can be done to maintain heartworm prevention in the face of spreading ML-resistance. These steps include more monitoring for resistance, paying particular attention to animals that are transported to new locations, and the use of the most effective preventive regimes. The latter involves the choice of ML used, dose rate, route of administration and full prevention compliance during periods when mosquitoes may acquire and transmit the parasite. Further steps are needed to reduce mosquito transmission of *D. immitis* in general, and particularly in the case of vectors which may acquire a resistant infection. Optimally this involves using medications with high potency against developing stages of *D. immitis*, including strains that show evidence of resistance to less potent ML formulations, but also preventive combinations with ectoparasiticides which prevent mosquito reproduction and/or repel mosquitoes during the interval between preventive administration. Finally, novel heartworm preventives which are not affected by ML-resistance, and are safe and convenient to administer, are needed.

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I-11. ML RESISTANCE IN EUROPE – FIRST IMPORTED CASE.

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Keywords: *Dirofilaria immitis*, Macrocytic Lactones, Resistance

The prevention of dog cardiopulmonary filariasis is crucial in enzootic areas, as treating dogs infected by *Dirofilaria immitis* is extremely laborious and risky. For this purpose, Macrocytic Lactones (MLs) are administered according to different chemopreventative schemes to dogs at risk of infection. In the past years various strains of *D. immitis* resistant to MLs have been described in Southern USA and, to date, it is hard to define their precise geographic distribution. The raising concerns for possible introduction, emergence, or spreading of *D. immitis* ML-resistant strains outside USA have fostered different studies that proved no evidence for their presence in Europe until 2023, when a dog

infected with a ML-resistant *D. immitis* strain was identified in Italy. This dog arrived in Italy from Louisiana, USA, in the first half of 2023 and less than 6 months after its arrival in Italy, it tested positive for heartworm circulating antigen and microfilariae, despite it having received monthly a macrocyclic lactone. The microfilariae suppression test and the genetic make-up of microfilariae carried out with a droplet digital PCR-based duplex assays targeting four marker positions at single nucleotide polymorphisms (SNP1, SNP2, SNP3, SNP7) proved that it was a resistant strain. A series of biological and epidemiological reasons clearly indicate that the dog was already infected when imported from USA to Europe.

This case demonstrates the realistic risk of ML-resistant *D. immitis* strains being imported and possibly transmitted outside USA. There is the need to increase the vigilance against ML-resistance to minimize the risk of importing resistant *D. immitis*. Therefore, travelling dogs must be always carefully monitored and examined for *D. immitis*, especially when they originate from areas where ML-resistance is known.

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I-13. PROPOSAL FOR USING TTE (TRANSTHORACIC ECHOCARDIOGRAPHY) AND VU (VASCULAR ULTRASOUND) AS AN ULTRASOUND GUIDING TECHNIQUE IN CSS (CAVAL SYNDROME SURGERY) FOR GP (GENERAL PRACTITIONER).

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Keywords: Heartworm, Caval Syndrome Surgery, Transthoracic Echocardiography, Vascular Ultrasound guidance

Introduction

Caval Syndrome (CS) represents a potentially fatal emergency caused by the sudden onset of hemolysis in dogs infected with *Dirofilaria immitis* due to the presence of adult worms at the level of the tricuspid valve. CS is diagnosed based on a constellation of signs: hemoglobinuria, anemia, and clinical signs of reduced cardiac output such as lethargy, weakness, and right-sided congestive heart failure (Atwell and Buoro, 1988; Kitagawa et al., 1986; Venco, 1993). CS should not be confused with Superior Vena Cava Syndrome (SVC)/Vena Cava Syndrome or PreCaval Syndrome, which are caused by severe obstruction or occlusion of the SVC.

The "Gold Standard" for performing CS surgery involves both fluoroscopic and ultrasound image guidance (American Heartworm Society Canine Heartworm Guidelines, Revised 2024). Hybrid imaging (fluoroscopy and ultrasonography) is widely used in human medicine, but its use in veterinary medicine is still limited, mainly due to financial considerations (IAEA Safety Report for Veterinarians using Radiation). Using a combined Transthoracic Echocardiography (TTE) and Vascular Ultrasound (VU) as image-guiding techniques in Caval Syndrome Surgery (CSS) may extend the procedure beyond the Veterinary Interventional Radiology Laboratory (VIRL), making CSS accessible to general practitioners (GPs)

Methods

These recommendations are based on the experience gained from performing more than 50 CS surgeries (from both published and unpublished data). The surgical procedure was performed using TTE guidance in 31 cases, while the remaining procedures employed hybrid TTE and fluoroscopy guidance in the Veterinary Interventional Cardiology Laboratory "Doctor's Vet Univers". This study included 45 cases diagnosed with intracardiac worm masses over a 9-year period (2013- 2022). Of these 45 cases, 36 were diagnosed with Caval Syndrome. Total intravenous anesthesia was administered using chemical restraint protocols (benzodiazepine and butorphanol), along with a dissociative agent (ketamine administered as an IV bolus, continued as a constant rate infusion (CRI) for maintenance and pain control).

The surgical technique involved right external jugular vein venotomy (surgical "cut-down"), which served as the venous access point for the introduction of embolectomy instruments (retrieval basket, tripod grasping forceps, Clear-It

flexible alligator forceps, rigid alligator forceps, or single/multiple wire semiflexible loops). Filariae from the right atrium were extracted, and the jugular vein was either ligated or preserved.

Results

After reviewing the data obtained from these cases, we identified the major difficult surgical steps in CSS (Fig. 1), particularly when fluoroscopic guidance is not used (or available) or when improper surgical techniques are applied. These challenges include:

1. Inability to reach the right atrium-right ventricle site due to improper patient positioning.
2. Introduction of the embolectomy instrument into the venous confluence or tributary of the Superior Vena Cava.
3. Insertion of the embolectomy instrument into the Inferior Vena Cava.
4. Failure to guide the instrument into the Right Ventricle (Fig. 2 and 3) due to a prominent Intervenous Tubercule.
5. Failure to remove the embolectomy instrument at the level of the thoracic inlet (due to a large number of worms entrapped).

We also identified several anatomical landmarks that can make guiding the embolectomy devices more difficult: External Jugular Vein, Superior and Inferior Brachiocephalic Confluence Area (including Cephalic vein, Omobranchial vein, Subclavian vein-Axillary vein, Costocervical vein, Internal Thoracic vein, Axillobrachial vein), Intervenous tubercule, Azygos vein, Inferior Vena Cava, Coronary Sinus (Fig. 4-9).

Conclusions and discussions

Considering that fluoroscopy is generally not available in general practice and taking into account the invasive nature of continuous radiation exposure of the medical team, using alternative image guidance during CSS represents a useful tool for general practitioners (GPs). Combining TTE with Vascular Ultrasound (VU) may reduce the reliance on X-ray-based image guidance during the procedure. Using TTE and VU can minimize radiation exposure to the medical team, even in the VIRL, potentially reducing exposure to zero minutes.

VU during the procedure is achieved by placing the linear ultrasound probe in the jugular fossa (Fig. 10), a triangular depression at the base of the neck, which communicates internally with the axilla. This is within the thoracic inlet (an oval opening bounded by the sternal manubrium, the first pair of ribs, and the thoracic vertebrae). This can be described as the “Vascular Ultrasound Suprasternal Jugular Fossa View” (VUSJF). Difficulties in passing the embolectomy tool from the level of the external jugular vein to the right atrium-right ventricle can be overcome by using combined Vascular Ultrasound and TTE.

The speed and outcome of the surgery are highly dependent on the ultrasonographer’s skills, but adding VUSJF can make this type of surgery accessible to GPs. This method (TTE-VUSJF) allows proper visualization of the parasites and embolectomy retrieval devices, ensuring the extraction of the filariae without causing damage to the tricuspid valve. Precise “arming” of the embolectomy device without dislodging any pre-existing ulcero-vegetative or thrombotic endocarditis (if present) is also achieved.

The beneficial role of dual image guidance (TTE and VUSJF) needs further exploration to reduce radiation exposure and make CS surgery more accessible to GPs.

I-14. (AHS) MANAGEMENT OF SYMPTOMATIC HEARTWORM DISEASE IN DOGS.

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Most dogs diagnosed with heartworm disease (HWD) do not have clinical signs (or have only mild signs such as intermittent cough) and go through adulticide therapy without complication. Clinical signs usually reflect the pulmonary vascular and parenchymal injury caused by *Dirofilaria immitis*. This lecture will review several clinical presentations of HWD and discuss therapy. (Table 1). Note: A uniform treatment recommendation for all conditions below is activity restriction.

Table 1. Approach to the symptomatic heartworm patient

Condition & Signs	Time of Onset and Comments	Treatment
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Pneumonitis Cough, tachypnea, dyspnea	<ul style="list-style-type: none"> -Timing varies -Associated with death of Mf or presence/death of adult worms -Radiographic findings: perivascular inflammatory bronchial and/or interstitial infiltrates -Note: this is not cardiogenic edema and furosemide is <i>not</i> indicated -Timing varies 	<ul style="list-style-type: none"> -Oxygen -Dexamethasone (0.2 mg/kg, IV) -Oral corticosteroids (0.5 mg/kg PO q12h, tapered as clinical signs allow)
HW-associated thrombosis/PTE Tachypnea, dyspnea, cyanosis, collapse, cough, hemoptysis	<ul style="list-style-type: none"> -May occur spontaneously at any time; risk likely greatest 3-21 days after melarsomine administration (peak worm death) -Injury/death of worms leads to thrombosis -Risk of severe thrombotic event increases with exercise or rapid worm kill (2-dose melarsomine protocol) 	<ul style="list-style-type: none"> -Oxygen -Sildenafil (1 to 2 mg/kg PO q8h) -Corticosteroid therapy, route, and agent determined by severity and patient's need for rapid treatment
Right-sided CHF Abdominal distension, jugular distention, tachypnea, dyspnea, weight loss	<ul style="list-style-type: none"> -Usually associated with chronic, untreated HWD -Pulmonary hypertension often present -Can initiate 'run-in' to adulticide therapy at the time of diagnosis (preventive and doxycycline) -Melarsomine should not be started until the dog's CHF is medically controlled 	<ul style="list-style-type: none"> -Furosemide ('at home' dose typically 1 to 3 mg/kg PO q12h) -Pimobendan (0.25 mg/kg PO q12h) -Sildenafil (1 to 2 mg/kg PO q8h) -Spironolactone (2 mg/kg PO q24h) -Centesis -Therapy can sometimes be reduced or discontinued after successful HW treatment
Caval syndrome Pallor, murmur, pigmenturia, collapse Signs of low cardiac output ± right-sided CHF	<ul style="list-style-type: none"> -Usually associated with chronic, untreated HWD or when a very large worm burden matures simultaneously -Can initiate 'run-in' to adulticide therapy at the time of diagnosis (preventive and doxycycline) -Melarsomine should not be started until the dog is stable 	<ul style="list-style-type: none"> -Stabilization: fluid resuscitation, ± vasopressors, ± blood products to normalize coagulopathy -Heavy sedation or general anesthesia, jugular venotomy, and worm extraction with forceps^a, gooseneck snare, or endovascular snare -Prompt HW removal is ideal (if not possible, can try oxygen therapy, pimobendan, and sildenafil) -See above for therapy of R-CHF

CHF, congestive heart failure; HW, heartworm; HWD, heartworm disease; PTE, pulmonary thromboembolism; Mf, microfilaria; ^aAvalon Medical, ClearIt® Heartworm Removal Device, Stillwater, MN 55082, USA

I-15. AMERICAN HEARTWORM GUIDELINE UPDATES: WHAT WE KEPT AND WHAT WE CHANGED AND WHY.

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The American Heartworm Society (AHS) periodically revises their guidelines for diagnosis and treatment of canine heartworm. The most recent update included a comprehensive review of the literature. While many aspects of the guidelines remained the same, information about the expanding number and range of mosquito intermediate hosts

was updated. This presentation will focus on two key recommendations with regards to diagnosis and treatment. For diagnosis, both testing for adult female uterine antigen, using a commercially available test, and microfilariae are recommended. The reason for this is that immune complexing of parasite antigens and host antibodies has been documented and can result in false negative antigen tests. By checking for microfilariae by either visual or molecular methods, we will have a greater chance of diagnosing positive animals, even when antigen tests are false negative because of immune complex formation. We can confirm a positive microfilariae result in these cases by “heat-treating” serum, thus breaking antigen/antibody complexes. The now “free” antigen will now be available to bind with the antibodies on the testing platform. The second recommendation is regarding treatment and the 30-day period between the end of doxycycline administration and the beginning of melarsomine dihydrochloride injections. The AHS recommended treatment for heartworm includes consistent administration of a macrocyclic lactone preventive, 28 days of doxycycline at a dose of 10 mg/kg BID for *Wolbachia*, followed by a 30-day period. At the end of this period one injection of melarsomine is given, followed 30 days later by two injections 24 hours apart. The need for the 30-day period between the end of doxycycline and beginning of melarsomine has been debated on the basis that there may be an increase in the pathologic changes in the host associated with heartworm during this time. Recent research has shown that there are no further pathologic changes during this period and furthermore that the amount of *Wolbachia* present in adult worms is reduced to almost zero. Since the presence of has been associated with increased clinical signs secondary to pulmonary thromboembolism, a greater reduction of *Wolbachia* prior to melarsomine treatment is considered beneficial to the animal.

ORAL PRESENTATIONS

8th ESDA Days
and the
2025 Annual EVPC Scientific Meeting

Thessaloniki, Greece
May 22-24, 2025



O-1. EVALUATING THE USE OF SALIVARY ANTI-CARLA IGA TESTING TO REDUCE GASTROINTESTINAL PARASITISM IN CANADIAN PASTURED SHEEP.

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Keywords: gastrointestinal nematode, carbohydrate larval antigen, salivary IgA, sheep, Canada

Gastrointestinal nematode (GIN) parasitism is common in Canadian sheep flocks, and managing GINs through the selection of sheep with superior immunity is of growing interest. The CARLA[®] Saliva Test measures salivary IgA against the carbohydrate larval antigen (CarLA) found on third-stage larvae of all GIN species. Salivary anti-CarLA IgA exceeding 1.0 U/ml is associated with 20–30 % lower fecal egg counts (FEC) in sheep under New Zealand grazing conditions (1), but there has been limited application of the CARLA[®] Saliva Test elsewhere (2). To address this gap, this study explored the utility of the CARLA[®] Saliva Test under Canadian grazing conditions. In Year 1, eighteen sheep farms in Ontario were enrolled and 25 ewe lambs per farm, on average, were randomly selected after grazing pasture for at least 60 consecutive days. The body condition, fecal consistency, FAMACHA[®] score, weight, packed cell volume, FEC, and salivary anti-CarLA IgA level were recorded for each study animal in Year 1. Study animals returned to pasture in Year 2 and were re-sampled 4 weeks after turnout. Multivariable linear regression modeling demonstrated that the salivary anti-CarLA IgA response in Year 1 predicted the salivary anti-CarLA IgA response in Year 2 ($\beta = 0.213$; $p < 0.001$). In addition, salivary anti-CarLA IgA in Year 1 was negatively associated with FEC in Year 2 ($\beta = -0.167$; $p = 0.025$). These data indicate that salivary anti-CarLA IgA measurements may be useful for identifying replacement sheep with superior immune responses to GIN infection in Canada.

Funding: Canadian Sheep Breeders' Association, Ontario Sheep Farmers, Ontario Ministry of Agriculture, Food and Agribusiness, and the National Science and Engineering Research Council of Canada.

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O-2. SPECIES DIVERSITY AND ZONOTIC SUBTYPES OF CRYPTOSPORIDIUM IN CALVES, DENMARK.

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Keywords: *Cryptosporidium parvum*, *Cryptosporidium bovis*, *Cryptosporidium ryanae*, 18S, gp60

Introduction

Cryptosporidium infection is a worldwide cause of gastrointestinal disease in especially young and immunocompromised humans and animals. The most reported species in calves, *Cryptosporidium parvum*, includes several subtypes that have been described as zoonotic, while the zoonotic potential of *Cryptosporidium bovis* and *Cryptosporidium ryanae* is considered limited. Species and subtype identification can aid in understanding the epidemiology and in source attribution in disease outbreak investigations.

The purpose of the current study was to investigate *Cryptosporidium* species and subtypes in calves in Denmark.

Methods

Faecal samples and metadata were collected from 140 calves from 13 farms in Denmark. Faecal DNAs were tested by genus-specific real-time PCR, next-generation sequencing of nuclear small subunit rDNA (18S) for species identification and 60-kDa glycoprotein gene subtyping of *C. parvum*. Associations between metadata and laboratory results were assessed by logistic regression with farm as random effect.

Results

Real-time PCR identified 101 *Cryptosporidium* positive samples. A total of 62 calves were positive for *C. parvum* by subtyping and/or 18S analysis. Subtyping revealed three different *C. parvum* subtypes (IIaA15G2R1 [n=37], IIaA16G3R1 [n=11], IIaA16G1R1 [n=5]). Analysis of 18S data revealed 11 samples with *C. bovis*, six *C. ryanae* positive samples, and four mixed species infections. Diarrhoea, observed in 60 calves, was associated with real-time PCR cycle threshold (CT) value (odds decreased 12.5% per increase in CT value, p=0.0215) and detection of *C. parvum* (odds of diarrhoea was 4.76 times higher with *C. parvum*, p=0.0009). *C. parvum* was associated with younger age (odds of *C. parvum* decreased 10.8% with each day of age, p<0.0001). *C. bovis* and *C. ryanae* were only detected in calves ≥ 27 days of age.

Conclusions

Cryptosporidium was commonly detected in calves in Denmark. *C. parvum* subtypes detected in human infections in Denmark were observed.

O-3. BESNOBIT - UPDATES ON BOVINE BESNOITIOSIS IN ITALY: NOVEL INSIGHTS, PERSPECTIVES, AND FUTURE DIRECTIONS OF A NEGLECTED DISEASE

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Keywords: *Besnoitia besnoiti*, cattle, Apicomplexa, guidelines, questionnaire survey

BESNOBIT project focuses on bovine besnoitiosis (BB), a parasitic disease caused by *Besnoitia besnoiti*, a cyst-forming Apicomplexa, considered (re-)emerging in Europe. Despite its recognized importance, significant gaps remain in our understanding of this parasite and the disease: its economic impact on cattle production is often underestimated. In Italy, epidemiological studies on BB are scattered, and current data do not confirm the disease's endemicity in bovine population.

BESNOBIT aims to clarify the biological and epidemiological aspects of *B. besnoiti* infection and to develop guidelines for integrated, sustainable, and effective control of BB in Italy, through three main actions: i) a seroepidemiological survey on dairy/beef cattle, and sympatric domestic animals (water buffaloes and donkeys), and a molecular study on potentially competent definitive hosts (red foxes, wildcats); ii) a longitudinal entomological survey in case-study farms to assess the role of hematophagous insect vectors; iii) a molecular analysis of genetic markers of *B. besnoiti* isolates. Additionally, an online questionnaire survey titled "Bovine Besnoitiosis: The Bovine Veterinarian on the Front Line" was implemented to gather information on BB knowledge among livestock veterinarians. This short, anonymous questionnaire was disseminated through the provincial veterinary medical associations' mailing list and promoted through the project's social media channels. It is divided into three sections based on the respondent's level of expertise (no knowledge of BB, knowledge without diagnosis experience, clinical experience) and includes multiple-choice, rating, or open-ended questions on the disease's theoretical knowledge, and diagnostic measures or control practices in infected farms. Within two weeks of its launch, over 80 Italian veterinarians had responded, and the preliminary results will be presented and discussed.

The expected outcomes of BESNOBIT will contribute to the development of sound guidelines for BB control in Italy, emphasizing traceability systems and biosecurity measures, that could be adapted to other livestock European contexts.

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O-4. SPREADING OF *Parafilaria bovicola* CASES IN SWITZERLAND

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Keywords: Cattle, Parafilariosis, cox1 sequencing

Bovine parafilariosis caused by *Parafilaria bovicola* is a fly-borne disease, characterized by cutaneous haemorrhagic exudations or 'bleeding spots'. Adult parasites are localised in subdermal nodules, which ulcerate and produce haemorrhagic exudate containing eggs and microfilariae. This can result in severe eosinophilic inflammation, which may affect adjacent muscle tissues and lead to partial carcass condemnations. The disease has been described in Africa, Asia, and the Americas, and seems to be emerging in Europe, where the face fly (*Musca autumnalis*) is the main vector. In Switzerland it was first reported in 2019 and, more recently, a case of an atypical intraocular localization in a heifer has been published.

Since 2023, six *P. bovicola* cases from different Swiss Cantons were diagnosed at the Institute of Parasitology in Bern. In 2023, two cases were recorded: one including several animals showing skin nodular lesions with bloody exudation (Jura), and the other involving a 3-year-old cow with skin bleeding without apparent injury (Zurich). In 2024, four cases were registered: an adult cow with bleeding spots (St. Gallen), and three different cases with intraocular localization of adult nematodes: a 2-year-old cow (Fribourg), an adult cow (Vaud), and a 3-year-old alpaca (Bern). All six samples (bloody exudate or worms) were processed by DNA extraction and PCR-sequencing for the cytochrome oxidase subunit 1 gene fragment of Filariid nematodes. All resulted in a 649 bp sequence (primers trimmed) with a 99.8-100% identity amongst each other, and with several *P. bovicola* GenBank sequences. The distribution in different cantons and the increase of detected cases over time suggest that parafilariosis is spreading in Switzerland. All three typical clinical cases were detected in May and June, and the three intraocular *P. bovicola* cases were detected in October. This is the first report of a *P. bovicola* infection in an alpaca.

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O-5. NONPRURITIC CLINICAL MANIFESTATION OF *SARCOPTES SCABIEI* ON DISTAL LIMBS OF A HORSE.

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Keywords: *Sarcoptes scabiei*, horse, mange, distal leg, nonpruritic

In November 2024, a 14-year-old Haflinger gelding, in Slovenia, was presented with painful, nonpruritic skin lesions on the distal part of the left front and both hind legs. Two lesions (lateral fetlock of the left front and medio-caudally below the tarsus of the right hind) were open wounds, surrounded by hyperkeratotic and crusted skin. Other lesions appeared dry with hyperkeratotic skin and alopecia. The horse was individually stalled and turned out in paddocks surrounded by forest. None of the other nineteen horses, nor the two dogs present at the barn were affected.

A total of six deep skin scrapings were taken from the borders of all lesions. After 10 min at 100°C in 10% KOH, samples were screened for the presence of mites under a light microscope. Two samples were positive for *S. scabiei*.

The horse was treated orally with 0.2 mg/kg ivermectin in combination with 1.5 mg/kg praziquantel (Noromectin Praziquantel Duo, Norbrook Manufacturing Ltd., Ireland) given twice two weeks apart, and local application of 30 g of a 50 mg/g permethrin-based cream (Infectoscab 5%, Infectopharm Arzneimittel GmbH, Germany) around the wounds and on the skin lesions, twice, two days in a row. A third treatment with ivermectin at a dose of 0.2 mg/kg (1%

Ivomec, Merial, France) was given off-label by deep i/m injection in the neck by a local veterinarian at week 4 after initiation of treatment. Four weeks after the last treatment, the owner stated that all skin lesions were completely healed.

Because of the rare nature of *S. scabiei* infestation in horses in Europe, cases are usually associated with infestation from other animals (1), which in this case could have been subclinically infected dogs or wildlife, such as foxes. The presented case serves as an important reminder that several samples must be taken to confirm the presence of mites, while a negative result might not exclude them. *Sarcoptes scabiei* may be an underreported etiology of skin diseases of horse distal limbs.

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O-6. COMPARATIVE EFFICACY OF TOPICAL PYRETHROIDS AND BENZOYL PEROXIDE FOR TREATING CHORIOPTIC MANGE IN SPANISH-BRETON HORSES.

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Keywords: Horse, Mange, *Chorioptes bovis*, Topical treatment, Benzoyl peroxide, Pyrethroids.

Chorioptic mange is a challenging condition to treat due to the superficial locations of the non-hematophagous mite *Chorioptes bovis*, and while topical acaricides are recommended, the clinical features relapses are frequent. In a double-blinded clinical trial, three randomised groups of Spanish-Breton horses ($n=32$) naturally infected with *C. bovis* on their legs were evaluated over a period of 70 days.

All treatments were applied once every 14 days for three treatments. Before treatment and on Day (D)10, D25, D37, D56 and D70, each leg per animal was scored according to equine pastern dermatitis clinical presentations and severity (1=mild, 2=exudative, 3=chronic proliferative), and mite reduction was calculated using a 2 cm × 6 cm adhesive tape counting total mites on D36, D56 and D70. The trial aimed to assess the clinical improvement and efficacy of a topical pyrethroid emulsion alone (group 1), and in combination with benzoyl peroxide (group 2), compared to a control group (group 3). The trial also included environmental disinfection. Acaricide efficacy was determined using Abbott's formula.

Our analysis reveals no adverse reactions attributable to the treatment, yet lesions showed limited clinical improvement. Both treatment groups exhibited mite reduction compared to the control. The mite reduction on the evaluated days was 14.58%, 47.62% and 55.77% for group 1, and 85.42%, 88.10% and 78.85% for group 2, respectively. The mite reduction was significant in group 2 on D56 and D70 ($p<0.0156$) compared to the pre-treatment and superior to group 1 ($p<0.0229$) at the end of the study (D70). Age and mite numbers showed no significant connection. In horses with higher clinical scores, there was no significant changes, most probably due to the short length of the study.

The combination of benzoyl peroxide with topical pyrethroids can reduce the numbers of *C. bovis* mites and it can be considered as an alternative therapeutic option in horses.

O-7. A SURVEY OF DIPTERA INVOLVED IN TRANSMITTING DISEASES TO HORSES IN GALICIA (NS SPAIN).

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Keywords: Diptera, Culicoides, *Culex*, Horses, northern Spain

Diptera-borne diseases are a major concern for Public and Animal Health, with 249 million cases globally. Equestrian centres are a suitable environment where horses and humans are in close proximity and there are specific risks that can compromise their health, like the presence of hematophagous diptera, associated with irritation, severe allergic reactions, and the transmission of several diseases. With the aim of continuing the entomological surveillance in equestrian centres of northern Spain, between January and October of 2024, periodically captures were done using CDC-UV and BG-Sentinel traps together with the dipping technique.

The presence of diptera of sanitary interest was confirmed in 92% (23/25) of equestrian centres. A total of 1194 specimens were identified as belonging to genera *Culicoides* (88,27%), *Culiseta* (3,18%), *Culex* (2,43%), and *Anopheles* (0,42%). In Europe, *Culicoides* biting midges, the most frequent genera in this study, may not spread equine viruses, but they still pose a significant health risk to horses as their bites can cause severe allergic reactions, leading to serious skin problems. Moreover, these midges are responsible for transmitting African Horse Sickness, a devastating disease that has already led to outbreaks in Spain, resulting in substantial economic losses due to the high mortality rate of infected horses. *Culex pipiens*, the most frequent culicid species, are known for being the vector of West Nile Virus, an endemic zoonoses in Spain that, until now, have not yet been reported in Galicia, where 23 of 25 equestrian centres were located.

It is confirmed the continuous presence of hematophagous dipteran of sanitary interest in equestrian centres where favourable conditions are found for both mature and immature stages. Therefore, the importance of dipteran surveillance is essential for the early detection and prevention of the entry of dipteran-borne pathogens with implications in Public and Animal Health in Galicia.

Acknowledgement

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O-8. INCREASED PRESENCE OF *HYALOMMA MARGINATUM* IN THE NW IBERIAN PENINSULA: ONE HEALTH INFERENCE.

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Keywords: ticks, *Hyalomma marginatum*, NW Iberian Peninsula, spreading, ReGaViVec

The presence of the tick *Hyalomma marginatum*, the primary vector of the Crimean-Congo haemorrhagic fever virus (CCHF) in Europe, was investigated in the NW Iberian Peninsula from 2019 to 2024. Ticks were collected through environmental sampling and collaboration with Health Centres, Official Veterinarians, Veterinary Clinics, Wildlife Recovery Centres, Field Veterinarians, and private individuals as part of the Galician Vector Surveillance Network (ReGaViVec, Spain).

A total of 63 adult ticks were identified as *H. marginatum* using morphological keys, with prevalence increasing from 1.6% in 2019 to 57.1% in 2024. Regarding host origin, 60.3% of ticks were collected from humans, 28.6% from cattle, 4.8% from horses, 3.2% from dogs, 1.6% from a goat, and 1.6% from the environment. According to Köppen's climatic zones, 82.5% of ticks were found in warm summer Mediterranean areas (Csa), 1.6% in temperate oceanic locations (Cfb), and 15.9% in cool summer Mediterranean regions (Csb). Seasonal distribution showed over half of the ticks were collected in spring, with numbers decreasing in summer and almost disappearing in autumn; no ticks were collected in winter.

Among humans, 18.4% of ticks were attached to the head, 34.2% to the trunk, 21.1% to the lower extremities, 10.5% to the pelvic area, 5.3% to the upper extremities, and 10.5% were found moving freely on the skin or hair.

It is concluded that *H. marginatum* ticks are increasingly present in the NW Iberian Peninsula, emphasizing the need for ongoing surveillance, particularly in ungulates grazing in open wooded areas from late winter onwards. The effective collaboration between health care centres, veterinarians, and local authorities highlights the importance of a coordinated One Health approach.

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O-9. PARASSESS, A digital tool based on algorithm to assess parasite risk for dogs and cats.

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Parassess is an interactive digital risk checker based on the most up-to-date understanding of parasite risk factors and control recommendations from scientific associations such as ESCCAP, TROCCAP, CAPC and WAAVP. The major parasites of dogs and cats are included in this assessment, fleas, ticks, mites, roundworms, hookworms, whipworm, tapeworms, lungworms, heartworm and *Leishmania infantum*. The risk factors are related to each parasite biology and epidemiology, and to each dog or cat, such as their sex and age, and other external factors, like location, behavior, feeding, and antiparasitic treatments. One algorithm has been built for dogs, another for cats. It is based on 13 to 20 questions, some being qualitative, others quantitative including single or multiple answers. A few questions refer to the possible presence of clinical signs like pruritus, fatigue, coughing, diarrhea and more. The algorithm calculates scores and provides a final score from 0 to 100 for each parasite. The final scores correspond to a risk assessment for the most common parasites. The digital questionnaire is easy to fill out in a few minutes. Parassess is not product related but only based on the biology and epidemiology of parasitic diseases. It can be adapted to specific geographies by adding or removing certain parasites. It offers veterinarians and pet owners the opportunity to better discuss and understand the risks, and to tailor the parasite prevention strategies.

O-10. FELINE DIROFILARIOSIS DIAGNOSIS IN TWO CATS FROM ITALY: EXPECTING THE UNEXPECTED.

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Keywords: Cats, diagnosis, Feline dirofilariosis, Heartworm disease

Feline cardiopulmonary dirofilariosis by *Dirofilaria immitis* occurs where the infection is endemic in dogs, the principal host species. Here we describe two cases of *D. immitis* infections in cats from Central-Northern Italy, where heartworm disease is historically endemic in dogs. Case 1, a 13-year-old male castrated domestic shorthair (DSH) cat was investigated for progressive gastrointestinal and respiratory signs including cough, nasal discharge, dyspnoea and reverse sneezing. After failure of symptomatic treatments, thoracic radiography was performed, showing pulmonary alterations suggestive of dirofilariosis, further confirmed by a positive rapid heartworm antigen test. In contrast, Case 2, a 7-year-old male entire free-roaming colony cat with a fractured right tibia tested positive on a rapid heartworm antigen test during pre-surgical screening, further confirmed by thoracic x-ray, but the cat did not present any respiratory signs. While microfilariae were not detected on peripheral blood smears in either cat, both were seropositive for *Dirofilaria* spp. anti-IgG. A duplex real-time PCR for the simultaneous detection of *D. immitis* and *D. repens* was performed and was positive for *D. immitis* DNA in Case 1, and negative in Case 2. Conventional PCR and sequencing of partial *cox1* and *12S* rRNA genes confirmed *D. immitis* infection in Case 1. Echocardiography displayed the presence of adult heartworms in the right heart and main pulmonary artery in both cats.

These two cases of feline cardiopulmonary dirofilariosis, confirmed on echocardiography, highlight the importance of i) considering *D. immitis* infection in the differential diagnosis of respiratory signs and/or vomiting in cats living in endemic areas, ii) performing *D. immitis*-screening in exposed cats that are not receiving heartworm prophylaxis. Overall, the imaging investigations performed proved to be crucial for reaching a definitive clinical diagnosis.

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O-11. GASTROINTESTINAL PARASITES OF SHELTER DOGS IN ISRAEL.

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Keywords: canines, endoparasites, Israel, toxocarosis.

Although the companion dog population is well-established in Israel, infection with gastrointestinal parasites may cause ill health in affected animals as well as humans including their owners. A cross-sectional prevalence study of endoparasite infection among Israeli shelter dogs from pounds in the north, center and south of Israel was performed by flotation and molecular PCR of sampled feces. Infection prevalence according to region of shelter, dog age and season was recorded and significant differences in parasite infection rates were analyzed between groups. Overall, 92 of 393 (23.4%) dogs surveyed were infected with at least one endoparasite. According to fecal microscopy, twenty-nine of 393 dogs (7.4%) were infected with *Cystoisospora* spp.; 28/393 (7.1%) with *Giardia intestinalis*; 4/393 (1.0%) with *Toxocara canis*; 12/393 (3.1%) with *Toxascaris leonina*; 10/393 (2.5%) with *Sarcocystis* spp.; 9/393 (2.3%) shed taeniid eggs; 1/393 (0.3%) *Spirocerca lupi* eggs; 1/393 (0.3%) hookworm spp. eggs and 1/393 (0.3%) shed *Hammondia heydorni* oocysts. After performing PCR screening for *Echinococcus granulosus* and *Taenia* spp., *Strongyloides* spp., and *Cryptosporidium* spp., 7/393 (1.8%) were diagnosed with *Taenia hydatigena*, and 2/393 (0.51%) with *Taenia* spp. eggs. Several epidemiologically significant points were identified; *Ta. leonina* was more prevalent in dogs from the north of Israel, while *Sarcocystis* spp. ($p = 0.035$) and *Taenia* eggs ($p=0.001$) were more prevalent in the south. In addition, infection with *Ta. leonina* was found to be significantly increased in summer compared to other seasons ($p= 0.017$). This is the first large scale study of endoparasites in dogs from Israel, with results showing presence of zoonotic parasites, such as *T. canis*, *Giardia* spp. and hookworms. These results should serve to increase awareness of veterinarians, human infectious disease specialists, and dog shelter personnel, of relevant parasites to plan better prevention, diagnosis and treatment schemes in dog shelters and to look for suggestive signs of endoparasite-related disease in a One Health framework.

O-12. ENTERIC HELMINTH INFECTION IMPAIRS THE ANTIGEN-SPECIFIC CD8+ T RESPONSE TO VACCINATION.

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Keywords: Helminth infection, vaccination, immune modulation

Current vaccines against viral diseases of both animals and human are primarily designed to elicit an effective antibody response, yet robust T lymphocyte activation and establishment of immune memory are crucial for a durable protection. Gastro-intestinal (GI) nematodes induce an immune-regulatory response, which has been associated with poor vaccine efficacy. Infections with GI nematodes are highly prevalent, affecting millions of people in (sub)tropical regions as well as companion animals, equids, and ruminants in northern regions. Despite increasing evidence that helminth infections impair vaccine responses [1], the mechanisms underlying this immunomodulation remain poorly understood.

To investigate how GI nematode infection influence the establishment of memory T cell responses to vaccination, we employed the mouse enteric nematode *Heligmosomoides polygyrus bakeri* (Hpb) and tested experimental vaccines, including a recombinant Vesicular Stomatitis Virus (rVSV) vector and an mRNA-based vaccine. Mice were first infected with Hpb, followed by intramuscular vaccination with either rVSV or mRNA, both encoding the model antigen chicken ovalbumin.

We observed that vaccination of Hpb-infected mice resulted in a significant reduction of the number of total and antigen-specific CD8⁺ T cells in peripheral blood and spleen, when compared to the vaccination of healthy mice. Furthermore, when vaccinated mice were challenged with a recombinant strain of murid gammaherpesvirus 4 expressing the same ovalbumin antigenic peptide, the recall of memory T lymphocytes was impaired in Hpb-infected mice, which also had an increased viral load.

Ongoing investigations aim to elucidate the mechanisms by which parasitic infections modulate vaccine efficacy. These findings hold important implications for enhancing vaccination strategies in broader populations, including companion animals, equines, and ruminants. Understanding how parasitic infections interact with immune responses could pave the way for developing targeted vaccination approaches, ultimately providing improved protection against both viral and parasitic diseases in diverse clinical contexts.

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O-13. GASTROINTESTINAL HELMINTH INFECTIONS IN DOGS IN SHEEP AND GOAT FARMS IN GREECE: PREVALENCE, INVOLVEMENT OF WILD CANID PREDATORS AND USE OF ANTHELMINTICS.

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Keywords: Anthelmintic treatment, Canid predator, Farm dog; Hookworm; Praziquantel

The objective was the investigation of gastrointestinal parasitic infections in dogs in small ruminant farms in Greece. This study was carried out in 444 small ruminant farms in Greece. Faecal samples were collected from the rectum of farm dogs and processed by a sedimentation-flotation technique.

Helminth eggs were detected in samples from 72.6% of the farms. The main helminth eggs detected were those of hookworms (*Uncinaria/Ancylostoma*) and *Toxocara canis*, in 68.6% and 51.3% of the farms, respectively. In our multivariable analyses, an association emerged between the presence of canid predators near a farm and the detection of these helminths in faecal samples: in 76% and 60% of the samples, respectively, versus in 58% and 39% of the samples from farms with no canid presence. Of farmers with dogs, 16.0% reported that they omitted the administration of anthelmintics to the animals. In multivariable analysis, the semi-extensive or extensive management system applied in the farm, the lower annual milk production per animal and the lack of collaboration with a veterinary practice were the significant predictors for the omission of anthelmintic administration to the farm dogs. There was also a clear association in the omission of anthelmintic administration to the dogs and to the livestock on the farm. The most frequently administered anthelmintic was praziquantel, which was used in 93.6% of the farms.

O-14. OCCURRENCE OF ANTIBODIES AGAINST ENCEPHALITOZON CUNICULI IN HORSES IN AUSTRIA.

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Keywords: equine, *Encephalitozoon cuniculi*, Microsporidia, antibodies, indirect immunofluorescent antibody test

Introduction

Infections with the microsporidian *Encephalitozoon cuniculi* occur in a wide range of mammalian and avian hosts. The development of clinical disease in different hosts is strongly influenced by natural innate species-specific susceptibility and the immune status of the individual. Infection in animals is usually subclinical but abortion, encephalitis and colic in horses have been described. Less is known about the infection rate of *E. cuniculi* in horses in general, and in Austria data is completely lacking. Therefore, this study was conducted to fill the data gap on the current seroprevalence of the pathogen in the Austrian horse population.

Methods

Clinically healthy horses were convenience sampled by veterinarians throughout Austria after public announcement of a study on infectious diseases in the Austrian horse population. Throughout Austria 588 horses were sampled. An in-house indirect immunofluorescence antibody test (IFA) was used to detect serum antibodies against *E. cuniculi* with a cut-off of 1:40.

Results

Of 588 tested horses, 211 (35.9%; CI95%=32.0-39.8) had an antibody titer against *E. cuniculi* of 1:40 or more, with maximum titers of up to 1:2560. The seropositive horses were located all over Austria.

Conclusion

Due to the unexpectedly high prevalence rates of antibodies against *E. cuniculi*, further studies are needed to address the seropositive horse population, especially with relevance to clinical diseases and diagnosis.

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O-15. WILD HOSTS, HIDDEN THREATS: TICK-HOST ASSOCIATIONS AND TICK-BORNE PATHOGENS IN WILD CARNIVORES FROM NORTHWESTERN ITALY.

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Keywords: Tick, carnivores, wildlife, tick-borne pathogens

Wild carnivores, living in environments populated by ticks, are continuously exposed to these ectoparasites throughout their lifespan. Additionally, their role as predators or scavengers, interacting with other mammals, further enhances the probability of tick transmission.

In this study, we characterized the tick fauna parasitizing wild carnivores and transmitted tick-borne pathogens (TBPs) in the Piedmont region (North-west Italy) in the years 2023-2024 (within project GR-2021-12374932). Ticks were morphologically identified [1] and molecularly analysed in pool for *Borrelia burgdorferi* sensu lato complex, spotted fever group (SFG) rickettsiae, *Anaplasma* spp., tick-borne encephalitis virus and *Coxiella burnetii* [2]. We examined 107 individuals, comprising 63 foxes, 27 badgers, 15 wolves and two stone marten, from which we identified 380 ticks, belonging to the genera *Ixodes* (97.6%), *Dermacentor* (1.3%), *Haemaphysalis* (0.5%) and *Rhipicephalus* (0.5%). *Ixodes hexagonus* and *I. ricinus* were the most frequently identified species. Among investigated TBPs, SFG rickettsiae were the most prevalent, accounting for an overall pool prevalence of 19.0% (95% CI= 13.0–26.3) out of

147 analysed pools. *Rickettsia helvetica* was found infecting both *I. ricinus* and *I. hexagonus*, while *R. monacensis* was detected only in *I. ricinus*. Infections by *C. burnetii* were detected in nine pools, involving pools of *I. hexagonus* (n= 6), *I. ricinus* (n= 2) and *Hae. erinacei* (n= 1). Only one pool of *I. ricinus* females tested positive to *B. burgdorferi* s.l., while no infections by tick-borne encephalitis virus and *Anaplasma* spp. were detected.

Our results highlight the diverse tick fauna parasitizing wild carnivores in Piedmont region, with *I. ricinus* and *I. hexagonus* identified as the most prevalent and widespread species, and a non-negligible prevalence of SFG rickettsiae. These findings demonstrate the use of wildlife as effective sentinels for tick surveillance and emphasize the need for targeted control strategies to mitigate the risk of tick-borne diseases.

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O-16. CYSTOISOSPORA SPP. INFECTIONS AND OTHER INTESTINAL PARASITOSEs IN YOUNG DOGS AND CATS WITH DIARRHEA.

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Keywords: coccidiosis, diarrhea, dogs, cats, copromicroscopy, clinical pictures

Data on the occurrence of different species of *Cystoisospora* in young dogs and cats with diarrhea is scant. This study has evaluated the presence of *Cystoisospora* spp. in diarrheic dogs (n. 117) and cats (n. 118) of <2 years.

For each animal, (i) a fecal sample was collected and examined *via* flotation and sucrose concentration, and (ii) a fecal and a clinical score were determined based on fecal consistency and clinical signs. Statistical associations between the occurrence of coccidia and/or other intestinal parasites and the severity of clinical signs were evaluated (binomial logistic regression, Fisher's exact test).

Seven dogs were positive for *Cystoisospora canis* (6.8%), 3 for *Cystoisospora ohioensis* (3.4%) and 1 (0.8%) for both species (0.9%), while 33 (28%) and 6 (5.1%) cats were infected with *Cystoisospora felis* and *Cystoisospora rivolta* respectively, and 1 with both (0.8%). *Giardia*, hookworms, *Trichuris vulpis* and roundworms, were recorded in 24 (20.5%), 11 (9.4%), 11 (9.4%), and 8 (6.8%) dogs, while ascarids, *Giardia*, cestodes and ancylostomatids were found in 34 (28.8%), 7 (5.9%), 4 (3.3%) and 3 (2.5%) cats.

All dogs infected by coccidia had very soft feces. Watery/hemorrhagic diarrhea was detected in 4/21 (19%) and 2/5 (40%) cats with monospecific infection by *C. felis* and *C. rivolta* respectively. Mild and moderate disease were respectively present in 2/3 (66.7%) and 1/3 (33.3%) dogs infected with *C. canis* only. Two dogs infected only with *C. ohioensis* showed mild disease and the dog co-infected with both displayed severe illness. In cats, 4/21 (19%), 13/21 (61.9%) and 4/21 (19%) with *C. felis* only infection displayed very mild, mild, and moderate disease, while 2/5 (40%) cats with monospecific infection by *C. rivolta* had a mild disease, and the remaining three had very mild, moderate and severe disease respectively. The Fisher's exact test revealed significant associations between reduced attitude and positivity to *C. canis* (p=0.021) and presence of very soft feces and positivity to Ancylostomatidae (p=0.007) in dogs. In cats, positivity to hookworms was associated with vomitus (p=0.04) dehydration (p=0.009) and watery/hemorrhagic diarrhea (p=0.007).

This study highlights the frequent occurrence of *Cystoisospora* spp. in young dogs and cats with diarrhea, with a greater incidence in cats. A higher pathogenicity of *C. canis* and *C. rivolta* in dogs and cats respectively is here suggested.

O-17. THREE VETERINARY CASES OF ABERRANT PARASITE LOCATIONS.

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Keywords: Liver fluke, horse strongyle, oestrids, uncommon parasite presentations

Dicrocoelium dendriticum in a Japanese Chin dog

Dicrocoelium dendriticum can appear as gastrointestinal passage of eggs in dogs. A Japanese Chin dog presented with diarrhoea and weight loss, with *D. dendriticum*-like eggs found in faeces. Infection was confirmed, ruling out other possibilities. Treatment with a high dose of praziquantel (100 mg/kg BW) resulted in negative faecal samples and resolution of clinical signs.

Strongylus vulgaris in the hoof abscess of a donkey

Strongylus spp. rank amongst the most pathogenic internal parasites in equids. A donkey presented with lameness and a single specimen of *Strongylus vulgaris* was recovered from a hoof abscess. *S. vulgaris* infection was confirmed, necessitating anthelmintic treatment. Follow-up examinations showed sustained parasite control.

Oestrus ovis and Cephemyia stimulator in dogs

Oestrus ovis and *Cephemyia stimulator* infestations in domestic dogs are rare and poorly documented. We report two cases of botfly infestations in dogs presenting symptoms such as sneezing, coughing, and choking. Endoscopic examination revealed larvae in nasal cavities and larynx with parasitological/molecular analysis confirming infestations by *Oestrus ovis* and *Cephemyia stimulator*. Treatment included Moxidectin (2.5 mg/kg BW) and Imidacloprid (10 mg/kg BW) in a spot-on formulation (Advocate®). Clinical signs eventually resolved with additional supportive care.

O-18. BIRAGO PROJECT - WHAT HAS HAPPENED FORTY YEARS AFTER THE INTRODUCTION OF MACROCYCLIC LACTONES IN A HISTORICALLY ENDEMIC AREA FOR DIROFILARIA IMMITIS?

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Keywords: *Dirofilaria immitis*, *Dirofilaria repens*, Epidemiology, Prevention

The first description of *Dirofilaria immitis* in dogs was made by Birago in 1626, a nobleman living in Northern Italy. Since the 1980s, scientific studies have identified Northern Italy, particularly the area around the Po River, as a highly endemic region for heartworm disease, with prevalences ranging from 31% to 98%. After the introduction of ivermectin in 1988 and other macrocyclic lactones in the following years, parasitic pressure gradually decreased, leading to a reduction in the prevalence of heartworm disease in this area. However, in recent years, this reduction has led to some practitioners to pay less attention to the disease, including in terms of diagnosis and prevention.

The aim of the study was to assess, approximately 40 years after the introduction of macrocyclic lactones preventives, the prevalence of *D. immitis* in Northern Italy.

The inclusion criteria were stray dogs or those without microchips referred to shelters or veterinary facilities, older than 10 months, and untreated with macrocyclic lactones for heartworm prevention. Approximately 2 ml of blood was collected from each dog. An identification form was completed for each animal (location of recovery, age, breed, sex, weight, clinical signs). Each blood sample was analyzed using Knott's test and an antigen test.

Data from 510 dogs examined with Knott's test, from Lombardy and Emilia-Romagna, showed a *D. immitis* prevalence of 27%. In particular, 16% were positive for *D. immitis*, 7% for *D. repens* and 11% mixed infection. The antigen prevalence was 31%.

The data show that even 30 years after the introduction of chemoprophylaxis, in a historically endemic area such as Northern Italy, parasitic pressure has remained high. These data underline the importance of continuing to make a correct diagnosis and prevention for *D. immitis* and also for *D. repens*, and how practitioners must not lower their attention towards these parasites.

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O-19. DIROFILARIA IMMITIS AND ANGIOSTRONGYLUS VASORUM IN WILD CARNIVORES FROM ROMANIA: OVERVIEW AND NEW DATA.

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Keywords: *Angiostrongylus vasorum*, carnivores, *Dirofilaria immitis*, Romania, wild carnivores, zoo

Angiostrongylus vasorum and *Dirofilaria immitis* are two important nematodes affecting carnivores with significant health implications. Wild carnivores serve as important reservoir hosts for *A. vasorum* and to a lesser extent also for *D. immitis*, being responsible for the spreading and maintenance of their life cycles. Considering the increasing overlap between domestic and wild animals due to urbanisation, surveillance of these parasites in wildlife is important for assessing their epidemiological impact on domestic animals or human health.

In Romania, the first data concerning *Dirofilaria* spp. in wild carnivores dates back to 1935, when it was reported in red foxes (*Vulpes vulpes*) and gray wolves (*Canis lupus*), but with no prevalence values given, or species mentioned. In later studies (after 2014), *D. immitis* was reported in red foxes (0.33%), golden jackals (*Canis aureus*, 9.26%), wildcats (*Felis silvestris*, 10%), raccoon dogs (*Nyctereutes procyonoides*, single case report), one Eurasian badger (*Meles meles*, 0.87%) and one otter (*Lutra lutra*, 16.67%). The French heartworm was reported for the first time in red foxes (4.2%) from Romania in 2017.

Considering these, the aim of this report was to investigate the diversity of wild animal species infected by these two nematodes and to determine the current prevalence and distribution.

A total of 92 wild carnivores were examined by necropsy, including canids (22 red foxes, 9 golden jackals and 1 gray wolf), felids (n=4), mustelids (10 badgers, 5 otters, 8 small mustelids), and ursids (n=30). Additionally, 1 leopard and 2 lions originating from zoos were necropsied as well. Fecal samples were collected from 35 captive wild carnivores originating in 5 different zoos and examined using the larval concentration method.

D. immitis was identified by necropsy in red foxes (9%), golden jackals (33.33%), one brown bear (*Ursus arctos*, 3.33%) and a leopard. *A. vasorum* was identified by necropsy in red foxes (27.27%), golden jackals (55.55%) and in the wolf. One red fox was co-infected with both *D. immitis* and *A. vasorum* (4.54%). Among zoo animals, *A. vasorum*-like larvae were identified in feces collected from the only wolf examined (100%), originating from Târgu Mureș Zoo.

Understanding the epidemiological role of wild carnivores in the transmission of *A. vasorum* and *D. immitis* is essential for the development of effective control strategies and for reducing risks in both veterinary and public health contexts.

O-20. LONG-TERM SURVEILLANCE OF DIROFILARIA IMMITIS IN WILD CARNIVORES FROM SERBIA (2013–2025).

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Keywords: *Dirofilaria immitis*, Serbia, wild carnivores, golden jackal

Introduction:

Dirofilaria immitis is a parasitic nematode primarily affecting domestic dogs but also found in wild carnivores. This study builds on previous findings ¹, to further assess the prevalence of *D. immitis* in wild carnivores in Serbia and explore potential transmission risks to domestic dogs.

Methods:

A total of 962 wild carnivore hearts were obtained in collaboration with hunters or as a roadkill. The hearts were macroscopically examined, and the *Dirofilaria immitis* specimens from the positive hosts were stored in ethanol for further analysis.

Results:

During the research period from 2013 to 2025, total of 82 wild animals were positive for *D. immitis*. Infected species included 76 golden jackals (*Canis aureus*)², 1 wolf (*Canis lupus*), 1 otter (*Lutra lutra*)³, and 4 red foxes (*Vulpes vulpes*). No infection was found in beavers, wild cats, beech martens, pine martens, badgers, weasels, or brown bears. A higher prevalence in golden jackals was observed, likely due to their habitat preferences in areas of lower altitudes, and riverine regions where mosquito vectors are abundant.

Conclusions:

The high prevalence of *D. immitis* in golden jackals suggests that they may represent an important reservoir for the parasite, especially in Serbia, where jackals have preference toward anthropogenically modified habitats. These habitats, which are often located in lower altitudes, and riverine areas, likely provide an abundance of mosquito vectors that facilitate the transmission of *D. immitis*. This finding highlights the increased risk of transmission to domestic dogs, particularly in regions with high jackal populations, and emphasises the need to monitor *D. immitis* in both wildlife and domestic dogs to prevent zoonotic transmission.

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O-21. DIROFILARIA IMMITIS IN A RED FOX (VULPES VULPES) IN AUSTRIA – IS D. IMMITIS ESTABLISHING IN EASTERN AUSTRIA?

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Keywords: *Dirofilaria immitis*, red fox, Austria

Dirofilaria repens and *D. immitis* are filarioid helminths with domestic and wild canids as main hosts and mosquitoes as vectors. They are causative agents of zoonotic diseases. Both *D. immitis* and *D. repens* are known as potential invasive species, and their distribution seems associated with climate change. Until very recently, both species were nonendemic in Austria (Fuehrer et al. 2016; Fuehrer et al. 2021). The first autochthonous case of *D. immitis* was documented in a cat in the most easternmost province of Austria, Burgenland, in 2020 (Kulmer et al. 2021). Since then no further autochthonous cases were reported which can be associated with the way of dog keeping in Austria (dogs are not staying outside overnight).

In January 2025 a male red fox (*Vulpes vulpes*) was shot in the region of Parndorf – province of Burgenland approx. 20 km from the Hungarian border. At necropsy a single male adult *D. immitis* was found. The parasite was specified morphologically and with molecular tools. Moreover, this worm will be included in a genome project and further analysed.

Cases of the canine heartworm *D. immitis* in Austria are still limited to imported cases in dogs but our findings in a red fox and cat indicate its spread from Hungary to Austria. Moreover, establishment in Austria is discussed as realistic with the establishment of new competent day-biting mosquito vectors – tiger mosquitoes (*Aedes albopictus*) which establishment process is ongoing in Austria.

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O-22. DETECTION RATES OF DIROFILARIA IMMITIS AND OTHER CANINE FILARIAL INFECTIONS IN GERMANY (2019-2023.)

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Keywords: *Dirofilaria immitis*, *D. repens*, heartworm, Germany

Canine heartworm and other canine filarial are emerging pathogens that have been detected more frequently in previously non-endemic areas. In Central Europe, migration of wildlife host species, relocation of dogs from endemic regions and climate change contribute to this phenomenon. The present study aimed to estimate the occurrence of *D. immitis* infections in dogs in Germany, where autochthonous heartworm infections have not yet been reported.

A total of 15,997 serum samples and 9,099 blood samples from dogs from Germany were submitted to a private diagnostic laboratory for *D. immitis* antigen detection as well as a modified Knott test between 2019 and 2023. Furthermore, the species specific detection of *D. immitis* DNA as well as other canine filarial DNA (*D. repens*, *Acanthocheilone-ma reconditum* A. *dracunculoides*), which is consistent with microfilaria in the peripheral blood, was carried out by real-time PCR on 5,990 canine blood samples in the years 2022 and 2023. Information on travel history of the patient was not available with the laboratory submissions. However, a travel history to and/or from endemic areas of the patient was usually the reason for sample submission.

In total, heartworm antigen was detected in 661 (4.13%) serum samples and microfilaria were detected in 248 (2.73%) blood samples. Both tests were carried out simultaneously on samples from 8,560 animals with 391 (4.57%) cases of positive antigen detection. Among those submissions microfilaria were detected in 123 (1.44%) samples. In 2022 and thereafter the Knott test was almost completely replaced by filarial DNA detection in blood samples. The heartworm antigen detection as well as the simultaneous detection of filarial DNA including a PCR specific for *D. immitis* were carried out in samples from 5,808 animals, yielding 200 (3.44%) antigen positive samples and 87 (1.50%) samples, which were positive in both testing methods, heartworm antigen and *D. immitis*-PCR. Among other canine *Dirofilaria* spp., microfilaria of *D. repens* were detected in 126 cases.

These data show that a substantial number of animals with filarial infections reside in Germany. This, in conjunction with the presence of the vectors and climatic conditions that support larval development in Germany underline the importance of filarial testing schemes, especially in animals with a travel history.

O-23. EMERGING HUMAN DIROFILARIA REPENS INFECTIONS: NEW CASES IN SOUTHERN ITALY.

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Keywords: Human dirofilariosis; southern Italy; zoonotic infection; *Dirofilaria repens*-gravid female;

Dirofilaria repens is the primary cause of human dirofilariasis in the Old World, with Italy reporting the highest number of cases in Europe. This study describes two recent infections identified in southern Italy, where canine dirofilariasis is highly endemic.

The first case involved a 33-year-old man from Caserta (Campania) who presented with a subcutaneous mass on the upper eyelid. Surgical excision revealed an immature female *D. repens* measuring 14 cm, with no microfilariae detected in either the worm's uterus or the patient's peripheral blood. The second case was a 67-year-old man from Pozzuoli (Metropolitan City of Naples) who had an oval subcutaneous nodule on the left frontal scalp. A live gravid female worm measuring 15–16 cm was extracted, with no peripheral microfilaremia and no male worm identified. PCR analysis confirmed a 100% sequence identity with *D. repens*, and both patients tested positive for *D. repens* IgG antibodies.

These cases emphasize the ongoing spread of human dirofilariasis in southern Italy and highlight the diagnostic challenges posed by its diverse clinical presentations. The presence of a gravid female worm in the absence of microfilaremia hints at potential complexities in the parasite's life cycle within humans, raising questions about whether humans truly serve as dead-end hosts.

Given the increasing prevalence in both humans and dogs, a comprehensive epidemiological investigation is warranted. Furthermore, classifying dirofilariasis as a notifiable disease would enhance case detection and tracking, ultimately improving monitoring and control of this zoonotic threat.

O-24. PERSISTENCE OF DIROFILARIA REPENS MICROFILAREMIA IN A PATIENT FROM CROATIA.

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Keywords: *Dirofilaria repens*, human, microfilaremia

Humans are considered accidental hosts in which *Dirofilaria repens* rarely reaches sexual maturity. Previous studies have demonstrated the presence of microfilariae, but the persistence of microfilariae in blood has never been demonstrated. In the present report, we describe a case of persistent microfilaremia of *D. repens* in a 54-year-old patient from the Croatian coastal area. In January 2024, the patient underwent surgery for a nodule on the left forearm in which a parasite was described histologically and was discharged home after surgery. In April, she had an eosinophilia of 14.2% and was treated with albendazole for seven. Due a rash that travelled over the body for about a week and was accompanied by fatigue and myalgia, the patient was admitted to the Infectious Diseases Hospital in Zagreb on August 27, where she again showed an eosinophilia of 20%. Examination of the thick drop of blood revealed three microfilariae that morphologically matched those of *D. repens*. In October, *D. repens* was confirmed by PCR and sequencing of the COI gene segment from the paraffin block of the nodule collected in January and *Wolbachia* sp. was confirmed as the endosymbiont of *D. repens*. At the second control examination on October 24, one microfilaria per ml of blood was detected using the Knott method, and later on December 6, one microfilaria per 3 ml of blood was detected. During this time, the patient described feeling as if something was moving and tickling all the time. The patient was treated with doxycycline for two weeks, and a follow-up examination of five ml of blood using the Knott test on January 15, 2025, revealed no microfilariae.

It is not entirely clear whether the microfilaremia was present from February, when the parasite was removed, but it was certainly present from the end of August until December 6. However, with this case we have shown that microfilariae can persist in human blood, albeit in low numbers.

O-25. HUMAN CASES OF DIROFILARIASIS IN NORTHEASTERN EUROPE.

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Keywords: *Dirofilaria repens*, zoonosis, dirofilariasis, co-infection

Northeastern (NE) Europe became one of the new regions of *Dirofilaria* spp. expansion in the beginning of 21st century. *Dirofilaria repens* has successfully spread in Poland and Lithuania and became endemic in these countries for the last 15 years. Similarly, the human cases started to appear in these countries.

The aim of this review was to assess if dirofilariasis in humans is a constant problem in NE Europe despite the control of *Dirofilaria* spp. infection in dogs.

Review of literature has yielded the increasing number of reports on human cases in Poland (31) and Lithuania (11-20). In addition to known localizations and manifestations, also new localization were reported, and new manifestations (i.e. MALT lymphoma). Also co-infections by more than one *D. repens* nematode were reported recently from Poland.

Conclusion: Despite the increasing awareness and decreasing prevalence in owned dogs, the number of human cases rises constantly in NE Europe.

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O-26. HUMAN EXPOSURE TO DIROFILARIA IMMITIS FOLLOWING A CANINE HEARTWORM DISEASE ELIMINATION PROGRAM IN LINOSA ISLAND.

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Keywords: *Dirofilaria immitis*, ELISA, Humans, Italy.

A new epidemiological scenario for *Dirofilaria immitis* was recently described in Italy with highly endemic foci of heartworm disease (HWD) detected in southernmost regions. Linosa island (Sicily, Italy) represents a paradigmatic example of that scenario, with previous reports showing *D. immitis* infection in 58.9% of dogs and a parasitic exposure in 7.9% of islanders. These findings prompted a targeted elimination program, leading to HWD eradication by 2022 (1). This study aims to assess antibody response and kinetics among Linosa residents, one year after implementing the canine HWD elimination program.

In 2023, blood samples were collected from 97 volunteer residents and 39 dogs. Participants completed a questionnaire with demographic and clinical data, including symptoms and relevant risk factors. Human sera were tested for *D. immitis* exposure using an in-house ELISA (2). Dog samples were analyzed using Knott's test and a commercial ELISA rapid test (SNAP 4DX, IDEXX). Additionally, previously infected dogs underwent echocardiographic evaluation.

Genomic DNA was extracted from all human and dog samples and tested by PCR using *Dirofilaria* spp. and *Wolbachia*-specific protocols (2).

Serological analyses revealed 24.7% of the volunteers seropositive for *D. immitis*, marking an increase of seropositive people compared to 2020 (8%).

None of human and dog samples tested positive for *Dirofilaria* spp. or *Wolbachia* by PCR.

Despite the successful elimination of *D. immitis* in dogs, the increased seroprevalence in humans suggests a stable level of human exposure even in the absence of canine infection. It is possible that some of the new seropositive cases may represent previously exposed individuals who had not yet seroconverted at the first testing in 2020. Conversely, it is also plausible that some of the new seropositives resulted from recent exposure to infected mosquito bites. Long-term studies on antibody persistence and vector surveillance are needed to improve elimination efforts.

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O-27. PROGNOSTIC FACTORS IN CANINE ANGIOSTRONGYLOSIS COMPLEMENTED WITH A QUANTITATIVE SYNTHESIS OF THE EXISTING DATA ON CLINICAL SIGNS.

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Keywords: *Angiostrongylus vasorum*, prognostic factors, laboratory parameters, pulmonary surfactant-associated protein B, quantitative meta-analysis

Canine angiostrongylosis, a potentially life-threatening parasitic disease, is widespread in Europe. While reliable diagnostic tools are available, there are limited prognostic variables.

We investigated the relationship between laboratory findings and clinical manifestations in *Angiostrongylus vasorum*-infected dogs presented at the University Animal Hospital of the Vetsuisse Faculty in Zurich, aiming to identify factors linked to disease severity and outcomes. In addition, serum samples from 71 naturally and 12 experimentally infected dogs and 16 wild red foxes were analysed for pulmonary surfactant-associated protein B (PSAPB) and chitinase 3-like protein 1 (CHI3L1) using commercial ELISA kits because quantitative proteomics had revealed that these proteins were upregulated in *A. vasorum* affected dogs.

Infected dogs exhibited a variety of clinical signs, with respiratory symptoms being the most common (62.0%), followed by nonspecific signs (46.5%), bleeding disorders (42.3%), neurological symptoms (17.0%), and cardiovascular abnormalities (9.9%). A comparison with the literature revealed higher frequencies of bleeding, neurological, and gastrointestinal signs in referral cases. Notably, a combination of respiratory and nonspecific signs was frequently observed in this study. The presence of nonspecific signs, often indicative of systemic inflammatory processes, suggests a more severe disease course. Blood analyses revealed that non-survivors had lower haemoglobin and higher eosinophil and alanine transaminase levels compared to survivors, reflecting different facets of the inflammatory response. These parameters demonstrated moderate to strong prognostic value, with area under the curve (AUC) values exceeding 0.7. Elevated PSAPB levels were detected in infected dogs, especially non-survivors, highlighting its potential as a prognostic marker (AUC 0.7). CHI3L1 levels were higher in infected dogs but showed limited prognostic potential (AUC 0.6).

These findings underscore the importance of integrating clinical signs, haematological markers, and serum biomarkers in the assessment of canine angiostrongylosis. Therefore, we will complement the existing knowledge of clinical data on angiostrongylosis in dogs with a quantitative analysis.

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O-28. ANGIOSTRONGYLUS VASORUM COMPROMISES ENDOTHELIAL INTEGRITY AND VASCULAR HEALTH.

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Keywords: *Angiostrongylus vasorum*; transcriptome; coagulopathies; vascular endothelium; vascular integrity

Background: Dogs infected with the lung- and heartworm *Angiostrongylus vasorum* are at high risk to die because of the associated bleeding pathologies. Our knowledge of the pathomechanisms of canine angiostrongylosis is limited and the clinical management mostly relies on case studies. Hence, to date, the elements we have are not sufficient to understand, prevent, and efficiently manage coagulopathies due to *A. vasorum*.

Methods: Since blood is also “the pipeline of the immune system”, we compared the blood transcriptomic profiles from i) *A. vasorum* naturally infected dogs presenting hypocoagulability (Infhypo; n = 5), ii) infected dogs showing normal coagulation values (Infnorm, n = 5), and iii) uninfected animals (UC, n = 12). In addition to classical hematology and coagulation parameters, including rotational thromboelastometry, a global blood cell transcriptomic profile was generated, allowing identification of differentially expressed genes following infection and coagulation status.

Results: Fibrinogen levels were significantly lower in Infhypo than in Infnorm. All Infhypo dogs showed out-of-range prothrombin times. Matrix metalloprotease transcripts were strongly upregulated among infected dogs, regardless of their coagulation status; excessive metalloproteinase activity might amplify vascular disruption and inflammation. Analysis of downregulated transcripts in infected animals compared to UC suggests a loss of endothelial integrity, especially at the level of the lungs. Endothelial injury is a known trigger of disseminated intravascular coagulation and consumption of coagulation factors, which may ultimately lead to coagulation disorders.

Conclusion: Our data suggests that vascular endothelial integrity might be compromised, along with an enhanced metalloproteinase activity, and both likely play decisive roles in the development of coagulopathies. Our study supports both some form of DIC – perhaps sustained by excessive metalloproteinase activity - and hyperfibrinolysis to explain *A. vasorum*-induced coagulopathies.

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0-29. SUCCESSFUL TREATMENT OF ANGIOSTRONGYLUS VASORUM INFECTIONS IN DOGS AFTER A SINGLE ADMINISTRATION OF A FLURALANER, MOXIDECTIN, AND PYRANTEL CHEWABLE TABLET.

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Keywords: lungworm, treatment, moxidectin

Two masked, randomized and GCP-compliant dose confirmation studies were conducted to evaluate the efficacy of an oral combination product for dogs at the target dose of 10 mg fluralaner + 0.025 mg moxidectin + 5 mg pyrantel/kg

of body weight (BW) in the treatment of infections with *Angiostrongylus vasorum* after artificial inoculation of dogs with third stage larvae (L3). In the two studies, 20 and 16 dogs were inoculated with 250 or 200 L3 on Day -57 or -60, respectively. On Day 0, dogs in group 1 were treated with the combination product at the target dose and dogs in group 2 were left untreated. Before inoculation, before treatment (for randomization) and weekly after treatment, all dogs were evaluated for lungworm infection: fecal larvae examinations (Baermann technique), clinical and respiratory examinations, and antigen and antibody blood test for the detection of *A. vasorum* infection.

In both studies, an adequate infection was achieved in both study groups pre-treatment and in the control group post-treatment. The reduction of fecal larvae count based on geometric means was >99.9% in weeks 3 and 4 after treatment with significantly different mean fecal larval counts between groups ($p < 0.0001$). Antibody and antigen titers as well as respiratory assessments were consistent with the larval count results. Most adverse events were observed in the control group and related to lungworm infection.

In conclusion, in these studies a single administration of the fluralaner, moxidectin, and pyrantel combination chewable tablet was safe and effective in the treatment of *A. vasorum* infections in dogs as determined 3 and 4 weeks after treatment.

O-30. SUCCESSFUL PREVENTION OF ANGIOSTRONGYLOSIS IN DOGS AFTER A SINGLE ADMINISTRATION OF A FLURALANER, MOXIDECTIN, AND PYRANTEL CHEWABLE TABLET.

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Keywords: lungworm, prevention, moxidectin

Two GCP-compliant studies were conducted to determine the dose and confirm the efficacy of a fluralaner (FLU), moxidectin (MOX) and pyrantel (PYR) chewable tablet to prevent angiostrongylosis after artificial inoculation of dogs with third stage larvae (L3). In a dose determination study, 32 dogs (n=8 per group) were inoculated once with approximately 200 infective *Angiostrongylus vasorum* L3 larvae each on study day (SD) -31. Dogs of Groups 1, 2 and 3 were treated orally on SD 0 (31 days post inoculation) at the target doses of 5 mg/kg FLU+ 0.0125 mg MOX/kg + 2.5 mg/kg PYR (Group 1), 10 mg/kg + 0.025 mg/kg + 5 mg/kg (Group 2), and 20 mg/kg + 0.05 mg/kg + 10 mg/kg (Group 3). Eight dogs remained untreated and served as the control (Group 4).

The infection was considered adequate as at least five adult worms were recovered at necropsy on SD 33, 34 and 35 (64 to 66 days post inoculation) from all eight control dogs. Efficacy based on geometric means (arithmetic means) was 92.2% (90.8%), 99% (98.7%), 100% (100%) for Groups 1, 2 and 3, respectively.

Fecal larval counts, antibody-, antigen titers, and clinical findings confirmed efficacy. All three doses were safe and effective at preventing infections with *A. vasorum*.

The target dose of 10 mg/kg FLU + 0.025 mg MOX /kg + 5 mg/kg PYR was confirmed in a second study with non-terminal endpoints: where the primary efficacy (reduction of fecal larvae count) was above 99% in the treated group based on geometric mean ($p < 0.0001$). Secondary parameter results (antigen and antibody ELISAs, Computer tomography and respiratory assessments) confirmed efficacy.

In conclusion, a single oral administration of a chewable tablet at a dose rate of 10 mg/kg FLU, 0.025 mg/kg MOX and 5 mg/kg PYR was safe and efficacious in preventing canine angiostrongylosis.

O-31. RAINFALL AND TEMPERATURE DRIVEN EMERGENCE OF NEURAL ANGIOSTRONGYLOSIS IN EASTERN AUSTRALIA, 2020-2024.

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Keywords: Rat lungworm, *Angiostrongylus cantonensis*, climate, canine, qPCR, ELISA, Australia

Neural angiostrongyliasis (NA), caused by rat lungworm (*Angiostrongylus cantonensis*), is an emerging zoonotic disease on Australia's east coast. The number of cases has risen since 2010. This study investigated the spatial and temporal dynamics of canine NA (CNA) and the genetic diversity of *A. cantonensis* in affected dogs. We analysed cerebrospinal fluid samples from clinically suspected cases (2020-2024, n = 180) using AcanR3990 qPCR, confirming the disease in 93 cases. Cases occurred around Brisbane and Sydney, with peak incidence in 2022 (32 cases). Multiple logistic regression modelling demonstrated that CNA incidence depends on immediate and long-term rainfall (1, and 10-12 month lags) and medium-term temperature changes (5-7 month lags). Partial cox1 sequencing revealed Ac13 as the dominant haplotype (9/15). Comparison with an established ELISA showed substantial agreement ($\kappa = 0.66$) across 50 randomly selected samples. With many cases likely remaining undiagnosed, NA poses an ongoing One Health issue in Australia.

O-32. MICROSCOPIC LESIONS IN RATS NATURALLY INFECTED WITH ANGIOSTRONGYLUS CANTONENSIS MIRROR THAT OF DIROFILARIA IMMITIS IN DOGS.

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Keywords: rat lungworm, heartworm, canine, rat, histology

Angiostrongylus cantonensis, the Rat Lungworm (RLW), is a metastrongyloid nematode using rats as definitive hosts, with adults in the right ventricle and pulmonary arteries. Rats have been shown to harbor large numbers of worms before health is severely impacted, with multiple organ damage caused by infections. In 2024, rats from Florida, United States, were collected and evaluated for the presence of RLW. Hearts and lungs were removed and evaluated grossly and histologically for the presence of adult and larval worms. Grossly, 2-54 entire or fragmented adult worms were recovered from the right ventricle and pulmonary artery of 4 naturally infected rats. Microscopically, the lungs of each rat had numerous larvated ova and first stage larvae (L1) embedded throughout the pulmonary capillaries and arterioles, with associated, marked granulomatous to lymphoplasmacytic interstitial pneumonia and perivascularitis. Large pulmonary arteries exhibited a spectrum of changes including moderate to severe villous myointimal proliferation, medial hypertrophy and fibrosis, arteritis, and occasional thrombosis in response to luminal nematode adults. The arterial findings mirror those in dirofilariasis (i.e. *Dirofilaria immitis*, canine heartworm), in which this distinct intimal proliferation and atherosclerotic changes are the hallmark of disease and representative of significant pulmonary hypertension. The similarities between the two parasites may provide a better understanding of angiostrongyliasis in the rat host.

O-33. COTTON-TOP TAMARINS (SAGUINUS OEDIPUS) MAY DEVELOP-FATAL INFECTIONS AND ARE DEFINITIVE HOSTS OF THE METASTRONGYLOID RODENT PARASITE ANGIOSTRONGYLUS DUJARDINI.

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Keywords: Lungworms, Non-human primates, Definitive host, *Angiostrongylus dujardini*

Angiostrongylus dujardini inhabits primarily the pulmonary arteries and the right heart of murid and cricetid rodents. Female worms produce eggs that embolize and embryonate in the pulmonary capillaries, in which first-stage larvae (L1) hatch, cross into the alveoli and are carried up along the airways, swallowed, and shed with faeces. L1 are ingested by gastropod intermediate hosts, mainly aquatic snails, in which they develop into infective L3. *A. dujardini* infections have caused the death of captive Callitrichid monkeys and meerkats in zoos from France and Italy. However, no shedding of L1 was observed in these species as it occurs in rodents; therefore, they were so far considered as accidental hosts.

A 5.5-year-old female cotton top tamarin born in captivity in Swiss zoo was found dead in the enclosure without previous clinical signs. At necropsy, multifocal-confluent pulmonary reddening was observed. Histological examination of the lungs revealed multifocal-confluent, large, granulomatous areas composed of numerous epithelioid macrophages, multinucleated giant cells, lymphocytes, plasma cells, and fewer neutrophils, surrounding and accompanying necrotic areas admixed with hemorrhages involving alveoli and interstitial spaces. Numerous larvae and embryonated eggs were observed in the alveoli, bronchioles, and some blood vessels within these areas. Coproscopical analysis by flotation and Baermann methods revealed metastrongylid larvae. A PCR targeting the ITS2 plus 5.8S and 28S rDNA flanking regions of lungworms was performed on these larvae. Sequencing of the amplified product showed 99.6% (481 bp) identity with GenBank sequences of *A. dujardini*. The percentage of identity with sequences from other *Angiostrongylus* species was lower than 82.2%. *A. dujardini* has been recently found in a coconut lorikeet (*Trichoglossus haematodus*) from the same zoo.

Conclusion: This is the first report of *A. dujardini* infection with shedding of larvae in a non-human primate, confirming that they may act as definitive hosts of this parasite.

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O-34. EVALUATION OF THE EFFICACY OF PERMETHRIN AND FIPRONIL (FRONTLINE TRI-ACT®) SPOT-ON IN REDUCING THE TRANSMISSION OF DIROFILARIA IMMITIS IN DOGS UNDER NATURAL PARASITIC EXPOSURE.

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Keywords: *Dirofilaria immitis*, dogs, prevention, permethrin

Introduction: *Dirofilaria immitis* represents a significant threat to canine and feline health worldwide. It is one of the most common canine mosquito-borne diseases and is endemic in the Mediterranean region. This study aims to evaluate the efficacy of a topical combination of permethrin and fipronil in reducing *D. immitis* transmission to dogs under natural field exposure in Greece.

Methods: The study included 24 Beagle dogs negative for heartworm infection, 12 as a negative control group, and 12 as a treated group. All were housed in similar conditions, and naturally exposed to flying insects in a high endemic region for heartworm disease in Northern Greece. Treated dogs received Frontline Tri-Act® spot-on administration every 28-days interval during the mosquito season from May to November (i.e. Phase 1 of the study). Control dogs remained untreated. After the 7-month exposure period, all dogs were monitored monthly with health check and blood sample collection used for *Dirofilaria* spp. antigen test and mod. Knott test for microfilariae circulation for a period of 5 months from December to April (Phase 2 of the study). The follow-up period was designed to detect all dogs that could be infected from the start to the end of the phase 1, considering the prepatent period of *Dirofilaria immitis*. Despite low risk of mosquito activity during that winter phase, all dogs in both groups were fitted with a deltamethrin collar and their environment was sprayed with insecticides.

Results: All dogs in the Frontline Tri-Act[®] group remained *Dirofilaria immitis* free during the whole period of the study while a significant number of dogs in the negative control group tested positive (IDEXX 4Dx and mod. Knott) during the second phase of the study, corresponding to an infection during the mosquito season.

Conclusions: This study confirmed a significant prevention of the risk of *D. immitis* thanks to the repellent activity of permethrin against mosquitoes in the tested combination. It suggests that Frontline Tri-Act[®] spot-on can be used in prevention programmes against canine heartworm disease.

O-35. POPULATION GENOMICS REVEALS AN ANCIENT ORIGIN OF HEARTWORMS IN CANIDS.

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Keywords: Glaciation refugia, Human-mediated dispersal, Dog domestication, Whole-genome sequencing, SNP, disease

Heartworms (*Dirofilaria immitis*) are parasitic nematodes causing significant morbidity and mortality in canids worldwide. These parasites are widely believed to have dispersed globally with domesticated dogs. To test this theory, we conducted the largest population genetics study of heartworms to date, using whole-genome sequencing data for 127 modern adult individuals collected from mammalian carnivore hosts across four continents. We reveal distinct genetic differences between continental populations, indicating a deeper, more ancient origin and dispersal of heartworms in canids than previously recognised. We identify an Asian origin for Australian heartworms, consistent with the arrival

of dingoes thousands of years ago. Additionally, we find genetic relatedness between European and Central American heartworms, likely reflecting modern dispersal via domesticated dogs during the European colonisation of the Americas. In short, our findings shed light on the global population dynamics and evolutionary history of heartworms, which can aid future surveillance and control efforts for this important parasite.

O-36. NEW INSIGHTS INTO THE PHYLOGEOGRAPHIC HISTORY OF DIROFILARIA IMMITIS IN THE CANARY ISLANDS.

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Keywords: *Dirofilaria immitis*, PCR, Dogs, Gran Canaria (Spain), Phylogeny.

Dirofilaria immitis, a nematode that causes heartworm disease, is transmitted by mosquito vectors such as *Culex* spp., *Aedes* spp., and *Anopheles* spp.. It is prevalent worldwide, with increasing cases in Europe, particularly due to factors like globalization, pet movement, and climate change. In Spain and Portugal, the disease has high incidence variability, with islands like Gran Canaria showing particularly high prevalence rates. Although control measures have reduced the impact, reservoirs of the disease persist, necessitating ongoing monitoring. Diagnostic techniques, including serology and molecular methods, are crucial for identifying infections in hosts and vectors. Despite advancements in molecular diagnostics, genetic markers need further optimization to improve the understanding of the disease's impact and predict future transmission patterns. Previous studies on the genetic diversity of *D. immitis* have shown low variability, but more research is needed in hyperendemic areas like Gran Canaria to better understand the disease's evolution [1]. This study aims to analyze the genetic diversity in *D. immitis* from infected dogs in Gran Canaria, using mitochondrial and nuclear markers. Following primer optimization, we generated >100 new sequences and >40,000 bp from 21 worms present in different dogs across veterinary clinics. The genetic diversity and structure are low at the global level if we compare it with the results obtained for its sister species *D. repens* [2]. On the island of Gran Canaria, new minor haplotypes appear in the mitochondrial marker COI that seem to indicate that the disease originates there or comes from an introduction from the continent in historical times. Comparative studies with data from massive sequencing of endemic areas are needed to obtain a more robust view of their evolutionary history, structure and diversity at the genomic level. The data obtained here can help in the implementation of surveillance and control measures for this region.

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O-37. EFFECT OF DIROFILARIA IMMITIS EXCRETORY/SECRETORY AND SOMATIC ANTIGEN ON THE EXPRESSION OF ANGIOGENESIS-RELATED PROTEINS AS A SURVIVAL MECHANISM.

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Keywords: *Dirofilaria immitis* excretory/secretory antigens, *Dirofilaria immitis* somatic antigens, angiogenesis, HUVECs.

The relationship of *Dirofilaria immitis* with the angiogenic process as a possible survival mechanism has been previously described in investigations in which it has been demonstrated that the excretory/secretory antigens (DiES) promoted the expression of proangiogenic factors in addition to stimulating related cellular processes, while the somatic antigens (DiSA) only promoted the expression of vascular endothelial growth factor (VEGF). Thus, this study aims to deepen our understanding of the relationship of *D. immitis* with this process by evaluating the effect of DiES and DiSA on the expression of angiogenesis-related proteins.

For this purpose, we used an in vitro model of human umbilical vein endothelial cells (HUVEC) that were treated for 24 hours with the following stimuli: DiES, DiSA, VEGF-A, DiES+VEGF-A, DiSA+VEGF-A and unstimulated cells that were used as a control group. Subsequently, the supernatant was collected and a mass spectrometry study was performed, identifying between 360-450 proteins in all groups.

Gene ontology analysis was performed on the identified proteins to evaluate the effect of the different stimuli on the protein expression of the cells. The treatment of cells with DiES+VEGF-A produced significant differences in the expression of 143, 121 and 84 proteins compared to the control group, DiES-treated cells and VEGF-A-treated cells respectively, of which 26, 15 and 10 are related to the angiogenic process. Regarding DiES+VEGF-treated cells, significant differences were observed in the expression of 91, 71 and 31 proteins compared to the control group, DiES-treated cells and VEGF-treated cells, respectively, of which 14, 8 and 3 are related to angiogenesis.

Stimulation with DiES+VEGF-A and DiSA+VEGF-A interacts with the angiogenic pathway by producing changes in the expression of proteins related to this process. Thus, and based on previous studies, angiogenesis is a potential survival mechanism of *D. immitis* in the host.

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O-38. REEVALUATING DOXYCYCLINE DOSAGE IN CANINE HEARTWORM DISEASE: IS LESS MORE?

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Keywords: *Dirofilaria immitis*, *Wolbachia*, C-reactive protein, anti-rWSP antibodies, doxycycline

Several aspects of heartworm disease adulticide treatment require further investigation, particularly regarding *Wolbachia pipientis* elimination. This study evaluated the acute phase response, specifically C-reactive protein (CRP) concentrations and anti-*Wolbachia* surface protein (r-WSP) antibody response, before (D0) and after (D30) treatment with oral ivermectin (6-12 µg/kg, monthly) and doxycycline for 30 days at three dosages: Group A (n=10, 10 mg/kg BID), Group B (n=16, 10 mg/kg SID), and Group C (n=13, 5 mg/kg BID). CRP concentrations were generally elevated at D0 (Group A: 15.3±22.7 mg/L; Group B: 27.6±45.3 mg/L; Group C: 13.9±14.2 mg/L) and slightly decreased at

D30 except in Group A (Group A: 25.9±63.3 mg/L; Group B: 17.7±15.7 mg/L; Group C: 12±9.4 mg/L). Anti-rWSP antibody response remained stable in Group A but significantly increased in Groups B and C at D30 ($p<0.05$). The increase in anti-WSP antibody optical densities may result from Wolbachia release during microfilarial death following ivermectin administration at D0 in microfilaremic dogs. Additionally, lower doxycycline doses in Groups B and C may have resulted in a minor Wolbachia reduction over 30 days. Also, it should be noted that the immune response is not well known and the serological persistence of these antibodies is unknown. Elevated CRP values at D0 confirm the strong inflammatory component of heartworm disease. By D30, slight CRP reductions in Groups B and C suggest decreased vascular inflammation due to Wolbachia elimination and microfilariae reduction. However, Group A showed increased CRP levels at D30, which may be due to other inflammatory factors or spontaneous adult worm death induced by antibiotic and macrocyclic lactone treatment. These findings support previous studies suggesting lower doxycycline doses may be sufficient for Wolbachia elimination in *Dirofilaria immitis*-infected dogs, and encourage further studies in this line of research.

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O-39. USE OF CORTICOSTEROIDS DURING ADULTICIDE TREATMENT IN DOGS WITH HEARTWORM: IS IT A RISK OR A BENEFIT?

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Keywords: *Dirofilaria immitis*, dogs, corticosteroids, pulmonary thromboembolism, D-dimer

D-dimer, a biomarker resulting from fibrin degradation, is a key protein used in the diagnosis of pulmonary thromboembolism (PTE). PTE is a life-threatening complication in dogs infected with *Dirofilaria immitis*. However, there are conflicting results regarding the effect of glucocorticoid therapy on these patients. This study aims to investigate the effects of low dose of corticosteroids on D-dimer levels in dogs with heartworm disease before, during, and after adulticide therapy.

Sera of 48 dogs with *D. immitis* undergoing adulticide therapy were evaluated on days 0, 30, 60, and 90. Additionally, parasite burden, presence of pulmonary hypertension, and microfilariae were assessed on day 0. Of them, 19 dogs (group B) received prednisone at decreasing doses (starting 0.5 mg/kg/BID) from day 0 during all study.

Dogs not receiving corticosteroids (group A) showed higher D-dimer concentrations on day 0 (0.13 ± 0.15 µg/mL), which decreased on day 30 (0.10 ± 0.04 µg/mL) and remained similar thereafter. Dogs receiving corticosteroids started with slightly lower D-dimer concentrations (0.09 ± 0.00 µg/mL), which remained at similar levels to group A for the rest of the study.

D-dimer levels were found to be higher in older dogs, in dogs with high parasite burden, presence of microfilariae or symptoms, but not with pulmonary hypertension. There were no statistically significant fluctuations of D-dimer values between groups, suggesting that there was no increased risk of PTE due to the use of corticosteroids during adulticide treatment.

The results showed that the use of anti-inflammatory doses of corticosteroids did not produce significant variations in D-dimer concentrations during the adulticide treatment, contradicting previous results. Furthermore, anti-inflammatory doses of prednisone improved general symptoms, reduced local inflammation at the melarsomine inoculation site, and contributed to a faster recovery. Therefore, the benefits of corticosteroid administration may outweigh the potential risks, although further studies with larger sample sizes are necessary to confirm these findings.

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O-40. APPLICATION OF THE SLOW KILL PROTOCOL AGAINST CANINE HEARTWORM DISEASE IN GREECE.

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Keywords: Canine Heartworm Disease, *Dirofilaria immitis*, dog, Slow kill protocol, Advocate® (Elanco)

Canine Heartworm Disease (CHD, *Dirofilaria immitis*) is a widespread parasitic infection of canids around the world. It is common in the Mediterranean countries, particularly in areas with suitable climatic conditions that favour mosquito abundance and filarial larva development. In severe cases, they can progress to weakness, dyspnoea and death. CHD represents a major concern, because in contrast to the easy low cost prevention, the treatment up to now relies on melarsomine. However, melarsomine is unavailable in several countries, and therefore in many areas it has been practising the slow-kill protocol, being the only available alternative adulticide treatment option. This protocol includes the use of doxycycline and a topical formulation of moxidectin 2.5 % + 10 % imidacloprid. The aim of our study was to evaluate this slow-kill protocol in Greece. Towards this end, a group of 25 naturally infected dogs, without any severe clinical sign (stage 1 and 2), aged between 2 to 5 years old and of both sexes were used to evaluate the slow kill protocol for one year long. The protocol (doxycycline and a topical formulation of moxidectin 2.5 % + 10 % imidacloprid) was followed as described by Savadelis et al. (2017), Genchi et al. (2019) and Paterson et al. (2020). Every month a blood sample was collected from each dog and tested for the presence of parasite antigen using the SNAP® 4Dx® Plus (IDEXX) and the DiroCHEK (Zoetis) read by TECAN freedom (A23). The study was approved by the Ethics Committee of the Aristotle University of Thessaloniki (306362/2021). Descriptive statistics were estimated, while results before and after treatment were analysed using McNemar's test to evaluate the treatment's efficacy. All dogs became negative to both tests between 9 to 12 months after the start of the slow kill protocol. It was concluded that the use of doxycycline and a topical formulation of moxidectin 2.5 % + 10 % imidacloprid (Advocate®, Elanco) could be considered as an effective alternative treatment of CHD, when access to melarsomine administration is not possible.

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O-41. DIROFILARIA SPP. EXTRACELLULAR VESICLES AND MIRNAS AS GAME-CHANGERS IN DIAGNOSIS AND HOST-PARASITE DYNAMICS.

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Keywords: *Dirofilaria*, extracellular vesicles, microRNAs

The zoonotic nematodes *Dirofilaria* spp., responsible for canine heartworm disease and subcutaneous filariasis, are vector-borne parasites endemic in Europe. In humans, infections manifest as pulmonary nodules, ocular pathologies, or subcutaneous lesions, frequently misdiagnosed as tumors. Current diagnostic and therapeutic methods show limitations, highlighting the need for innovative approaches. This study explores extracellular vesicles (EVs) and microRNAs (MiRNAs) from different *Dirofilaria* spp. developmental stages (adults [AD], microfilariae [MF], third-stage larvae [L3]), with the aim to enhance understanding of infection dynamics in both vertebrate and vector hosts.

AD, MF, and L3 extracted from a naturally infected dog, dog blood, and experimentally infected *Aedes albopictus* mosquitoes, respectively, were used for culture and identification using Knott test and *cox1* PCR. EVs were isolated through size-exclusion chromatography and precipitation-based methods, then characterized using nanoparticle tracking analysis, dynamic light scattering, and transmission electron microscopy. MiRNAs were isolated from parasite tissues, their EVs, and from sera of infected humans and dogs and the presence of miRNAs (i.e. miR1, miR100, miR34, miR71) was investigated using stem&loop RT-PCR.

PCR confirmed the Knott's test identification of *D. immitis* and *D. repens*, reflecting their endemicity in Italy. Coinfections with *Acanthocheilonema reconditum* (excluded from the analyses) were recorded. EVs from AD and MF exhibited diameters averaging 142 nm, concentrations of 10^8 particles/mL, and zeta potentials of $-10,5$ mV, indicative of charged bioactive vesicles. L3 EVs isolation is still under optimization. Preliminary miRNAs data confirmed the presence of conserved nematode miR1 in all *D. repens* L3, MF and their EVs, in *D. immitis* MF but not in AD and serum samples.

This is the first study aimed to characterize *Dirofilaria* EVs addressing their physical and molecular features. Despite challenges in parasites manipulation, advancements in this field are crucial to cope the emerging of drug resistance and improve diagnosis and prevention.

This study was funded by the PRIN 2022 Next Generation EU "Nemexit" Prot. 2022PX9HTE

O-42. IDENTIFICATION OF DIROFILARIA IMMITIS, DIROFILARIA REPENS AND ACANTHOICHEILONEMA RECONDITUM MICROFILARIAE THROUGH MATRIX ASSISTED LASER DESORPTION/IONIZATION TIME- OF-FLIGHT MASS SPECTROMETRY (MALDI-TOF MS).

Napoli, E.^{1*}, Taranto, C.¹, Gabrielli, S.², Baldaccini, A.³, Leo, P.³, Cavallero, S.², Pombi, M.², Varcasia, A.⁴, Nonnis, F.⁴, Tamponi, C.⁴, De Benedetto, G.¹, Sturiale, E.,¹ Fraggetta, F.⁵, Brianti, E.¹

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Keywords: *Dirofilaria immitis*, *Dirofilaria repens*, *Acanthocheilonema reconditum*, MALDI-TOF MS, speciation

Canine filarioses, caused by *Dirofilaria immitis*, *Dirofilaria repens*, and *Acanthocheilonema reconditum*, are widespread in dog populations. Diagnosis is performed by detecting microfilariae using Knott's test or PCR. However, morphological identification requires expertise, and PCR can be time-consuming and challenging to perform. Recently, MALDI-TOF MS has emerged as a promising technique for pathogen identification. This study aimed at evaluating the use of MALDI-TOF MS for the identification of microfilariae.

Blood samples from ten naturally infected dogs per each filarial species, i.e. *D. immitis*, *D. repens* and *A. reconditum*. Infection was diagnosed by Knott's test and confirmed by PCR. Microfilariae were extracted from samples, added with 25% formic acid, centrifuged, and analyzed via MALDI-TOF MS. The protein extracts were deposited on a steel

target plate, air-dried, and analyzed at 200 Hz within a mass range of 2–20 kDa. Each sample was tested 12 times. Spectral data were obtained using the MicroFlex LT instrument with the MBT_Standard method and analyzed by MALDI Biotyper® Sirius One IVD System and FlexAnalysis software.

MALDI-TOF MS analysis of protein extracts revealed high-intensity peaks (7–17 kDa) but with varying intensities, while distinct peaks (2–9 kDa) enabled species differentiation. Briefly, the spectrum of *A. reconditum* is characterized between the 2 and 5 kDa by small peaks that never exceed the intensity of $0.4 \cdot 10^4$ a.u., while *D. immitis* spectrum is characterized by a peak at 7.044 kDa that exceeds the intensity of $2 \cdot 10^4$ a.u. Finally *D. repens* spectrum is characterized by a series of five peaks between 2.460 and 2.510 kDa and by a peak at 8.723 kDa (intensity of $1.5 \cdot 10^4$ a.u.).

MALDI-TOF MS proved to be a reliable tool for distinguishing *D. immitis*, *D. repens*, and *A. reconditum*, enabling accurate and rapid microfilariae identification.

This project was funded by the PRIN Project “Nemexit” Prot. 2022PX9HTE

O-43. RADIOGRAPHIC DIAGNOSTICS OF THE CANINE HEARTWORM DISEASE: OLD AND NEW METHODS.

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Keywords: Heartworm disease, Radiography, Dogs

Diagnosis of heartworm (HW) infection in dogs is established by blood tests which demonstrate the presence of circulating microfilariae and/or adult antigens in serum or plasma samples. However, further diagnostic procedures, such as radiography and echocardiography, are required to determine the severity of HW disease, and thus prognosis and treatment options. The most objective method of assessing the severity of cardiopulmonary disease secondary to HW infection is radiography. In order to describe and define radiographic changes in cardiovascular and pulmonary tissues caused by pathogenesis of the HW infection, radiographs can be assessed both subjectively and objectively. Subjective assessment of radiographs is based on the identification of the vascular, alveolar or interstitial pattern of a dog's lung field, and the remodelling of the cardiac silhouette. Objective radiographic assessment mainly involves observing certain parameters in relation to various skeletal structures.

In this article, the published literature on the subject of radiographic diagnostic methods suitable for HWD diagnosis, both subjective and objective, is reviewed with emphasizing their advantages and disadvantages. In addition, we retrospectively analyzed radiographs of dogs naturally infected with HW (N=32), recorded in laterolateral (LL) and dorsoventral (DV) positions. Having analyzed the results derived from different radiographic methods, their statistically significant differences and their mutual correlation, we recommended modifications of certain methods in order to overcome their potential disadvantages and improve their clinical feasibility. The subjective evaluation describes an increased sternal cardiac contact, a reversed “D” shape of the heart, and the right caudal lobar artery exceeding the size of the analogous vein, as the most prevalent features. The objective methods define the following values of the cardiac size and shape in these dogs: vertebral heart size (VHS-LL=10.41±0.98; VHS-DV=10.72±1.13), manubrium heart size (MHS-LL=5.23±1.97; MHS-DV=5.34±2.01), sternbral heart size (SHS-LL=9.05±0.93; SHS-DV=9.38±0.88), and cardiac size index (CSI-LL=0.86±0.08; CSI-DV=0.74±0.06).

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O-44. FROM INCIDENTAL DISCOVERY TO CLINICAL RELEVANCE: THE JOURNEY OF DIROFILARIA IMMITIS.

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Keywords: *Dirofilaria immitis*, Heartworm disease, Pulmonary hypertension, Parasite-host relationship

Canine heartworm disease, caused by *Dirofilaria immitis*, was first described in 1626 during a necroscopy of a greyhound in Pavia, Italy (*Francesco Birago: "Trattato cinegetico, overo della caccia"*). Initially considered incidental finding, interest in this parasite increased during and after World War II, when scientific investigations demonstrated that pulmonary hypertension (PH) is the main cause of symptoms associated with the disease.

This case exemplifies an unusual host response, with severe PH surprisingly well-tolerated by the infected dog. It could reflect a pattern where the parasite, with a lower worm burden and a chronic course, coexisted with its host with minimal harm. Factors such as different mosquito populations, less urbanization, dogs density and rural settings, supported peaceful parasite-host relationships, facilitating its global spread.

An 8-year old, 8 Kg, male, mixed breed dog was referred to the cardiology department of the Anicura Istituto Veterinario di Novara for a positive heartworm antigen test and suspected parasite in the pulmonary artery. The patient was otherwise healthy, with a BCS of 3/5 and normal clinical parameters. Physical examination revealed a 2/6 mid-systolic murmur, with PMI at the left apex. Echocardiography confirmed parasites in the pulmonary artery lumen and a high probability of PH, assessed following the ACVIM guidelines.

This case highlights the adaptive strategy of *Dirofilaria immitis*, which relies on host survival to complete its life cycle. The absence of overt clinical signs demonstrates the parasite's capacity to sustain a delicate coexistence, ensuring long-term host viability. However, modern changes, including urbanization, increased dog density, and climate shifts, have enhanced transmission dynamics, resulting in more severe disease presentations. These findings underscore the importance of early detection and monitoring in asymptomatic cases. Understanding the evolving host-parasite relationship, enables veterinarians to better address heartworm disease, balancing the parasite's biological strategies with proactive management to mitigate disease progression.

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O-45. CAN DOGS BE INFECTED WITH DIFFERENT LINEAGES OF DIROFILARIA IMMITIS? WHOLE GENOME SEQUENCING HAS THE ANSWER.

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Keywords: *Dirofilaria immitis*, Genetic diversity, WGS, Haplotypes

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Dirofilaria immitis are nematodes that are vectored by mosquitoes and infect mainly members of the canine family causing the lethal disease pulmonary dirofilariosis. The disease is widespread across the world existing in both the Old and New World with certain affinity to tropical climates. Due to climate change the parasite is currently spreading into new regions and is now endemic in numerous regions, mainly southern and central European countries such as Spain, Italy, Greece, Romania and Hungary. Due to increasing prevalence and abundance of *Dirofilaria* infection, the question regarding the possibility of a single host being infected with different lineages (haplotypes) of the nematode arises? This question was not answered by population genetics studies on filarial nematodes so in our work we intend to utilize the Whole Genome Sequencing (WGS) to answer this question. Multiple nematodes were extracted from a single host (cases with high numbers of adults in the heart) and individually sequenced to establish their full genome and possible SNPs. Samples were obtained from the endemic country Romania, five blood samples stored in EDTA in -20 Celsius from canine hosts with high parasitemia were chosen, DNA was extracted from all MF blood samples and sequenced with WGS by a private company, where we obtained 6G with 150 bp paired-end reads and map them against a Reference genome, FASTAQ files were processed by experienced bioinformaticians to properly assign all haplotypes and differentiate between lineages, preliminary results show that it is possible for a single host to be infected with different haplotypes of *Dirofilaria immitis*, answering this important question can help establish the direction of spread of the parasite, especially if the parasite is being introduced into a region from multiple directions and overcome the limitation where DNA amplification with PCR can be biased towards the most dominant lineage of microfilaria inside the blood sample.

O-46. TREND IN THE CIRCULATION OF DIROFILARIA IMMITIS DNA IN MOSQUITOS OF EMILIA-ROMAGNA REGION (ITALY) IN YEARS 2022-2023-2024 CAPTURES.

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Keywords: *Dirofilaria immitis*, mosquitos, Italy, DNA

Dirofilaria immitis, the parasite responsible for heartworm disease in dogs and cats, is spread by mosquitoes and is increasingly found across Europe, including areas once considered free of the infection. Successful transmission depends on the presence of suitable mosquito vectors and a climate that supports mosquito survival, which is essential for the development of infectious larvae L3 from ingested microfilariae. This study aims to update our understanding of the species composition of competent mosquito vectors in the northern Emilia-Romagna region, an area traditionally endemic for both *D. immitis* and *D. repens*.

Mosquitoes were collected from 2022 to 2024 by the IZSLER, as part of the regional surveillance plan for West Nile Virus (WNV) in peri-urban and rural areas, and by MF in 2024 in more urban areas and near municipal kennels in Parma. Mosquitos were captured with CDC-CO2 traps in the second two weeks of August. Mosquitoes were stored at -20°C, then sorted by capture zone and species identified following the dichotomous keys. DNA from female mosquitoes was extracted and analyzed for the presence of *D. immitis* and *D. repens*. From the positive pools, RNA was also extracted and analyzed for the presence of WNV.

The capture trends highlighted that the majority of species identified belonged to *Culex pipiens* species followed by *Aedes caspius*, *Ae. vexans*, *Ae. albopictus*. A total of 8248 mosquitos were captured in 2022, 15746 in 2023 and 19310 in 2024. Of these, 140 pools (~20 mosquitos/each) in 2022, 133 and 98 in 2023 and in 2024, respectively, have been analyzed. *Ae. caspius* (27/35) has proven to be the species most frequently positive for the presence of *D. immitis* DNA, followed by *Ae. vexans* (6/35) and *Ae. albopictus* (2/35) in all three years considered, especially in the area of Ferrara and Bologna provinces. None of the pools was positive for *D. repens* neither WNV. The three-years trend confirmed the importance of *Ae. caspius* as a vector for *D. immitis* and additional in-depth studies should be conducted using different types of traps, with a particular focus on urban areas.

O-47. BEWARE OF DIROFILARIA RISK WHEN TRAVELLING WITH YOUR DOGS!

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Keywords: Zoonosis, Sardinia, Pet travel, Heartworm.

Among canine filarioids, *Dirofilaria immitis* and *Dirofilaria repens*, the causative agents of canine heartworm disease and subcutaneous dirofilariosis, respectively, are the most well-known species due to their veterinary and public health significance. Given the peculiar endemicity of Sardinia for these species and its role as major touristic destination that raises concerns about the potential threat for dogs travelling with owners, a study was conducted to update prevalence data and assess associated risk factors.

A total of 741 owned and sheltered dogs were examined. Data on sex, age, size, night sheltering, cohabitation, lifestyle, preventive strategies, ownership status and habitat were collected. All dogs were over 12 months old with no prior chemoprophylactic treatment against dirofilariosis. Blood samples obtained from enrolled dogs were analyzed for circulating microfilariae using Knott's test and for *D. immitis* antigen via ELISA rapid test (SNAP 4DX, IDEXX).

The overall microfilaremia prevalence was 15.2% (113/741), with *D. immitis* being the most detected species (9.9%, 73/741), followed by *D. repens* (5.5%, 41/741). Additionally, *Acanthocheilonema reconditum* microfilariae were identified in 3.1% (23/741) of the samples. Mixed infections were observed in 3.2% (24/741) of dogs, while 12.2% (88/721) tested positive for *D. immitis* antigen. Statistical analysis revealed significant risk factors for *D. immitis* infection, including absence of ectoparasite prevention ($\chi^2 = 21.863$; $p < 0.001$), shelter dog ($\chi^2 = 6.512$; $p = 0.011$), and urban/suburban residence ($\chi^2 = 64.725$; $p < 0.001$). Male sex ($\chi^2 = 4.904$; $p = 0.027$) and small size ($\chi^2 = 5.450$; $p = 0.020$) were associated with higher *D. repens* infection risk, as observed for *A. reconditum* ($\chi^2 = 5.205$; $p = 0.022$).

These findings confirm that dirofilariosis remains endemic in Sardinia, emphasizing the importance of integrated control strategies, including preventive measures for resident and traveling dogs to safeguard animal and human health.

Acknowledgements

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O-48. AN ANALYSIS OF THE CURRENT RISK OF TRANSMISSION OF ANIMAL AND HUMAN DIROFILARIOSIS IN PORTUGAL AND SPAIN MONTH BY MONTH THROUGH ECOLOGICAL NICHE MODELING.

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Keywords: *Dirofilaria* spp., *Culex pipiens*, *Culex theileri*, Ecological Niche Model, Spain, Portugal.

Spain and Portugal are considered endemic countries for dirofilariosis. Previous studies have observed a prevalence of *D. immitis* in Spain of 6.47% in dogs and 9.4% in cats. In Portugal, the mean prevalence for dogs was 5.9%, with higher percentages in the south. As it is a zoonotic disease, it is important to carry out control measures for both animal and human populations. One of them is the analysis of the risk of infection by means of colour maps to alert the population. Previously, a study has been carried out to analyse the risk of infection in the Iberian Peninsula, the Canary Islands and the Balearic Islands with average annual temperatures. The aim of this study was to carry out infection risk maps for *Dirofilaria* spp. month by month, analysing the vector's dispersal capacity through ecological niche models (ENM) and implementing the number of generations of the parasite. Habitat suitability models for vector transmitting dirofilariosis (*Cx. pipiens* and *Cx. theileri*) were performed with the maximum entropy algorithm (Maxent) optimised with the KUENM package of R based on bioclimatic conditions, distribution of irrigated crops, human footprint, water bodies and rivers. For the risk model, the annual and monthly generations of *Dirofilaria* spp. were used as a variable and multiplied to the final ENM of the vectors. In winter, the risk of infection is low practically throughout the peninsula, except in the coastal areas of the southwest and in the Balearic Islands, where winters

are mild and wet, and in the Canary Islands. Between June and September, the risk of infection reaches its highest values mainly in the centre-south of the peninsula and on the Mediterranean coast, followed by the north and central coast with high values. Therefore, it is necessary to apply control measures and pay special attention to the areas and months with the highest risk of infection.

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O-49. AN ANALYSIS OF THE CURRENT RISK OF TRANSMISSION OF DIROFILARIA IN UNITED STATES OF AMERICA AND ITS FUTURE PROJECTION UNDER CLIMATE CHANGE SCENARIOS

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Keywords: Dirofilariosis, *Aedes vexans*, *Aedes albopictus*, Ecological Niche Model, USA.

In the United States of America (USA), heartworm disease is of great veterinary and economic importance due to its impact on the health and welfare of both domestic and wild animals (mainly canids and felids). The presence of *Dirofilaria immitis* depends on bioclimatic and environmental factors that determine the survival of its vectors (culicid mosquitoes). Therefore, our objective is to map the risk of infection in USA by modelling the ecological niche of two of the most widely distributed vectors in the country: *Ae. albopictus* and *Ae. vexans*. ArcMap 10.8 was used to process both bioclimatic variables (related to temperature and precipitation) and environmental variables (water bodies, human footprint, herbaceous and shrub density, etc.). Once the relevant predictor variables were obtained, the ecological niche models of *Ae. albopictus* and *Ae. vexans* were elaborated using the MaxEnt algorithm automated by the Kuenm R package. In addition, we also calculated the number of generations of *Dirofilaria* spp. both annually and month by month as a function of temperature using a custom R script. The areas with the highest risk of infection correspond to the eastern states of the country, which are more humid and warmer (at least for a certain period of the year). To the west, the risk decreases mainly in the Rocky Mountains, Sierra Nevada and the deserts of Arizona and Nevada, mainly. Regarding the risk of infection by month, during the winter months the risk is very low practically everywhere in USA, increasing progressively month by month, reaching the highest risk values in summer. Finally, the projection to 2080 indicates an increase in the risk of infection mainly in the north of the country and towards areas with higher altitudes.

POSTER PRESENTATIONS

8th ESDA Days
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P-1. ASSESSMENT OF THE SPEED OF TRANSMISSION OF EHRLICHIA CANIS, ANAPLASMA PHAGOCYTOPHILUM, AND BORRELIA BURGdorFERI SENSU STRICTO BY INFECTED TICKS THROUGH AN IN VITRO EXPERIMENTAL SYSTEM.

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Keywords: Tick-borne pathogens; speed of transmission; *Ehrlichia canis*; *Anaplasma phagocytophilum*; *Borrelia burgdorferi* s.s.; in vitro feeding system

Canine vector-borne diseases (CVBDs) have significant clinical and public health implications. This experimental study used an adapted version of the USDA-developed continuous flow in vitro feeding system (CFIFS) for ticks to investigate the speed of transmission (SOT) of three tick-borne pathogens (TBPs): *Ehrlichia canis* by laboratory reared infected *Rhipicephalus sanguineus* (18.3% tick infection rate), *Anaplasma phagocytophilum* by laboratory reared infected *Ixodes ricinus* (56%), and *Borrelia burgdorferi* sensu stricto by laboratory reared infected *I. ricinus* (76%). All ticks began to attach and feed three hours after being introduced in the feeding system. PCR tests were used to detect the presence of pathogens in the blood flow collected every three hours. Swab samples from the inner face of the feeding membrane were also collected and tested every six hours during the *B. burgdorferi* study. In this experimental in vitro design, *Ehrlichia canis* had a SOT of 6-9h, *A. phagocytophilum* of 15-18h, and *B. burgdorferi* of 45-48h in blood but only 3-6h on inner membrane swabs. The early detection of *Borrelia* spirochetes on the membrane indicates a delay in their bloodstream entry. This in vitro system allows to test and compare many tick pathogen transmission pathways. The findings of this study highlight the transmission time of tick-borne pathogens, emphasizing the the possible difficulty to prevent pathogen with high speed of transmission like *Ehrlichia* and *Anaplasma* using acaricides.

P-2. OCCURRENCE OF HOOKWORMS AND OTHER IMPORTANT INTESTINAL HELMINTHS IN DOG POPULATIONS FROM ROMANIA: RISK FACTORS AND ZONOTIC IMPLICATIONS.

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Keywords: hookworms, intestinal helminth infections, prevalence, dogs, Romania

Dogs are hosts for various helminth parasitic species that impact their health and welfare, but also with important relevance for humans since some of these parasite have a zoonotic potential. In this respect, knowledge about occurrence and prevalence of intestinal helminth infections in domestic carnivores are of high interest to identify the associated risk factors for animal and public health. Therefore, the present study aimed to investigate the occurrence and associated risks of hookworm infections and other intestinal helminths in different dog populations in Romania. For this, a copro-parasitological survey was carried out in dogs from nine counties in South-eastern Romania. In total, 321 faecal samples collected from owned (n=138), stray (n=72) and shelter (n=111) dogs were examined using the quantitative Mini-FLOTAC technique (sensitivity: eggs per gram of feces). The hookworm infection was analyzed also based on egg excretion, allocated into five intensivity classes. Overall, 29.6% (95% CI:24.74-34.87) of the tested samples were positive for hookworm infection, with a mean EPG shedding of 485.9 (Standard Deviation: 646.4; 10-2370). Higher hookworm infection rates, with statistic significance, were found in stray and shelter dogs, of 47.2% (95% CI:35.99-59.07) and 44.1% (95% CI:35.10-53.61), respectively, than in owned dogs (8.7%; 95% CI:4.97-14.75). The mean EPG varied among shelter, stray, and owned dogs, of 552.3 (SD: 703.2), 473.2 (SD: 598.9), and 349.5 (SD: 697.6;), respectively. Also, dogs from rural areas showed higher prevalence of hookworms, of 37.6% (95% CI:30.94-44.66) than urban dogs (16.9%; 95% CI:11.19-24.53). In addition to hookworms, other parasitic helminths, including zoonotic species, were detected, including: *Trichuris vulpis* (29.9%), *Toxocara canis* (22.12%), *Toxascaris leonina* (11.5%). These findings document on high prevalence and large distribution of hookworms and other intestinal helminth parasites in Romanian dog populations and highlight on the need for an adequate management of endoparasites in dogs, according to the risk category.

P-3. THE PREVALENCE OF CRASSICAUDA ANTHONYI IN CUVIER'S BEAKED WHALES (ZIPHIOUS CAVIROSTRIS) STRANDED IN GREECE AND ITS MOLECULAR CHARACTERIZATION.

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Keywords: *Ziphius cavirostris*, *Crassicaudas anthonyi*, parasitism, kidney, arteries, marine mammals, beaked whales, sonar exercises, conservation

Cuvier's beaked whale (*Ziphius cavirostris*) a deep-diving cetacean, Vulnerable (VU) under the Greek RedList, is a beaked whale species frequently found in the Greek marine areas. It is known to host various parasites, including nematodes of the genus *Crassicauda* that primarily inhabit vascular, renal, and urogenital structures. The last decades, several single or mass strandings of Cuvier's beaked whales have been reported in the Mediterranean Sea raising a great concern about the threats and the survival of the species. More precisely, 167 Cuvier's beaked whales stranded with different conservation code (1-4) between 1996-present in different locations in Greece. For monitoring and research purposes, the cause of death was investigated in 59 of them by "ARION"-Cetacean Rescue and Rehabilitation Center and the School of Veterinary Medicine-AUTH. The necropsy revealed, among others, light to heavy parasitism of kidneys and urinary tracts as well as the arterial walls. Adult parasites (>80 cm long) were collected from the kidneys of all animals for morphological and molecular identification. The histopathological examination showed that the parasites caused vascular occlusion and kidney failure. Abdominal vasculature presented fibrosing arteritis with mineralization and metaplasia, affecting blood supply. Molecular analysis was carried out using phenol/chloroform DNA extraction protocol and PCR amplification of the ribosomal DNA internal transcribed spacer [ITS] regions. Sequencing analysis displayed an over 99% similarity of the screened nematodes to the *Crassicauda anthonyi* species. Necropsy reports suggested that the cause of death was mainly related to naval mid-frequency active sonar exercises, although a high prevalence of *Crassicauda* infections was noticed. Given the deep-diving and elusive nature of this species, the full extent of the pathogenicity and ecological implications of *Crassicauda* parasitism in *Z. cavirostris* still remains unclear. This review highlights the importance of parasitological studies in deep-diving cetaceans, since chronic infections along with other environmental stressors contribute to long-term health deterioration, potentially affecting diving capacity, reproductive success, and survival of the species.

P-4. STOMOXYS CALCITRANS: A POTENTIAL MECHANICAL VECTOR OF ANAPLASMA PHAGOCYTOPHILUM? Bouhsira E.

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Granulocytic anaplasmosis is a zoonosis that affects various domestic species (domestic ruminants, horses, carnivores). It is widespread in European livestock farms, causing major economic losses such as milk production decrease, abortions and secondary infections. Its causative agent, the bacterium *Anaplasma phagocytophilum* (Aph.), is mainly transmitted by ticks of the genus *Ixodes*. However, other haematophagous arthropods, such as the stable fly *Stomoxys calcitrans*, could be involved in the transmission, particularly in areas where *Ixodes* are less prevalent but granulocytic anaplasmosis is present. *Stomoxys calcitrans* is implicated in the mechanical transmission of various infectious agents, including *Anaplasma marginale* (the agent of bovine anaplasmosis), equine infectious anaemia virus, enzootic bovine leukosis and lumpy skin disease. This mechanical transmission can be immediate, following an interrupted blood meal via the presence of residual blood on the mouthparts, or delayed, in the event of regurgitation of infected blood from the crop during a new meal.

The aim of our study was to explore the capacity of *S. calcitrans* to ensure mechanical transmission of Aph. using an artificial feeding system under experimental conditions. To do so, two types of infections were carried out: the first-one was an interrupted blood meal, exploring the immediate transmission to uninfected blood, mimicking the behaviour

of stable flies in the field due to host defence movements; the second one consisted in exploring the presence of Aph. in the stable flies during time after a complete infective blood meal.

DNA and RNA of Aph were detected in flies for the 24 hours post infection but were not detected in the uninfected recipient blood.

These preliminary results confirmed the carriage of Aph by stable flies after an infected blood meal, but did not suggest any transmission through the bite. Complementary experiments are needed to confirm these findings.

P-5. PREVALENCE OF HEPATOZOOM CANIS IN IBERIAN WOLVES (CANIS LUPUS SIGNATUS) FROM SPAIN.

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Keywords: wolf, *Hepatozoon canis*, Spain, tick,

Information about the prevalence of *Hepatozoon* spp. in canids from Spain is scarce and there are not reports about the prevalence of this pathogen in wolves (*Canis lupus*) from this country. The main objective of the present study was to determine the prevalence of *Hepatozoon* spp. in wolves from Northwestern Spain and to determine the influence of different variables on their prevalence.

A total of 129 spleen samples collected between 2008-2019 were included in the study. The presence of *Hepatozoon* spp. was molecularly assessed through a PCR targeting the 18S rRNA of this parasite; afterwards, a subset of positive samples was submitted to sequencing. The influence of several variables (study period, age and sex) on the prevalence of *Hepatozoon* spp. was determined using a generalized linear model (GML). In addition, a phylogenetic analysis was carried out using MEGAX software.

Hepatozoon spp. DNA was detected in 93.0% (120/129; 95% Confidence intervals: 88.6-97.4%) of the samples being *Hepatozoon canis* the only species identified after sequencing. The detected prevalence was higher in females (98.3%) than in males (93.8%); in young (100%) than in adult (97.5%) and subadult (90.9%) animals, and during the first half of the study period (2008-2013) (98.2%) compared to the second half (2014-2019) (89.2%). However, no significant differences were observed between categories in the GLM. The phylogenetic analysis revealed a high nucleotide diversity on *H. canis* sequences and the existence of three clades.

The high prevalence of *H. canis* together with the no existence of significant differences among the studied groups of animals suggest that this pathogen is probably endemic in the studied wolf population. In addition, the presence of several *H. canis* genotypes has been related to the existence of various infection routes highlighting the importance of performing epidemiological studies in wildlife from a “One health” approach.

P-6. ABERRANT MIGRATION OF SPIROCERCA LUPI IN THE URINARY BLADDER OF A DOG PRESENTED WITH HEMATURIA.

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Keywords: *Spirocerca lupi*, histopathology, urinary bladder

Spirocerca lupi is a parasitic nematode that primarily affects dogs in warm climates, transmitted via ingestion of coprophagous beetles (intermediate hosts) or paratenic hosts. While *S. lupi* typically targets the oesophagus, it can migrate to the thoracic aorta and vertebrae, stomach, intestines, lumbar fascia, lung, thymus, trachea, interdigital tissue, diaphragm, heart, kidney, subcutis and urinary bladder. This report presents a case of a urinary bladder mass caused by aberrant migration of *S. lupi*, identified by histopathological examination.

An eight-month-old intact female, mixed-breed dog, diagnosed with juvenile diabetes mellitus, presented with depression, anorexia, hematuria and bloody vulvar discharge. The dog had been adopted from an animal shelter five months prior, lived both indoors and outdoors and had not received prophylactic antiparasitic treatment. On admission, fever, tachycardia and tachypnea were recorded.

Complete blood cell count revealed neutrophilic leukocytosis, while biochemistry was unrewarding. Urinalysis identified glycosuria, hematuria, cocci, rod-shaped bacteria, degenerate neutrophils and activated macrophages. *Escherichia coli* was isolated. Abdominal ultrasound revealed a urinary bladder mass and an echogenic fluid accumulation on the caudal pole of the right kidney. Fluid cytology demonstrated a purulent exudate. Antibiotic treatment was ineffective. A right kidney nephrectomy and partial cystectomy for the mass removal led to clinical improvement. Histopathology revealed a perirenal abscess, a urinary bladder granuloma and intralesional sections of a coiled nematode. Based on morphological characteristics the nematode was identified as immature female *S. lupi*. Faecal examination and gastrointestinal endoscopy were negative for *S. lupi* eggs and additional granulomas, respectively.

In this case, *S. lupi* migration in the urinary bladder was presumably subclinical. However, the granuloma formation could have disrupted the mucosa integrity, facilitating a secondary urinary tract infection. The synergistic or exclusive effects of diabetes should also be considered. This report highlights the parasite's broad migratory potential and diagnostic challenges posed by atypical presentations.

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P-7. SEVERE CO-INFECTION OF TOXOPLASMA GONDII AND EHRLICHIA CANIS IN A DOG: A COMPELLING CLINICAL CASE.

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Keywords: *Toxoplasma*, *Ehrlichia*, Dog

Infections by vectorial pathogens are commonly diagnosed in domestic dogs, especially in endemic regions. In private practices, symptomatic animals are mainly diagnosed using rapid serological tests. *Ehrlichia canis*, a Gram-negative bacterium transmitted by *Rhipicephalus sanguineus*, has been detected in Romania, with local prevalence ranging from 2.1% to 11.1%. *Toxoplasma gondii* and *Neospora caninum* are major protozoan parasites of worldwide distribution and significance in veterinary medicine.

A 5-year-old female mixed-breed dog was referred to Pet Stuff Veterinary Hospital, a private referral hospital in Bucharest with altered mental status, apathy, and anorexia for the past week. Multiple ticks, mainly *Rhipicephalus* spp. and *Dermacentor* spp., were detected attached to the dog's skin. A blood smear was done and showed severe anemia and thrombocytopenia and revealed mild left-shift neutrophilia with toxic changes. Neutrophils contained multiple round to oval, "banana"-shaped structures, approximately 5µm in length and 1µm in width, resembling *Toxoplasma*-like tachyzoites. Additionally, within monocytes, granular basophilic microcolonies (morulae) consistent with *E. canis* were observed. Additionally, a serum ELISA for *T. Gondii* was positive for IgG, with a titer of 14 LE (cutoff titer: < 50), and negative for IgM, with a titer of 2.54 NTU (cutoff titer: < 9). A PCR blood test for *N. caninum* was sent to a referral laboratory and returned negative. Real-time PCR was also performed for *T. gondii* and *Ehrlichia canis* to assess the pathogen load using a semiquantitative Real-Time PCR approach. Both protocols returned positive results with a medium-to-low pathogen load and comparable Ct values. The dog's clinical condition severely deteriorated and the animal died. A necropsy was not performed.

This case reports a rare lethal infection of a dog with canine ehrlichiosis and toxoplasmosis diagnosed by microscopy, serology, and confirmed by PCR. This study highlights the rare detection of *T. gondii* tachyzoites and *E. canis* morulae in peripheral blood cells, providing new diagnostic insights. The presence of these parasites suggests widespread hematogenous spread, a rare phenomenon. This co-infection likely contributed to the dog's rapid health decline and fatal outcome.

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P-8. DAIRY GOATS HELMINTHOSIS AND ITS POTENTIAL PREDICTORS IN GREECE: FINDINGS FROM AN EXTENSIVE COUNTRYWIDE STUDY.

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Keywords: *Dicrocoelium dendriticum*, epg counts, lungworms, nematode, Trichostrongylidae

The objectives of the present study were the description of the prevalence of helminth infections from pooled faecal samples from goat herds across Greece and the evaluation of herd-related factors potentially associated with the presence of these infections in the herds.

A cross-sectional study was carried out in 119 goat herds around Greece and processed by conventional parasitological techniques.

Helminths were recovered from samples from 93.3% of herds: *Dicrocoelium dendriticum* (from 15.1% of herds), *Paramphistomum cervi* (0.8% of herds), *Moniezia* spp. (25.2% of herds), Trichostrongylidae (89.1% of herds), *Nematodirus* spp. (16.8% of herds), *Strongyloides papillosus* (5.0% of herds), *Trichuris* spp. (18.5% of herds) and lung-worms (23.5% of herds). Mean epg counts in all herds in the study were 219 epg. In multivariable analyses, for 'high (> 300) epg counts in faecal samples' the age of kid removal from their dams ($p=0.045$) was found to be a significant factor, for 'high proportion (> 64%) of *Teladorsagia* spp. in faecal samples' the month of the start of the kidding season ($p=0.045$) was a significant factor, for 'high proportion (> 27%) of *Haemonchus contortus* in faecal samples' nutritional modifications during gestation ($p=0.002$) and application of reproductive control practices in the farm ($p=0.013$) were the significant factors and for 'presence of *D. dendriticum* in faecal samples' the number of veterinary visits to the farm annually ($p=0.040$) was found to be significant.

P-9. CYSTOISOSPORA SUIIS IN THE REGION OF MURCIA: STUDY OF PREVALENCE IN SWINE FARMS.

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Keywords: *Cystoisospora suis*, Pigs, Spain

Cystoisospora suis is the etiological agent of neonatal cystoisosporosis, a significant disease affecting suckling piglets during the first three weeks of life¹. Oral uptake of sporulated oocysts is generally considered the primary route of infection in piglets, leading to high morbidity while mortality remain low². Previous studies have reported high prevalence rates in farms from Germany, (76%), Canada (70%) and China (66%)³. However, there is a lack of recent data on the prevalence and intensity of infection on farms in the Region of Murcia (Spain). In this context, we aimed to investigate the current qualitative (prevalence) and quantitative (number of oocysts per gram of faeces, OPG) status of cystoisosporosis in sow herds and piglets in the Region of Murcia.

A total of 824 sows and 70 piglets belonging to 6 different farms, subjected to common intensive farming conditions were selected for faecal samples collection for a year, and examined by flotation (qualitative) and MacMaster chamber (quantitative) methods.

In the prevalence study, 7 sows (0.85%) and 49 piglets (70%) diagnosed positive for infection. Also, prevalence varied widely among farms, for both sows and piglets (Figure 1). In the quantitative analysis, faecal OPG were higher in piglets than in sows across all farms, except for Farm 5 (Figure 2A and 2B), and the highest value was detected in sows from Farm 5, with 257.742 OPG (Figure 2B).

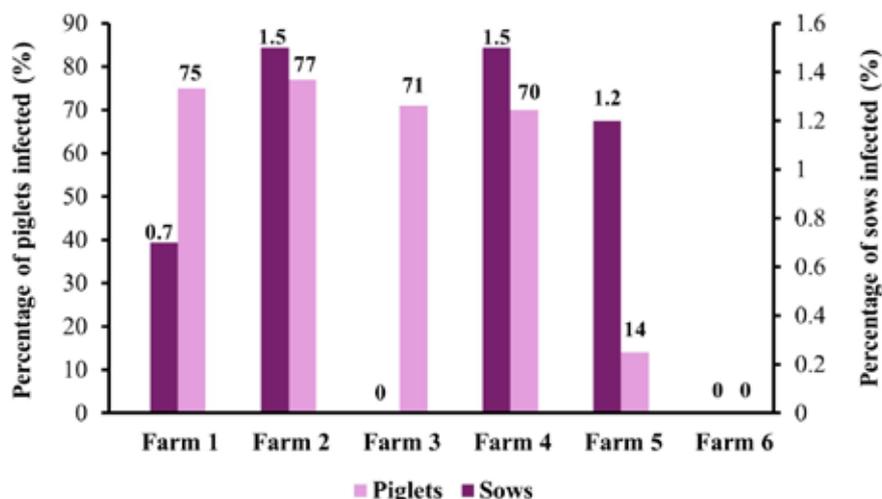


Figure 1. Prevalence of cystoisosporosis in sows and piglets from 6 farms in the Region of Murcia.

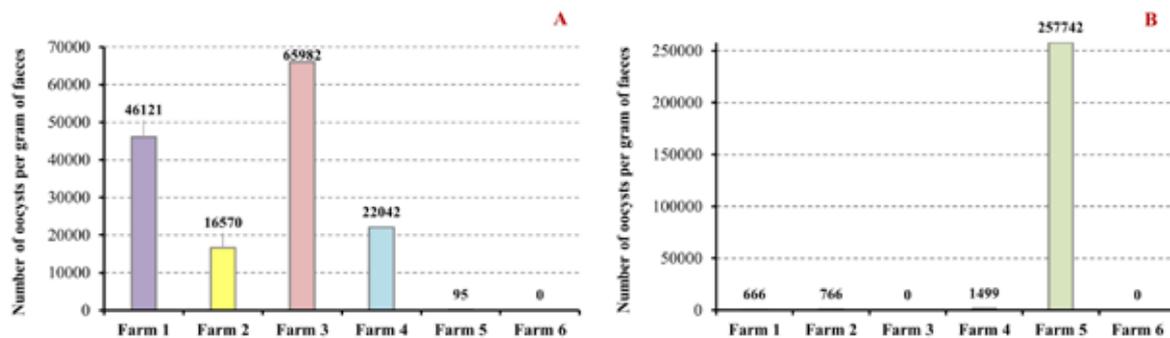


Figure 2. Number of oocysts per gram of faeces in piglets (A) and sows (B).

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P-10. EXPLORING THE IBERIAN WOLF'S DIET AND THE EPIDEMIOLOGICAL RISKS LINKED TO CESTODES.

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Keywords: Iberian wolf, Cestodes, Diet, Spain

The Iberian wolf (*Canis lupus signatus*) is a carnivore native to Iberian Peninsula which shares parasites with other wild and domestic canids, some of them with zoonotic importance. Therefore, its increasing presence in anthropized areas poses an epidemiological risk to both animal and public health. Among the most relevant parasites are cestodes, whose indirect life cycle provides valuable insight into the wolf's diet. This study analyzes the cestode species present in wolves from northwestern Spain.

Between 2009 and 2014, 82 wolves from the provinces of A Coruña, Lugo, Ourense, Pontevedra and Asturias, obtained by authorized hunting or found dead, were examined. After necropsy, cestodes were collected and identified through morphometry and the support of molecular biology techniques. Six species were identified by morphometry with the following prevalences: *Taenia krabbei* (55%), *T. hydatigena* (43%), *T. pisiformis* (5%), *T. crassiceps* (1%), *Mesocostoides* spp. (1%) and *Dipylidium caninum* (1%). By end-time PCR and further sequencing of amplified products, identification of *T. krabbei* and *T. hydatigena* was confirmed in 63 individual cestodes (74.6% and 25.4%, respectively).

The results differ from previous European studies, as neither *T. ovis* nor *T. multiceps* were found. *T. krabbei* was definitely identified with the combination of morphometry and genetic characterization, and its high prevalence is noteworthy since it has not been previously described in the study area. In addition, no species of the genus *Echinococcus* spp. were detected.

In conclusion, the Iberian wolf has a key role in the natural transmission of these cestodes in northwestern Spain. The high prevalence of *T. krabbei* suggests that wild cervids are a very important trophic resource in the studied wolves, playing a relevant role in the sylvatic cycle of this parasite. However, this carnivore does not seem to play an important role in transmission of cestodes of significant zoonotic relevance.

P-11. PARASSESS, A DIGITAL OWNER-FACING TOOL BASED ON ALGORITHM TO ASSESS PARASITE RISK FOR DOGS AND CATS.

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Keywords: Parasite, Risk assessment, algorithm, dog, cat

Parassess is an interactive digital risk checker based on the most up-to-date understanding of parasite risk factors and control recommendations from scientific associations such as ESCCAP, TROCCAP, CAPC and WAAVP. The major parasites of dogs and cats are included in this assessment, fleas, ticks, mites, roundworms, hookworms, whipworm, tapeworms, lungworms, heartworm and *Leishmania infantum*. The risk factors are related to each parasite biology and epidemiology, and to each dog or cat, such as their sex and age, and other external factors, like location, behavior, feeding, and antiparasitic treatments. One algorithm has been built for dogs, another for cats. It is based on 13 to 20 short and comprehensive questions to be answered by the pet owners. Some questions are qualitative, others are quantitative including single or multiple answers. A few questions refer to the possible presence of clinical signs like pruritus, fatigue, coughing, and diarrhea. The algorithm calculates scores and provides a final score from 0 to 100 for each parasite. The final scores correspond to a risk assessment for the most common parasites. The digital questionnaire is easy to fill out in a few minutes. Parassess is based on the biology and epidemiology of parasitic diseases and not product-related. It can be adapted to specific geographies by adding or removing certain parasites. It offers veterinarians and pet owners the opportunity to better discuss and understand the risks, and to tailor the parasite prevention strategies.

P-12. SEVERE FILARIASIS AND TRICHINELLOSIS IN A FREE-RANGING BROWN BEAR IN ALASKA – PARASITES KNOW NO BOUNDARIES.

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Keywords: *Dirofilaria ursi*, *Trichinella spiralis*, neurological, transboundary, reportable

We report an unusual case from a wild brown bear (*Ursus arctos horribilis*) on Chichagof Island in southeastern Alaska, which hosts the highest density of bears in the world. In June 2021, a 2-4 year old male bear with overt neurological clinical signs was euthanized for welfare reasons. On necropsy, the bear was emaciated and there was evidence of cranial trauma prior to euthanasia. Viral and toxicological causes of neurological disease were ruled out. On histology, we observed encysted *Trichinella* spp. larvae in skeletal muscle and tongue, and microfilaria in vasculature in the lung and brain. In the brain, there was lymphoplasmacytic choroiditis, and perivascular and perineuronal edema with neuronal necrosis and chromatolysis. On digestion of muscle, we recovered a high intensity of *Trichinella* spp. (380 larvae per gram of muscle). Using a novel metabarcoding assay and the traditional multiplex PCR assay, all larvae were *Trichinella spiralis*, and not *T. nativa*, T6, or *T. chanchalensis* as expected from wildlife in North America. *Trichinella spiralis* is eradicated in the USA and Canada, where it is a reportable animal disease to national authorities, and is rare in wildlife even where *T. spiralis* is endemic. Further genetic characterization is under way to determine potential origins, suggesting that this free-ranging bear consumed meat imported from outside North America. DNA extracted from microfilaria revealed *Dirofilaria ursi*, adults of which live in the subcutaneous and connective tissues, and are

generally considered non-pathogenic. We hypothesize that the neurological signs observed in this bear were secondary to filariasis. This case illustrates the need for surveillance in wildlife for biosecurity “breaks” in eradicated diseases, intra- and international cooperation for parasite diagnostics in remote regions, and monitoring climate-sensitive parasites for changes in transmission and pathogenicity in a rapidly warming, and increasingly globally connected, Arctic.

Please note that there are more than 6 authors!

P-13. THELAZIA CALLIPAEDA INFECTION IN IBERIAN WOLVES FROM NORTHWESTERN.

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Keywords: *Thelazia callipaeda*, wolf, Spain, epidemiology.

Introduction

Thelaziosis, caused by *Thelazia callipaeda*, is an arthropod-borne zoonotic disease that affects domestic and wild animals. In Spain, the first documented case of *T. callipaeda* infection in Iberian wolves (*Canis lupus signatus*) was reported in 2020, but no epidemiological studies have been conducted to elucidate the role these carnivores play in the transmission cycle. This study aimed to assess the prevalence of *T. callipaeda* and identify the epidemiological factors associated with its transmission in wolves from northwestern Spain.

Materials and Methods

From 2016 to 2024, 174 wolves from Galicia (A Coruña, Lugo, Ourense, and Pontevedra) and Asturias, which had died from natural causes or by car run over, were necropsied and epidemiological data (sex, age, body condition, year and parasitic load) was recorded. Eye worms were collected from the wolves' conjunctival sacs using sterile cotton swabs and/or by flushing with physiological saline solution (0.9% NaCl). The nematodes were morphologically identified, and molecular techniques were used for further confirmation in pooled samples.

Results and Discussion

The overall prevalence of *T. callipaeda* infection obtained in necropsied wolves was 17.2% (30/174), with higher rates in Galicia (18.5%) than in Asturias (8.7%). The highest prevalence was observed in Ourense (39.1%), while no cases were detected in A Coruña ($p < 0.01$). Infection rates were similar in males (16.3%) and females (18.7%) wolves, but females had a significantly higher parasitic burden, with 34.1 nematodes compared to 8.1 in males ($p = 0.03$). Young wolves showed higher prevalence and parasitic load (23.1% and 23.7) than adults (14.3% and 16.4) and pups (9.7% and 10). This could be due to higher activity and exposure to vectors in young and smaller eye size in pups. A significant association was found between parasitic load and body condition 4-5 ($p = 0.04$), showing lower rate of eye worms. These findings suggest that *T. callipaeda* does not pose a significant threat to wolf conservation. Prevalence showed a positive trend over the years, with increased parasitic load in 2019 and 2020.

Conclusions

The Iberian wolf plays a significant role in maintaining the sylvatic cycle of *T. callipaeda* in northwestern Spain, although additional studies are needed to better understand the epidemiological implications of these findings.

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Patrimonio Natural (Consellería de Medio Ambiente e Cambio Climático, Xunta de Galicia) for their authorization and support in facilitating the animal sampling process.

P-14. ESTABLISHMENT AND VALIDATION OF RED FOX (*VULPES VULPES*) AND DOMESTIC DOG (*CANIS LUPUS FAMILIARIS*) AIRWAY EPITHELIAL CELL CULTURES AT THE AIR-LIQUID INTERFACE AS A PLATFORM TO INVESTIGATE HOST-PARASITE INTERACTIONS.

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Keywords: Host-pathogen interaction, respiratory tract, in vitro model, wildlife reservoir, fox

The airway epithelium serves as a critical barrier against infectious pathogens and toxins while playing a key role in modulating immune responses in the upper respiratory tract. This is particularly important in red foxes (*Vulpes vulpes*), which are known reservoirs for zoonotic pathogens such as the fox tapeworm (*Echinococcus multilocularis*). In this study, we developed, established, and validated an air-liquid interface (ALI) model of the respiratory tract using primary airway epithelial cells isolated from the tracheas and main bronchi of hunted red foxes. For comparison, we applied the same methodology to develop a similar ALI model for domestic dogs (*Canis lupus familiaris*), enabling a cross-species evaluation of airway epithelial properties. The resulting ALI cultures for both species exhibited structurally differentiated, pseudostratified epithelia characterized by ciliated cells, mucus secretion, and tight junctions, as confirmed by histological and immunohistochemical analysis. Functional assessments, including paracellular permeability assays and transepithelial electrical resistance measurements, demonstrated the formation of tight epithelial barriers. To evaluate the models' utility for studying innate immune responses against respiratory parasites, cultures were exposed to lipopolysaccharide, phorbol-12-myristate-13-acetate, ionomycin, and nematode antigens. Quantitative PCR analysis revealed significant changes in the expression of pro-inflammatory cytokines, including TNF and IL-33 when ALI cultures were exposed to lipopolysaccharide of *E. coli*, phorbol-12-myristat-13-acetat/ionomycin, or *Angiostrongylus vasorum* first-stage larval antigen.

By comparing red fox and dog ALI models, this study provides novel insights into the respiratory biology of a canid wildlife reservoir and a corresponding domesticated species evidencing potential differences that may develop during co-evolution between the animal hosts and the parasite. These models represent an important advancement for studying innate immune responses and therefore host pathogen interactions and disease transmission and can also be applied for investigations on zoonotic diseases.

P-15. SEROPREVALENCE OF CEPHENEMYIA STIMULATOR IN ROE DEER FROM THE IBERIAN PENINSULA.

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Keywords: Roe deer; *Cephenemyia stimulator*; seroprevalence; Oestrinae; Iberian Peninsula.

Nasopharyngeal myiasis caused by *Cephenemyia stimulator* is very common in roe deer from northwestern Spain, although in recent years it has spread to other areas of the Iberian Peninsula. The visualization of larvae in the nasopharyngeal cavity is complicated and laborious, especially for first instars (L1), so the detection of antibodies against *C. stimulator* by indirect ELISA could be a feasible method to monitor the prevalence and geographical distribution of this myiasis.

Between 2022 and 2024, 314 roe deer from different regions in Spain and Portugal were analyzed. The diagnosis was made by the detection of antibodies by an indirect ELISA using a somatic antigen obtained from *C. stimulator* L1 (CsL1 ELISA) and the results were compared to necropsy, that was used as gold standard.

The overall prevalence by ELISA was 57% with a sensibility and specificity of 85% and 77.8%, respectively. Necropsy showed a prevalence of 55.4%; the degree of agreement between both techniques was good ($\kappa=0.69$). Absorbance values showed a positive correlation with the number of total larvae. Seroprevalence increased significantly with the age of the animals, so that in those under 2 years was 46.5%, 61.6% for adults 2-5 years and 62.5% in older than 6 years. When considering the origin of the animals, the seroprevalence was high in the northern most regions of Spain (Galicia, Asturias, Cantabria, Basque Country, Navarra; 60-100%); moderated in Castilla-León (58.4%), La Rioja (57.1%), Catalonia (54.5%) and Northern Portugal (50.0%) and low in Aragón (12.5%) and Castilla-La Mancha (3.2%). It is worth noting the absence of exposition to this myiasis in Valencian and Madrid communities.

Our results reveal that the CsL1 ELISA is a helpful tool in determining the prevalence and expansion of *C. stimulator* by the Iberian Peninsula.

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P-16. CASES OF LUNGWORM INFECTIONS IN HEDGEHOGS (*ERINACEUS EUROPAEUS*) IN GREECE AND THEIR THERAPEUTIC APPROACH.

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Keywords: Hedgehog, *Crenosoma*, *Capillaria*, NexGard® Combo, *Erinaceus europaeus*

The European hedgehog (*Erinaceus europaeus*) is one of the most common European wild mammalian species and it is widespread in Greece. Human related causes as well as infectious conditions have resulted in a declining tendency of their populations. Lungworm infections are the most common cause of respiratory disease in European hedgehogs. Lungworms are transmitted both through gastropods, which hedgehogs feed on, and also directly. They can cause severe pneumonia, which is usually complicated by bacterial infection and if left untreated, could become life threatening.

A group of 13 hedgehogs suffering from sarcoptic mange were admitted in a Greek wildlife rehabilitation center (ANIMA). One of them, with nasal discharge, passed away a few days after its admission. The necropsy and the lung histopathology confirmed the presence of lungworms and pneumonia lesions. Therefore, the rest of the animals (n=12) were included in the present study. Faecal samples were collected individually from all the animals and examined with a flotation method using ZnSO₄ (33.2%) in combination with the Baermann method in order to assess the presence of lungworm larvae. All (100%) of them had *Crenosoma* spp. larvae in their faeces and 6 (50%) of them also excreted *Capillaria* spp. eggs. The animals were treated using the combination of esafoxolaner, eprinomectin and praziquantel (NexGard® Combo, Boehringer Ingelheim) spot on at a dose rate of 0.2 ml/Kg BW. At 7, 10 and 14 days post treatment no parasitic elements were detected in the faeces of any of the animals.

The current study confirms for the first time common lungworm infections by *Crenosoma* spp. and *Capillaria* spp. in European hedgehogs in Greece. Given the high incidence of parasitic pneumonias in wild hedgehogs presented in wildlife hospitals worldwide, the treatment of lungworm infections plays a significant role in the conservation of this animal species. This study supports the efficacy and safety of the combination of esafoxolaner, eprinomectin and praziquantel against the lungworm infections in hedgehogs and offers an easy to apply treatment option.

P-17. PREVALENCE AND MOLECULAR IDENTIFICATION OF EIMERIA SPP. INFECTION IN PERI- WEANED DAIRY CALVES.

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Keywords: bovine coccidiosis, dairy calves, *Eimeria* spp., PCR, prevalence

Bovine coccidiosis, caused by the protozoa *Eimeria*, is an important parasitic disease that affects dairy calves' health and welfare, contributing to financial losses. The objective of the present study was to identify the different species of *Eimeria* infection in dairy calves, during peri-weaning period, through molecular approach and to determine their prevalence, for the first time to the best of our knowledge. From May 2024 to February 2025, 665 faecal samples were collected from peri-weaned dairy calves. Conventional polymerase chain reaction (PCRc) was used for the detection of *Eimeria* genus in faecal samples collected. The PCRc positive samples were, further, processed for *Eimeria* species identification. They were identified by multiplex PCR (PCRm), amplifying 18S RNA gene and confirmed by DNA sequencing. PCRc results showed that 308 (46.3%) of 665 faecal samples were positive for *Eimeria* spp. The prevalence of single *Eimeria* species infection was 49%. Among positive samples, PCRm identified five species of *Eimeria*, namely, *Eimeria bovis* (24.7%), *E. zuernii* (14.0%), *E. alabamensis* (5.8%), *E. ellipsoidalis* (3.6%), and *E. cylindrica* (1.0%), as single infections. Regarding mixed infections status (i.e., over 2 species of *Eimeria* identified in each faecal sample), the prevalence was 51%. DNA sequencing revealed the similarity of each *Eimeria* spp. with 96% – 100% nucleotide identity. The results confirmed the presence of *Eimeria* spp. among peri-weaned dairy calves. *E. bovis* and *E. zuernii* were the most predominant species in our study and the most pathogenic, according to literature review. These data could provide epidemiological information for developing future strategies to control bovine coccidiosis in Greece.

P-18. IN VITRO EFFECT OF PLANT EXTRACTS ON DIFFERENT DEVELOPMENT STAGES OF TRICHOSTRONGYLUS COLUBRIFORMIS AND HAEMONCHUS CONTORTUS OF SHEEP.

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Keywords: *Trichostrongylus colubriformis*, *Haemonchus contortus*, *In vitro* tests, Plant extracts

The nematodes residing in the abomasum (i.e. *Haemonchus contortus*) and the small intestine (i.e. *Trichostrongylus colubriformis*) reduce voluntary feed intake and nutrient absorption, impacting the production of small ruminants drastically [1]. The control of these gastrointestinal nematodes is traditionally achieved with the use of anthelmintic drugs, however due to regulations in organic farming and the rise in anthelmintic resistance (AR), alternatives are sought after.

The aim of the present study was to evaluate the *in vitro* anthelmintic effects of borage (*Borago officinalis*), wild mallow (*Malva silvestris*) and chicory (*Cichorium intybus*) extracts on *T. colubriformis* and *H. contortus* of sheep.

Extracts of three plants were tested *in vitro* on two development stages (eggs and infective larvae) of *T. colubriformis* and *H. contortus* using the Egg Hatch Assay (EHA) and the Larval Exsheathment Inhibition Assay (LEIA). The egg hatching rate was measured after incubation with each natural extract (concentrations: 150, 300, 600, 1200 µg/mL) for 48 h at 26 °C. Ensheathed infective larvae were incubated for 3h at 20 °C with each plant extract (concentrations: 150, 300, 600, 1200 µg/mL) and subsequently evaluated their ability to inhibit the exsheathment of the larvae [2].

The inhibitions in the development of *T. colubriformis* and *H. contortus* eggs using EHA were 100% and 100% for borage, 100% and 61.9% for wild mallow, 100% and 86.3% for chicory extracts, respectively. The inhibitions of exsheathment *T. colubriformis* and *H. contortus* larvae using LEIA were 100% and 100% for borage, 100% and 98.7% for wild mallow, 100% and 86.9% for chicory extracts, respectively. Results showed that plant extracts of borage, wild mallow and chicory exhibits *in vitro* anthelmintic activity, suggesting that, these natural products can also be an ally for *T. colubriformis* and *H. contortus* control in sheep.

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P-19. ECHOCARDIOGRAPHIC STRAIN IMAGING DOES NOT REVEAL MYOCARDIAL DEFORMATION IN DOGS WITH LEISHMANIASIS.

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Keywords: canine leishmaniasis, strain, echocardiography

Myocardial involvement in canine leishmaniasis (CanL) has been demonstrated (1,2). The aim of this prospective study was to evaluate by conventional and Strain echocardiography the myocardial involvement in dogs with CanL throughout the left ventricular systolic function assessment and the myocardial deformities study. Dogs with a diagnosis of leishmaniasis, were included before treatment. At time of inclusion (T0), a conventional echocardiographic examination was performed, followed by echocardiographic strain imaging. Apical longitudinal long axis strain was quantified using 4-chamber-view, 3-chamber-view, and 2-chamber-view (2C, 3C, 4C). Dogs were monitored for six months under meglumine or miltefosine combined with allopurinol treatment protocol. Clinical-pathological and echocardiographic data were collected at follow up at 1, 3 and 6 months after treatment starting (T1, T2, T3). Statistical analysis using Friedman test was performed. Nine dogs were included in the study. On clinical examination, no dog had murmurs or arrhythmias. Standard echocardiographic examination at T0 excluded cardiac diseases. Systolic dysfunction was registered in a single dog (C6), who showed a clear improvement after treatment. Among strain values, only strain 4C showed statistical significance over time and anyway values were within the reference ranges throughout the study. In general, both conventional and strain echocardiographic examination failed to reveal changes suggestive of systolic dysfunction in active leishmaniasis. Detection of myocardial involvement in dogs with leishmaniasis by using noninvasive echocardiographic methods was not possible in this preliminary study on a small population. Anyway, the statistical differences revealed on few conventional and strain parameters and the findings evaluated in a single dog suggest further investigations of this field of application.

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P-20. VARIATIONS IN THE HEMATOLOGY OF ASYMPTOMATIC HORSES EXPOSED TO BLOODBORNE PATHOGENS.

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Keywords: equine, asymptomatic, blood, *Anaplasma*, *Babesia*, NW Spain

Exposure to different pathogens can result in significant variations in several blood parameters, which in certain occasions have repercussions on equine health and activity. In order to estimate the possible influence of the exposure of Galician horses to intracellular blood pathogens, blood samples were collected from 186 individuals from the

Galician Autonomous Community, which were analyzed to establish the variations in the parameters of the white and red blood series. At the same time, the presence of IgG antibodies against *Anaplasma phagocytophilum* (granulocytic anaplasmosis) and *Babesia* spp. was investigated by using commercial tests. Data were considered regarding breed, management, aptitude and sex.

Significant differences were established in the white cell parameters regarding breed and sex, with the lowest values in autochthonous Galician Pure Breed (GPB), and in mares. The values in the red cells were significantly different according to breed, management and aptitude, with the lowest values among the GPB and the stallions.

The IgG seroprevalence to *Anaplasma* was 39% (95% CI 32, 46), and significant differences were demonstrated in the counts of erythrocytes, hemoglobin and hematocrit, which decreased in seropositive individuals. In addition, differences were observed concerning breed, management and aptitude but no sex.

A percentage of 27% (22, 32) horses had positive IgG values against *Babesia* spp., and differences with sex and management were probed in the red blood cell values.

These data highlight the need to analyze the possible exposure of horses to pathogens that can affect the health status of horses, decreasing their activity and even their chances of survival.

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P-21. TICKS AND TICK-BORNE PATHOGENS IN THE GALICIAN STAGES OF THE FRENCH ST. JAMES WAY.

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Keywords: *Anaplasma*, *Borrelia*, Galicia, *Rickettsia*, St. James Way

Ticks can act as vectors of a wide variety of pathogens for people and animals. French St. James Way (Camino de Santiago) is a network of pilgrimages leading to the shrine of the apostle James in the cathedral of Santiago de Compostela in Galicia (Northwestern Spain). Most of this route is composed by wooded areas with native vegetation and fauna leading a suitable habitat for the maintenance of tick populations. The main objective of the present investigation was to identify the tick species and to determine the presence of tick-borne pathogens in the French St. James Way in Galicia.

Ticks were sampled by flagging in a total of 44 transects of the seven stages of the French St. James Way. After identification, ticks were pooled and DNA was extracted to perform the detection and molecular characterization of five pathogen genera (*Borrelia* spp., *Rickettsia* spp., *Anaplasma* spp., *Babesia* spp. and *Theileria* spp.).

Ixodes ricinus (93.9%) was the most abundant tick species in all the sampled stages followed by *Ixodes frontalis* (3.3%), *Dermacentor marginatus* (2.3%) and *Rhipicephalus sanguineus* (0.5%). *Borrelia* genospecies associated with Lyme disease were detected in a high percentage of the *I. ricinus* pools (28.4%). In addition, zoonotic *Rickettsia* spp. (*Rickettsia felis*, *R. raoultii* and *Candidatus Rickettsia rioja*) were detected in *D. marginatus* (83.3%) and *I. ricinus* (12.5%) pools and one pool of *D. marginatus* and *I. ricinus* was positive to *Anaplasma ovis* (16.7%) and *Babesia venatorum* (0.97%), respectively.

These results are of great interest from a scientific and social point of view, since there is no data on the species of ticks and pathogens present in the areas included in the St. James Way and most of the detected pathogens are zoonotic. This knowledge will facilitate the implementation of control strategies by providing information on how to prevent bites.

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P-22. A PRELIMINARY STUDY TO DETERMINE THE RISK OF CULICIDS IN THE TRANSMISSION OF VECTOR-BORNE DISEASES IN WETLANDS FROM NW.

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Keywords: *Culex*, *Aedes*, *Anopheles*, Wetlands, Galicia

Wetlands are known for being used as migratory stopovers or breeding grounds by birds but also as ideal breeding environments for mosquitoes, due to the constant presence of stagnant water, essential for the development of immature stages. With the aim of identify culicids present in several wetlands in Galicia, northern Spain, between June and November of 2024, an entomological survey was performed using dipping technique and posing miniature CDC-UV light and BG-Sentinel traps running continuously for 48 hours. Samples were then transported to the COPAR laboratory, and immediately frozen at -20°C. Finally, culicids were sorted from another dipteran and identified using a taxonomic key (Becket et al., 2020).

The presence of culicids was confirmed in almost 86% of the sampling areas. A total of 331 specimens from four genera were identified: 194 *Culex*, 8 *Culiseta*, 40 *Anopheles* and 89 *Aedes*. The most frequent species was *Culex pipiens* with almost 53% of the total captures. The second most abundant genus was *Aedes* (26,89%) but species could not be identified due to the genitalia in males and females lead to contradictory results between *Ae. pullatus*, *Ae. detritus* and *Ae. mariaae*. Finally, *Anopheles maculipennis* (3,37%) and *An. claviger/petragnani* (1,28%) were identified and all the specimens of *Culiseta* belonged to *Cs. longiareolata*.

The presence of culicids and birds in wetlands constitutes a risk for the introduction and circulation of several zoonotic agents in Galicia. Birds are the main species in which mosquito-borne viruses circulate because of their role as reservoir and they are also considered important bridge vectors for other domestic animals, pets and humans. Entomological surveillance of wetlands is crucial for detecting the introduction of arbovirus such as West Nile Virus, Sinbdi virus and Usutu virus in Galicia, being the best-known tool for prevention and control programmes.

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P-23. ANALYSIS OF CURRENT RISK OF LEISHMANIA INFANTUM TRANSMISSION IN GREECE AND ITS PROJECTION.

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Keywords: *Leishmania infantum*, *Phlebotomus perfiliewi*, *Phlebotomus tobbi*, Ecological Niche Model, Greece.

Canine leishmaniosis is a vector-borne disease caused by the protozoan parasite *Leishmania infantum*. It is transmitted by different species of the genus *Phlebotomus*, with *Phlebotomus perfiliewi* and *Phlebotomus tobbi* being the most widely distributed species in Greece. In Greece, reported seroprevalence rates vary depending on the analyzed region, ranging from 0% to 53%. The overall seroprevalence of *L. infantum*-positive animals is 13.8%, with the highest incidences found in the geographical regions of Macedonia (62%), Thrace (34%), and Central Greece (23%). Ecological niche models are ecoinformatics tools that have already been successfully applied to model risk maps of other parasitosis according to the environmental variables necessary for their survival. This study aimed to develop a potential infection risk map for canine leishmaniosis in Greece by integrating ecological niche models of two disease vectors

(*Ph. tobbi* and *Ph. perfiliewi*) and the *L. infantum* infection rate in the vector (% of sandflies infected by *L. infantum*). Habitat suitability models for vectors were performed with the maximum entropy algorithm (Maxent) optimised with KUENM package of R based on bioclimatic conditions (variables related to temperature and precipitation), human footprint and density of shrubs and herbs. In turn, the infection rate was also calculated with a custom R script based on annual mean temperature values. The risk map presents a higher risk of infection in urban, coastal and low altitude territories. Central Macedonia, Thessaly, Attica and islands such as the Cyclades, Dodecanese and Crete, as well as some urban areas of Thrace close to the coast, present a high risk of infection. Mountainous areas in the interior of the continent, with a colder and drier climate, had a low risk of infection. The projected model for 2080 shows an increased risk of infection in the western territories of the country and towards higher altitudes, increasing by 9.6% compared to the current risk.

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P-24. TESTING THE EFFECT OF PLANT COMPOUNDS ON ASCARIDIA GALLI WITH AN IN VITRO LARVAL MOTILITY ASSAY.

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Keywords: ascariasis, poultry, plant-based anthelmintics

Introduction

Ascaridia galli infections have a major impact on poultry health and productivity. *In vitro* assays can be used to screen compounds for anthelmintic activity, reducing the need for *in vivo* studies. We present a method for assessing the effects of plant compounds on the motility of third-stage *A. galli* larvae (L3).

Methods

Live adult *A. galli* were incubated in saline or RPMI with and without antimicrobials (Penicillin-Streptomycin and Amphotericin B) at 37.5°C with 5% CO₂. Eggs were collected, cultured in 50% 1M H₂SO₄ at 22°C for three weeks, and stored at 5°C until use. Initial testing of selected plant extracts was performed. Eggs were hatched using chlorine (30% in water), washing and overnight incubation in HBSS, followed by centrifugation and Baermannization with HBSS. Larvae were transferred to a 96-well plate with RPMI

(~50 L3 per well). Two-fold dilutions of plant compounds, in DMSO or water, were added in duplicates. Negative (1% DMSO or 50% water) and positive (50 ug/mL Levamisole or Ivermectin) controls were included. Plates were incubated at 37.5°C with 5% CO₂. Motile larvae were counted before adding compounds and after 24 and 48 hours.

Results

Egg yield and embryonation suggested that saline without antimicrobials was most favourable for worm cultures. Larvae yield from hatching was >50% of embryonated eggs. Full effect of compounds was observed after 48 hours, where negative controls showed <10% motility loss, while plant compounds caused up to 100% motility loss. Dose-responses have been observed. Variation was observed in positive controls (motility loss 54-98%), which could be due to limited drug suspension stability.

Conclusion

The larval motility assay represents a simple method with low equipment requirements. Further validation of the method and investigations on various plant compounds are needed, but the method shows promise for initial screening of new treatment options for *A. galli*.

P-25. RELATIONSHIP BETWEEN ENVIRONMENTAL FACTORS AND OCCURRENCE OF CARDIOPULMONARY NEMATODES IN THE EURASIAN BADGER (MELES MELES) IN SEMI-ARID MEDITERRANEAN AREAS OF SPAIN.

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Keywords: Eurasian badger, cardiopulmonary nematodes, soil conditions

The Eurasian badger (*Meles meles*) is a mesocarnivore with wide distribution in Mediterranean areas, such as the Region of Murcia (SE Spain). Climatic conditions may lead to variations in badger's food sources, which could affect the species of nematodes parasitizing the badgers. This study aims to identify the cardiopulmonary nematodes in badgers from the Region of Murcia and to analyse the environmental variables that may influence the presence or intensity of these parasites.

From 2016 to 2019, the cardiopulmonary tracts of 37 badgers were collected (19 females and 19 males; 8 juveniles, 29 adults). Three consecutive techniques were used to extract the nematodes: opening of the cardiorespiratory tract, squeezing the lung parenchyma, and finally parenchyma digestion [1]. Prevalence, abundance, and intensity of nematodes were calculated [2]. The location of the individuals was determined by geographical coordinates for obtaining information about different environmental variables and to analyse the relationship between the presence or intensity of parasites and the environmental conditions in the study area. Significance threshold was established for p-value <0.05. A total of eight badgers were positive (P= 21.6%; 8/37). Three genera were identified, but only one to species level: *Vigisospirura* spp. (P=10.8%, 4/37, MI= 1-8), *Aelurostrongylus falsiformis* (P=2.7%, 1/37, 138 specimens) and *Angiostrongylus* spp. (P=2.7%, 1/37, one specimen). Statistically significant results were not observed between prevalence and host variables (sex and age category). Nevertheless, the environmental variables "acid pH soil" and "medium temperature" were negatively correlated with the occurrence of parasites found, while soil humidity and altitude were positively correlated. This study presented the first report of *A. falsiformis* in semi-arid Mediterranean areas of the Iberian Peninsula. Our study suggests that environmental factors likely influence the presence of intermediate hosts (mainly gastropods) of the detected parasites, and therefore, their occurrence in the badger.

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P-26. VECTOR-BORNE DISEASES IN PET DOGS FROM BUCHAREST, ROMANIA: INSIGHTS FROM A PRIVATE VETERINARY CLINIC.

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Keywords: Bucharest, dogs, Romania, serology, vector-borne diseases

Canine vector-borne diseases (CVBD) constitute a heterogeneous group of infections caused by bacterial, viral, and eukaryotic parasitic agents, transmitted through hematophagous arthropod vectors. The epidemiological significance of CVBD has increased in response to global climate changes, enhanced human mobility, and the zoonotic potential

of *Dirofilaria* spp., *Leishmania infantum*, *Ehrlichia* spp., and *Anaplasma* spp. Several vector-borne pathogens are known to exhibit dual infectivity in both canines and humans, thereby posing a significant public health concern. In endemic regions, domestic dogs act as major reservoir hosts, facilitating the maintenance and transmission of these pathogens within susceptible populations.

This study aimed to assess the prevalence of major CVBDs in domestic dogs referred to a private clinic, in the city of Bucharest, Romania's largest urban center. Serological diagnostic tests were performed over 5 years (2020-2024), for 317 dogs, using two rapid immunoassays: SNAP 4Dx Plus and SNAP *Leishmania*. The cumulative seroprevalence of CVBDs was 17%, with *D. immitis* exhibiting the highest prevalence, at 12.62%. The seroprevalence of *Ehrlichia* spp. displayed temporal fluctuations, peaking at 8.33% in 2022 and yielding an overall prevalence of 4.73%. A progressive increase in *Ehrlichia* spp. seropositivity over the five-year surveillance period correlated with the documented presence of *Rhipicephalus* spp. ticks in the region. Similarly, *Anaplasma* spp. exhibited a rising prevalence trend, with an overall seroprevalence of 4.42%, increasing from 2.94% in 2020 to 8.33% in 2024. In contrast, *Borrelia burgdorferi* demonstrated low seroprevalence, and no serological evidence of *Leishmania* spp. infection was detected. Overall, three cases of coinfection, representing 4.47% of positive dogs, were diagnosed.

This study establishes Bucharest as an endemic region for dirofilariosis and ehrlichial infections, highlighting their epidemiological relevance and potential public health risks, underscoring the necessity of targeted prevention measures and vector management programs.

P-27. REPEATED REPRODUCTIVE FAILURE OF A MARE DUE TO HALICEPHALOBUS SP. INFECTION.

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Keywords: *Halicephalobus gingivalis*, free-living nematodes, abortion

The genus *Halicephalobus* comprises free-living nematodes, which occasionally infect vertebrates. Reported equine, bovine and human cases were mostly fatal, with female nematodes, larvae and eggs identified in granulomatous lesions, predominantly in the central nervous system and the kidneys. Of the eight described species within the genus, only *Halicephalobus gingivalis* has been incriminated as causative agent of vertebrate infections, although the diagnosis was based on morphology in most cases.

Here, a case of repeated reproductive failure due to *Halicephalobus* sp. infection in a Connemara mare is described. In April 2024, a mature foal was stillborn. The mare did not show any clinical signs indicative of *Halicephalobus* infection and was inseminated again in August, but aborted in December 2024. Organs and placenta of the stillborn foal and the aborted fetus were subjected to post-mortem examination and histopathology. DNA isolated from tissue samples was subjected to PCR and sequencing of the 18S rRNA-internal transcribed spacer (ITS) 1-5.8S rRNA-ITS2 region for nematode species identification.

In both cases, histopathology showed granulomatous lesions in the placenta with presence of adult nematodes characterized by a rhabditiform oesophagus as well as nematode larvae. In the first case, multiple nematodes were also identified in the foal's kidneys and central nervous system. In the second case, nematodes could be demonstrated in the fetal liver and lungs, indicating a disseminated infection in both cases. PCR and sequencing of the nematodes' partial 18S rRNA-internal transcribed spacer (ITS) 1-5.8S rRNA-ITS2 region yielded a ~1000 bp sequence, 94% identical to a *H. gingivalis* isolate from Germany. Considering only the highly conserved partial 18S rRNA sequence, similarity with various *Halicephalobus* spp. sequences in Genbank ranged from 90-99%. The genetic data point towards a cryptic diversity among *Halicephalobus* spp. isolated from vertebrate tissues. These opportunistic pathogens may be a rare cause of equine reproductive failure.

P-28. PHYLOGENY OF THE SUBGENUS PHORTICA SENSU STRICTO (DIPTERA: DROSOPHILIDAE) FROM EUROPE.

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Keywords: fruit fly vector, *Phortica variegata*, *Thelazia callipaeda*

Drosophilid flies (Diptera: Drosophilidae) generally feed on fruits. However, in the genus *Phortica* – especially at members of the subgenus *Phortica sensu stricto* – several species are lachryphagous, feeding on tears from mammals. Through this behaviour, *Phortica okadai* and *P. variegata* are vectors of the Oriental eyeworm *Thelazia callipaeda* (Spirurida: Thelaziidae). From Asia there are over 80 *Phortica* species in the subgenus *Phortica s.s.* known while in Europe, only three species have been described. *Phortica variegata* and *P. semivirgo* are present in numerous European countries, while *P. erinacea* has only been described from Bulgaria. Furthermore, *P. oldenbergi* (subgenus *Allophortica*) is also present in Europe. In recent studies, *Phortica* spp. were detected in Austria and Italy, which could not be assigned to any of the known European species [1, 2].

To investigate the presence of unknown *Phortica* spp. in other European countries, over 300 *Phortica* specimens were analysed. These were either archived from previous studies or were collected for the purpose of this study. Samples could be obtained from Albania, Austria, Bulgaria, Czechia, France, Germany, Hungary, Italy, Luxembourg, Montenegro, Portugal, Romania, Sweden, Spain, Switzerland, and the United Kingdom. Collection efforts in Bosnia and Herzegovina, Poland, Slovakia, and Ukraine were unsuccessful. By screening two regions of the cytochrome c oxidase subunit I unknown *Phortica* spp. were detected in Austria, Czechia, Hungary, and Italy. A phylogenetic analysis of

European and Asian *Phortica s.s.* is presented to determine the relationship to other *Phortica*. In the future, the genetic marker 28S ribosomal RNA and NADH dehydrogenase 2 will be investigated in selected samples and morphological analyses of the unknown specimens will be performed.

Detailed knowledge about the present species and their phylogenetic relation is necessary to subsequently investigate vector competence of European *Phortica* spp. other than *P. variegata*.

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P-29. TREATMENT OF SARCOPTES MANGE IN LLAMAS AND ALPACAS WITH MOXIDECTI.

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An outbreak of sarcoptic mange was investigated in a herd of three female llamas (1, 2, and 4 years old) and four male alpacas (3-3,5 years old) in the Black Forest (Baden-Wuerttemberg, Germany). The diagnosis was made by clinical picture and detection of mites in skin and ear scrapings. At the beginning numerous of *Sarcoptes* mites were found in the scraping samples. The llamas and alpacas were treated with 0,2 mg/kg bdw. moxidectin (Cydectin® 1% inj.: Zoetis Deutschland GmbH, Berlin, Germany) subcutaneously (2 ml per llama, 1,5 ml per alpaca) every 21 d on days 0, 21, 42, 63, 84, 105, 126, 147, and 168. No other treatment or environmental decontamination was performed during the trial. Because of the slow recovery of the South American camelids it was necessary to repeat the treatment eight times. On days 0, 42, 84, 126, and 168, all animals were examined clinically, and epidermal debris were collected from both auricular areas and other body regions for microscopic examination. The alpacas recovered rapidly and mite counts declined steadily. Llamas showed a slower remission of mite counts and clinical condition. Clinical signs had subsided by day 126 in 3/4 alpacas and on day 168 in 2/3 llamas. All epidermal samples were negative by day 168. No adverse reactions were observed. Under the conditions of our trial, injectable formulation of moxidectin was a practical and well-tolerated means of treatment for sarcoptic mange in South American camelids.

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Conflicts of interest: Author is working as Area Veterinary Manager for Zoetis Deutschland GmbH

P-30. CIRCULATION AND DIVERSITY OF TRICHINELLA SPP. IN ROMANIAN WILDLIFE: MOLECULAR IDENTIFICATION AND NEW EPIDEMIOLOGICAL DATA.

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Keywords: *Trichinella* spp., wildlife, Romania, epidemiology

Trichinellosis is a parasitic disease that affects mammals, birds, reptiles, carnivores, and omnivores. In Europe, wildlife represents the main reservoir for *Trichinella* spp., acting as a source of infection for domestic pigs and, consequently, for other animals and humans. In Romania, wildlife, as well as domestic animal species, play an important role as a natural reservoir for *Trichinella* spp., and three species of the genus are present: *T. spiralis*, *T. britovi*, and *T. pseudospiralis*. The aim of this study was the identification and molecular characterization of *Trichinella* larvae isolated from the muscle tissue of wildlife collected in 28 counties of Romania. A total of 468 wildlife were examined, including: brown bears (*Ursus arctos*), wolves (*Canis lupus*), lynx (*Lynx lynx*), golden jackals (*Canis aureus*), red foxes (*Vulpes vulpes*), raccoon dog (*Nyctereutes procyonoides*), wildcats (*Felis silvestris*), badgers (*Meles meles*), pine martens (*Martes martes*), stone martens (*Martes foina*), European polecats (*Mustela putorius*), weasels (*Mustela nivalis*), otters (*Lutra lutra*), and wild boars (*Sus scrofa*). Muscle samples were examined by artificial digestion, and the larvae were identified to species level using multiplex PCR. *Trichinella* larvae were detected in 115/468 (24.57%) wild animals. Molecular identification of 115 larval isolates revealed the presence of three endemic species: *T. britovi* (93.91%), *T. spiralis* (5.21%, exclusively in wild boars), and *T. pseudospiralis* in a single jackal. It is well known that *T. spiralis* and *T. britovi* are circulated in the wildlife of Europe and Romania; however, *T. pseudospiralis* has been identified only recently in Romanian wildlife. This study provides new insights into the prevalence of *Trichinella* spp. in ten wild animal species. The identification of *Trichinella* spp. larvae in these wild hosts underscores their role as vectors of this zoonotic nematode and highlights their importance as a link between the sylvatic ecosystem and the peripheral domestic environment, posing a potential risk for human infection.

***P-31. PRELIMINARY INSIGHTS INTO THE GENETIC DIVERSITY OF HUMAN'S DIROFILARIA REPENS: A MOLECULAR ANALYSIS ON COX1 GENE.**

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Keywords: *Dirofilaria repens*, zoonosis, dirofilariasis, COX1

The genus *Dirofilaria* consists of parasitic nematodes responsible for dirofilariasis, a zoonotic disease affecting both animals and humans. Understanding the genetic diversity of *Dirofilaria* species is essential for epidemiological studies, disease control, and treatment strategies. The aim of this study to presents preliminary molecular analyses of *Dirofilaria repens* using three samples isolated from human cases in Poland. Genetic markers, including cytochrome c oxidase subunit 1 (cox1) was analysed to assess genetic variation. The phylogenetic analysis of *Dirofilaria* samples revealed two major clades. The first clade is divided into two subgroups: one representing *Dirofilaria repens* and the other *Dirofilaria immitis*, highlighting the genetic divergence between these two species. The second major clade consists of *Dirofilaria* sp., along with the outgroup *Setaria cervi*. Sequences isolated from human cases in Poland clustered within the *D. repens* subgroup, displaying slight genetic differences, indicating close similarity with other *D. repens* samples.

Conclusion: our findings confirm the presence of *D. repens* in human in Poland and there is no genetic diversity between the three analysed samples based on the cox1 gene.

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***P-32. HUMAN DIROFILARIASIS IN SLOVAKIA – EPIDEMIOLOGY AND CLINICAL MANIFESTATIONS.**

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Keywords: Dirofilariasis, Humans, Epidemiology, Clinical manifestations

Human dirofilariasis is considered an emerging parasitosis in Central Europe, including Slovakia, with globalization and climate change playing a significant role in its spread. The life cycle of *Dirofilaria* spp. involves carnivores, especially dogs, as definitive hosts and different species of mosquitoes as intermediate hosts or vectors of infection. The aim of the study is to summarize published and unpublished cases human of *Dirofilaria* infection in Slovakia, with an emphasis on their clinical picture and epidemiology.

The first human case of dirofilariasis in Slovakia was diagnosed in 2007, two years after the infection had been observed in the Slovak dog population. Since then, 34 human cases caused by *Dirofilaria repens* have been confirmed at the Institute of Parasitology SAS. Of them, 23 cases presented as subcutaneous form, in six patients ocular form was reported and in one case the worm migrated from subcutaneous to subconjunctival tissues. In addition, two patients suffered from rare pulmonary form of infection, in one case the parasite was located in the left testicle and in one patient a live worm was found in the sputum. In 2021, the first autochthonous case of human *D. immitis* infection manifesting as the formation of nodule in the right lung was confirmed in a woman from Trnava region (1). Moreover, several other cases of dirofilariasis were reported, but without sufficient details to confirm the diagnosis.

The majority of patients came from southern regions of Slovakia, which are considered endemic for *D. repens*. On the other hand, recent data from several European countries, including Slovakia, indicate dramatic increase of *D. immitis* in canine population. Therefore, we can expect an increase in both *D. repens* and *D. immitis* human cases in Slovakia in the future, highlighting the need for increased awareness of the disease among clinicians, dog owners and general public.

Acknowledgement

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*P-33. TRANSMISSION RISK MAPS OF CANINE ANGIOSTRONGYLOSIS CONSIDERING MULTIPLE POTENTIAL INTERMEDIATE HOSTS IN THE IBERIAN PENINSULA AND BALEARIC ISLANDS.

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Keywords: Ecological Niche Model, Europe, Dogs, Gastropod, Spain, Portugal.

The presence of certain gastropod species in the Iberian Peninsula and Balearic Islands (Spain) poses a certain risk for the transmission of canine diseases such as the “French heartworm”, caused by the parasite *Angiostrongylus vasorum*. The intermediate hosts responsible for spreading this parasite include land snails such as *Arion ater*, *Arion rufus*, *Arion vulgaris*, *Deroceras reticulatum*, and *Cornu aspersum*, which thrive in specific bioclimatic environments. These gastropods have contentious taxonomy and are widely distributed across regions with favorable climatic conditions, particularly in moist, temperate and northern areas of the Iberian Peninsula and the Balearic Islands. Environmental niche models (ENMs) are valuable tools in predicting the habitat suitability for these intermediate hosts by integrating bioclimatic, environmental and presence data. They also allow for the estimation of areas with high infection risk combining the preference of environmental conditions like temperature and humidity of different species [1, 2]. When considering multiple vectors or hosts, such as the various species of snails, conducting quantitative comparisons (ENM similarity) or weighting the contribution of each species to the overall infection risk is essential. This approach helps to generate more realistic risk maps that account for the ecological preferences and distribution of the host(s) and determine which host has the highest impact on the spread of the disease. By incorporating data from multiple vectors or hosts, researchers can produce more accurate and dynamic infection risk maps that predict potential outbreaks. This

data-driven approach aids veterinary professionals in targeting control measures more effectively, enhancing both prevention and management strategies for parasitic infections in dogs and other animals.

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*P-34. SEROLOGICAL INVESTIGATION OF VECTOR-BORNE INFECTIONS IN TURKISH DOGS WITH SPECIAL REFERENCE TO DIROFILARIA IMMITIS.

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Keywords: Dog, *Dirofilaria immitis*, *Ehrlichia*, *Anaplasma*, Türkiye

Vector-borne diseases (VBDs) (e.g. *Dirofilariosis*, *Anaplasmosis*, *Ehrlichiosis*, *Leishmaniosis*, *Babesiosis*) pose a significant health threat to canine populations worldwide. In Türkiye, all the VBPs mentioned above have also been documented in the past (1). One of the main causative agents of *Dirofilariosis* is *Dirofilaria immitis*, which has a wide distribution throughout many European countries (2). Studies have shown that the prevalence of *Dirofilariosis* ranged from 0.2% to 46% in dog populations in Türkiye, and infected dogs continue to be detected in new areas as the years progress. For example, while *D. immitis* was detected in only nine provinces until 2000, this number increased to 36 in the last quarter century.

A total of 661 dogs of both sexes, different ages and breeds, brought from more than 20 provinces across Türkiye to an Animal Rehabilitation Center in Bursa between December 2022 and December 2024, were the subject of this study. Dog blood samples were tested using four commercial rapid test kits for *D. immitis* antigen, *Ehrlichia* spp., and *Anaplasma* spp. antibodies. Results have shown that 144 (21.8%), 74 (11.2%) and 25 (3.8%) dogs were positive for *Ehrlichia* spp., *Anaplasma* spp. and *D. immitis*, respectively. *D. immitis* was more frequently positive in samples with *Ehrlichia* spp. compared to those with *Anaplasma* spp.. Over half of the dogs tested positive for *Dirofilariosis* (54.2%) displayed at least one of the following signs: coughing, dyspnoea, lethargy, cachexia, anaemia and exercise intolerance. Older age and larger animal size were identified as risk factors for *D. immitis*-infection. One of the *Dirofilaria*-positive dogs was from a province (Muğla) where *Dirofilariosis* had not been reported before. Given the zoonotic risks of *Dirofilariosis* and the lack of registered drugs (i.e. melarsomine) for dogs in Türkiye, it is important to conduct screening studies in untested regions. This will assist in identifying endemic areas and developing risk maps to enhance animal and public health.

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*P-35 EVALUATION OF THE INTERACTION OF ANGIOSTRONGYLUS VASORUM WITH THE HOST USING AN IN VITRO MODEL OF ENDOTHELIAL CELLS.

Collado-Cuadrado, M.¹, Sotillo, J.², Vázquez-Ávila, S.², Montero-Calle, A.³, Barderas R.^{3,4}, Infante González-Mohino, I.¹, Rodríguez-Escolar, I.¹, Balmori-de La Puente, A.¹, Alarcón-Torrecillas, C.⁵, Pericacho, M.⁵, Morchón, R.¹

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Keywords: *Angiostrongylus vasorum*, Cellular processes, Endothelial cells, Proteomic.

Knowledge of the parasite-host interaction is one of the key tools when dealing with a parasitic disease. Previously, studies have been carried out on the relationship of several parasites with the host and the cellular processes on which they intervene, as in the case of *Dirofilaria immitis*, where their effect on processes such as fibrinolysis or angiogenesis has been demonstrated. However, in the case of *Angiostrongylus vasorum* there are not many studies on the parasite-host relationship. Therefore, this work aims to evaluate the effect of *A. vasorum* in different processes in order to deepen the knowledge of the relationship between the parasite and its host.

For this purpose, a primary culture of human umbilical vein endothelial cells (HUVEC) treated for 24 hours with *A. vasorum* somatic antigen was used. Non-stimulated cells were used as a control group under the same conditions. Subsequently, cell lysates and supernatants were collected and a mass spectrometry study was performed, identifying between 12,000-12,300 proteins in the lysates and 1400-1500 proteins in the supernatants.

Gene ontology analysis of the identified proteins was performed and the differences in protein expression between the stimulated cells and the control group were evaluated. Stimulation of the cells with *A. vasorum* produced significant differences in the expression of 467 proteins in the cell lysates, 34 of which were related to the angiogenic process, 51 to cell migration, 48 to cell proliferation, 14 to coagulation and 3 to fibrinolysis. In relation to the supernatants, significant differences were obtained in the expression of 196 proteins, 24 of which were related to the angiogenic process, 35 to cell migration, 26 to cell proliferation, 8 to coagulation and 2 to fibrinolysis.

Thus, observing the proteins with significant differences in their expression allows us to see the effect of *A. vasorum* on cellular processes such as cell migration and proliferation, thus allowing us to deepen our knowledge of the parasite-host relationship.

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*P-36. RELATIONSHIP OF DIROFILARIA REPENS WITH THE HOST THROUGH THE STUDY OF THE EFFECT OF THE DR20/22 PROTEIN ON THE EXPRESSION OF ANGIOGENESIS-RELATED PROTEINS.

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Keywords: *Dirofilaria repens*, Dr20/22, angiogenesis, VEGF-A, endothelial cells.

Angiogenesis is a process by which new blood vessels are formed from pre-existing ones that can occur pathologically in response to stimuli such as hypoxia, where cells produce angiogenic factors, one of the main ones being vascular endothelial growth factor (VEGF). Taking into account previous investigations in which the angiogenic potential of *Dirofilaria repens* somatic antigen (DrSA) has been demonstrated, this study aims to evaluate the angiogenic capacity of the Dr20/22 protein from the same parasite.

For this purpose, an in vitro model of human umbilical vein endothelial cells (HUVEC) was used, which were treated for 24 hours with the following stimuli: Dr20/22, VEGF-A, Dr20/22 + VEGF-A and unstimulated cells that were used as a control group. Finally, cell lysates and supernatants were collected and a mass spectrometry study was performed, identifying between 4,300-4,400 and 570-620 proteins, respectively.

Gene ontology analysis of the identified proteins and a subsequent evaluation of the differences in the proteomic profile in each of the treatments were performed. Regarding cell lysates, treatment of cells with Dr20/22+VEGF-A produced significant differences in the expression of 553, 289 and 309 proteins in comparison with the control group, cells treated with Dr20/22 and cells treated with VEGF-A respectively, within which 35 are related to the angiogenic process in the comparison with the control group and 24 in comparison with cells treated with Dr20/22 and cells treated with VEGF-A. With respect to the supernatants the treatment with Dr20/22+VEGF-A produced significant differences in the expression of 102, 64 and 293 proteins with respect to the control group, cells treated with Dr20/22 and cells treated with VEGF-A respectively, within which 16, 10 and 32 are related to the angiogenic process.

Thus, taking into account the changes in the proteomic profile with respect to the expression of proteins related to angiogenesis, the Dr20/22 protein contributes to this process, thus facilitating the survival of the parasite in the host.

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*P-37. THE ROLE OF 4 RECOMBINANT PROTEINS OF DIROFILARIA IMMITIS IN THE ANGIOGENIC PROCESS AS A MECHANISM OF SURVIVAL IN THE HOST.

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Keywords: Recombinant Proteins, *Dirofilaria immitis* excretory/secretory antigens, angiogenesis, VEGF-A, endothelial cells.

The angiogenic potential of 4 recombinant proteins from *Dirofilaria immitis* excretory/secretory antigens [actin (rDiACT), galectin (rDiGAL), fructose biphosphate aldolase (rDiFBAL) and glyceraldehyde 3-phosphate dehydrogenase (rDiGAPDH)] has recently been demonstrated by stimulating the expression of proangiogenic factors in addition to other cellular processes. Thus, this study aims to evaluate the effect of rDiACT, rDiGAL, rDiFBAL and rDiGAPDH on the expression of angiogenesis-related proteins and to deepen the knowledge of the parasite-host relationship.

For this purpose, we used an in vitro model of human umbilical vein endothelial cells (HUVEC) that were treated for 24 hours with the following stimuli: rDiACT, rDiGAL, rDiGAPDH, rDiFBAL, VEGF-A, rDiACT+VEGF-A, rDiGAL+VEGF-A, rDiGAPDH+VEGF-A, rDiFBAL+VEGF-A and unstimulated cells as a control group. Subsequently, cell lysates and supernatants were collected for subsequent mass spectrometry study where between 4,600-4,800 and 550-600 proteins were identified respectively in all treated groups.

Gene ontology studies were performed to evaluate the processes involved in these proteins and to analyze the changes produced by the stimulations on protein expression. Stimulation with VEGF-A-supplemented proteins produced significant differences in the expression of 1618, 605 and 769 proteins in cell lysates compared with the control, cells stimulated only with proteins and cells stimulated with VEGF-A, respectively, of which 76, 40 and 50 were related to the angiogenic process. In the analysis of the supernatants, protein stimulation supplemented with VEGF-A produced significant differences in the expression of 565, 470 and 741 proteins compared with the control, cells treated only with proteins and cells treated with VEGF-A, of which 87, 53 and 61 were related to the angiogenic process.

Stimulation of the cells with the different proteins supplemented with VEGF-A produced changes in the expression of several angiogenesis-related proteins, supporting previous studies demonstrating the interaction of *D. immitis* with the angiogenic process as a possible survival mechanism.

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*P-38. CYPRUS: A HEARTWORM-FREE COUNTRY UNDER THE THREAT OF INFECTION INVASION.

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Keywords: *Dirofilaria immitis*, Cyprus, imported cases, epizootiology

Cyprus, an island in the eastern Mediterranean Sea, remains heartworm-free, as shown in a recent epizootiological study investigating the prevalence of *Dirofilaria* spp. infections in dogs¹.

Since then, at least four cases of canine heartworm infection have been recorded. These dogs tested seropositive, although none exhibited clinical signs consistent with heartworm disease. In addition to serology, one dog tested positive on both Knott's and PCR tests, while two tested positive on PCR alone. One seropositive dog was PCR-negative. Two of the dogs had a confirmed history of travel to a hyperenzootic area in Greece, while the travel history of the other two remains unknown. The three PCR-confirmed cases received a "slow kill" heartworm treatment. Only one was retested nine months later with an in clinic antigen test and was found negative.

Cyprus' abundant native and invasive mosquito populations, which include confirmed heartworm vectors, coupled with favourable climate conditions, create an environment conducive to heartworm transmission. The introduction of a critical number microfilaraemic dogs can easily change Cyprus' status to enzootic. More concerning is the potential introduction of macrocyclic lactone-resistant *D. immitis* strains, which would pose a significant threat². If such strains became established in a heartworm free area, they would be the dominant population, compromising the primary preventive measure against heartworm, i.e. macrocyclic lactone administration.

Given the potential risk of heartworm establishment in Cyprus, it is crucial for veterinarians and pet owners to remain vigilant. Screening (serology and Knott's test) for heartworm infection in dogs arriving to Cyprus from an enzootic area and the implementation of prophylactic treatment in case of travel to enzootic areas are essential. Veterinary professionals play a key role in educating pet owners about the risks associated with heartworm disease and the importance of preventive strategies, particularly in light of the emerging threat of macrocyclic lactone-resistant strains.

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*P-39. CO-INFECTION OF MOSQUITOES WITH DIROFILARIA SPP. AND THE WEST NILE VIRUS.

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Keywords: Mosquitos, co-infection *Dirofilaria* spp., West Nile virus

Co-infection of mosquitoes with *Dirofilaria* spp. and the West Nile virus is a complex ecological phenomenon that raises significant concerns for public and veterinary health. Mosquitoes, particularly those of the *Culex* genus, which are the primary vectors of the West Nile virus, can serve as hosts for various pathogens, including parasites like *Dirofilaria* spp. and viruses such as the West Nile virus.

Recent studies have shown that the presence of *microfilariae* in mosquitoes can increase the infectivity of arboviruses. The *microfilariae* could also introduce the virus into the mosquito's hemolymph, transforming a species that would normally not be competent for virus transmission into a species capable of transmitting it. Furthermore, the co-presence of these pathogens could accelerate the virus's development within the vector, speeding up the transmission process and allowing the mosquito to infect the final hosts in a shorter time than would normally occur.

In order to expand the limited data in our area, we are monitoring the presence of *Dirofilaria* spp. in mosquitoes, without targeting a specific species, captured in Emilia-Romagna during the entomological surveillance conducted by the "Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna (IZSLER)" in 2024. The positive pools are then tested for the presence of the West Nile virus. So far, 2 mosquito pools positive for *Dirofilaria immitis* and West Nile virus Lineage 1 have been found in the provinces of Reggio Emilia and Bologna.

*P-40. DIROGEN: Deciphering the Genetic Diversity of *Dirofilaria repens* and *D. immitis*: A Global and European Perspective.

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Keywords: *Dirofilaria immitis*, *D. repens*, Genetic diversity, Zoonosis, Metabarcoding

Dirofilariasis, a zoonotic disease caused by *Dirofilaria* nematodes, is expanding geographically due to climate change with dogs as main reservoir hosts. The two primary species, *D. repens* and *D. immitis* (canine heartworm), exhibit significant ecological and epidemiological differences, with heartworm being found in warmer regions than *D. repens* (Capelli et al. 2018, Alsarraf et al. 2021, Fuehrer et al. 2021). Humans can be infected by the range of *Dirofilaria* species, however, molecular identification of parasites from human cases is very limited and urgently needed. Despite the global distribution of *D. repens* and *D. immitis*, knowledge of their genetic diversity and transmission dynamics remains limited.

This study aims to assess the genetic diversity of *D. repens* and *D. immitis* across continents using metabarcoding and whole genome sequencing, identify species and haplotypes infecting humans to elucidate zoonotic transmission routes, and investigate genetic variation among adult worms infecting individual canine hosts. Samples from dogs,

cats, wildlife, and humans will undergo DNA extraction, PCR, and advanced sequencing (Sanger, NGS). Data will be analyzed using bioinformatics tools for population genetics and phylogenetics.

This research addresses critical gaps in *Dirofilaria* epidemiology, aiding global control efforts and understanding zoonotic risks.

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*P-41. ANGIOSTRONGYLUS VASORUM IN SLOVAKIA – WHAT WE KNOW, WHAT WE DON'T, AND WHAT'S AHEAD.

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Keywords: *Angiostrongylus vasorum*, Dogs, Foxes, Epidemiology, Clinical cases

Angiostrongylus vasorum is a parasitic nematode that, around the turn of the millennium, crossed the area of its historical endemic foci and spread to new territories in Europe. In Slovakia, the first cases of canine angiostrongylosis were reported in 2012 and 2013 in the eastern part of the country. A serological survey conducted a year later revealed a relatively high overall seropositivity rate of 6.22%. Specifically, 14 out of 225 dogs tested were found to have circulating antigens or specific antibodies for *A. vasorum*. Subsequent copro-epidemiological research revealed a relatively high prevalence of the parasite in dogs (4.13%) and red foxes (5.43%), with the distribution primarily clustered in the eastern regions of Slovakia. Although angiostrongylosis is usually presented with cardio-respiratory clinical signs, the majority of infected dogs identified within the study were asymptomatic. However, in recent years, there have been more reports of severe cases, including fatal outcomes or cases of ectopic localisation of the nematode.

It has been over a decade since comprehensive research on the geographical distribution of *A. vasorum* in Slovakia, and current data mainly comes from sporadic reports, typically of fatal infections. Therefore, in consideration of the indirect life cycle of the parasite, ongoing climate change that may alter the mollusc biology, and other factors such as the distribution of the main reservoir hosts – foxes, continuous monitoring essential to gather updated information on the current distribution of the parasite and the factors driving its transmission. Equally important is raising awareness among pet owners and veterinarians about this potentially fatal parasitic infection, disease, as well as promoting prevention, proper diagnostics, and effective treatment.

Acknowledgement

This work is supported by the ESCCAP SK&CZ.

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*P-42. MAPPING THE CURRENT RISK OF TRANSMISSION OF DIROFILARIOSIS IN COLOMBIA MONTH BY MONTH THROUGH ECOLOGICAL NICHE MODELING.

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Keywords: *Dirofilaria* spp., Risk of infection, Culicid mosquitoes, Ecological Niche Model, Colombia.

Dirofilariosis is a cosmopolitan disease found especially in tropical and subtropical climates, with temperature and humidity being the main factors favouring the presence and proliferation of its vectors, the culicid mosquitoes. There are few reports of canine and human dirofilariasis in Colombia, a country with very favourable conditions for the development of mosquito vectors. So far, mean prevalence values ranging from 0.91% to 16.12% have been reported in dogs in different areas of the country, including high altitude with cold climate. Regarding human pulmonary dirofilariosis only one case has been described in the country. Due to the veterinary importance of this disease, as well as the need to increase knowledge of it in Colombia, the aim of this study is to create an infection risk map to facilitate the implementation of control and prevention measures, as well as to determine the areas with the highest risk of infection and during which months of the year it occurs. For this purpose, we used the ecological niche modelling methodology to calculate the habitat suitability of the vectors from MaxEnt, optimising the process with the KUENM package of R. The predictor variables chosen to model the potential distribution of mosquitoes were variables related to temperature and precipitation, distribution of irrigated crops, human footprint, water bodies and rivers. In addition, the number of generations of *Dirofilaria* spp. was calculated as a function of temperature both annually and month by month from a custom script of R. The territories with the highest risk of infection correspond to the coastal area of the country, as well as the Amazon in the interior of the continent. The three major mountain ranges of the country (western, central and eastern), characterised by a cold climate due to the altitude, had a low risk of infection. Due to the tropical climate (with the exception of the mountains), the risk remains moderate to high throughout the year without great variation, as the thermal amplitude in the country is negligible.

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*P-43. NEW INSIGHTS INTO THE EPIDEMIOLOGY OF CANINE DIROFILARIOSIS AND OTHER SELECTED VECTOR-BORNE DISEASES IN ROMANIA.

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Keywords: *Dirofilaria immitis*, *Dirofilaria repens*, vector-borne diseases, dogs, Romania

Vector-borne diseases are rapidly spreading through Europe, including Romania, and represent significant threats to animal and public health. Of these, canine heartworm disease, caused by *Dirofilaria immitis*, is a major parasitic threat of veterinary importance, due to its impact on the health of companion animals. In addition, infection with *Dirofilaria repens*, the causative agent of subcutaneous dirofilariosis in dogs, is considered an emerging zoonosis in many parts of Europe. Therefore, the present study aimed to provide new insights into the epidemiology of canine dirofilariosis and other selected vector-borne pathogens, in Romania. For this, blood samples collected from dogs (n=507) originating from eight counties in South-Eastern Romania, were analyzed by the modified Knott's test, for presence of microfilariae, and by a commercial ELISA test for presence of *D. immitis* antigen and antibodies against *Borrelia burgdorferi* sensu lato, *Ehrlichia* spp., and *Anaplasma* spp. Overall, 29.38% (95% CI: 25.53-33.52) of the tested animals were positive for *D. immitis*: 25.05% of dogs were positive by both tests, while 4.33% showed occult infection. By dog originating area, the *D. immitis* infection rate varied from 19.1% (95% CI: 11.10-30.05), in Giurgiu county (south Romania), to 42.9% (95% CI: 23.27-64.56), in Tulcea county (south-eastern Romania). In addition, microfilariae of *D. repens*, in 4.5% (95% CI: 3.01-6.77), and *Acanthocheilonema reconditum*, in 0.8% (95% CI: 0.27-2.03), respectively, were detected by the Knott test. Moreover, eight dogs (1.6%; 95% CI: 0.75-3.11) showed mixed infections with *D. immitis* and *D. repens*. The ELISA test showed exposure to infections with other vector-borne pathogens, such as *Ehrlichia* spp. (3.0%), *Anaplasma* spp. (1.0%), and *Borrelia burgdorferi* s.l. (0.4%). In conclusion,

these findings show significant rate of *D. immitis* infection of dogs in South-eastern Romania and confirm high risks for zoonotic vector-borne pathogens, highlighting the importance for continuous monitoring and adequate control measures to be applied.

***P-44. COMPARATIVE PALATABILITY ASSESSMENT OF MILBEMYCIN OXIME AND PRAZIQUANTEL ORAL CHEWABLE FORMULATIONS AND TABLETS.**

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Keywords: palatability, chewable, Milbemycin oxime, Praziquantel

Introduction:

This study aimed to assess and compare the palatability of a novel Milbemycin oxime and Praziquantel chewable tablet and similar products from competing brands, providing evidence to support the level of spontaneous consumption by dogs.

Methods:

The study was a blind, randomized trial conducted over 4 consecutive days, with 30 adult dogs. Each dog received 4 oral formulations (one formulation per day): one tablet, one chewable of the reference product and the novel product given from two different batches in two separate days. Palatability was assessed by measuring the spontaneous capture and consumption rates from a bowl within 1 minute time and subsequent hand feeding for the same duration, if the initial bowl capture was refused. The intake was considered successful when the product was captured and fully consumed, either by bowl or by hand. Partial intake was considered as rejection.

Results:

In all cases, formulations that were rejected when presented in a bowl were also rejected by hand feeding. Therefore the spontaneous capture was equal the total consumption.

The results indicated that the novel chewable formulation of Miblemycin oxime and Praziquantel demonstrates high acceptance rates, with over 98% of the cases ending to successful capture and consumption by bowl. Results are comparable and slightly higher to the reference product and to a competitive tablet containing the same active ingredients.

Product	Capture by bowl (%)	Total Consumption (%)
Tablet A	96.7	96.7
Reference chewable	96.7	96.7
Novel chewable – mean of 2 lots	98.3	98.3

Conclusions:

The findings confirm the high palatability of the novel chewable, suggesting a favorable acceptance. This study supports its use in small animal practices, where palatability can significantly influence treatment success, increase compliance and reduce stress for pets and pet owners. Even small differences in product acceptance can result in better treatment adherence and outcomes.

***P-45. CANINE HEARTWORM DISEASE – AN EMERGING VETERINARY CHALLENGE IN SLOVAKIA.**

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Keywords: Heartworm Disease, *Dirofilaria immitis*, Dogs, Epidemiology, Diagnostics

Heartworm disease caused by the *Dirofilaria immitis* nematode should be considered a newly emerged problem and a significant challenge for both veterinary practitioners and dog owners in Slovakia today.

Long-term epidemiological research on dirofilariasis (2007-2024) conducted in the area of the Danubian Lowland revealed a relatively stable situation in the prevalence of *Dirofilaria repens* in the dog population, while showing a rapidly increasing trend in the occurrence of *Dirofilaria immitis*. Between 2007 and 2016, cases of infection with *D. immitis* were recorded only sporadically (a total of 11 cases). Currently, cardiopulmonary dirofilariasis, in the form of either mono-infection or co-infection with *D. repens*, represents up to 45% of all dirofilarial infections.

When diagnosing, it is important to take into account the relatively high occurrence of microfilaremic infected dogs, which accounted for more than 37% of diagnosed cases of cardiopulmonary dirofilariasis from 2022 to 2024. Nearly 45% of dogs infected with *D. immitis* came from shelters, which has significant epidemiological implications, as many of these dogs were intended for adoption in Western European countries, contributing substantially to the spread of the parasite to non-endemic areas.

From a therapeutic standpoint, two factors complicate the situation: still limited awareness among veterinarians about this infection and the absence of registered drugs effective against the adult forms of the parasite.

In conclusion, the study presented here confirmed that Slovakia, which has been endemic for *D. repens* since 2007, has also become endemic for *D. immitis* in recent years, with the endemic areas being identical for both species of dirofilariae.

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*P-46. DIROFILARIA SPP. IN MOSQUITOES FROM A CANINE DIROFILARIOSIS-ENDEMIC AREA.

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Keywords: *Dirofilaria immitis*, *Dirofilaria repens*, mosquitoes, southern Italy

Dirofilariosis caused by *Dirofilaria immitis* and *Dirofilaria repens* is an emerging mosquitoes-borne diseases, which pose an increased risk to both animals and humans health. Recent evidence from two dog shelters in Caserta (Campania region, southern Italy), each housing 260-300 dogs, indicated that over 60% of the canine population tested positive for *D. immitis* and/or *D. repens* infections. Therefore, this study aimed to investigate, monthly throughout the season of 2024 (May-November), the mosquito species present and their infections with *Dirofilaria* spp. in the dog shelters located in Caserta, an endemic area for canine dirofilariosis. Preliminary data presented here derive from a two-month (May and June) sampling, using BG-Sentinel traps (BioGents GmbH, Germany).

Briefly, a total of 136 female mosquitoes, such as 60 *Aedes albopictus*, 70 *Culex pipiens*, and 6 *Culiseta longiareolata* were tested for *Dirofilaria* spp. *Culiseta* specimens were tested individually, while *Ae. albopictus* and *Cx. pipiens* were analyzed in pools of five. PCR analysis revealed *D. repens* DNA in one *Cs. longiareolata* (16.7%), one *Cx. pipiens* pool (7.1%), and two *Ae. albopictus* pools (16.7%). Additionally, *D. immitis* DNA was detected in one *Ae. albopictus* pool (8.3%).

The detection of *D. repens* in *Cs. longiareolata*, an ornithophilic species whose vector competence is not well understood, raises questions about its potential role in *Dirofilaria* transmission. While *Cs. longiareolata* is primarily associated with avian pathogens and has been involved as a vector for viruses such as Usutu and West Nile, its role in *Dirofilaria* spp. transmission remains unclear.

Larger-scale, longitudinal studies analyzing the spatiotemporal dynamics of mosquito populations could clarify how *Cs. longiareolata* contributes to filarial parasite spread. Ultimately, integrated entomological surveillance, targeted vector control programs, and collaborative efforts between public health authorities and veterinarians are essential to mitigate the emerging threat of dirofilariosis in southern Italy.

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*P-47. PREVALENCE AND ETIOLOGY OF LOWER RESPIRATORY TRACT DISEASES IN CATS IN SPAIN.

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Keywords: Lower respiratory tract diseases, *Dirofilaria immitis*, *Aelurostrongylus abstrusus*, *Mycoplasma* spp., cats, Spain.

Lower Respiratory Tract Diseases (LRTD) in cats are a common reason for veterinary consultations. Due to overlapping symptoms like coughing, dyspnea, and wheezing, diagnosing the specific etiology can be challenging. This study evaluates the prevalence and causes of feline LRTD in Spain identifying inflammatory, parasitic and other infectious conditions to improve therapeutic approaches. This multicentric study included 458 cats not recently medicated or dewormed. Data collection included clinical evaluations (sex, age, breed, habitat, demographics, clinical symptoms) and diagnostic tests (blood serology, fecal analyses, radiological study, bronchoalveolar lavage, and pathological study). Diagnostic techniques assessed inflammatory diseases, parasitic infections (*Dirofilaria immitis* and *Aelurostrongylus abstrusus*), and bacterial infections (*Mycoplasma* spp.). Patients were categorized into those with LRTD (348/458; 75.98%) and those with other respiratory processes (110/458; 24.02%). Indoor-only cats were tested for parasitic infections highlighting potential exposure through intermediate hosts. All cats with LRTD exhibited radiological signs of bronchial or alveolar involvement. Chronic bronchitis (106/348; 30.46%) and feline asthma (96/348; 27.3%) were the most common conditions. Parasitic infections (107/348; 30.74%) included cases with *A. abstrusus* and *D. immitis* as the primary culprits [Heartworm-Associated Respiratory Disease (HARD) 41/348; 11.78%], adult *D. immitis* (8/348; 2.3%) and *A. abstrusus* (55/348; 15.8%). Regions like Ibiza and Gran Canaria exhibited high *D. immitis* prevalence (17.14% and 16.92% respectively) likely due to climatic conditions favorable for mosquito vectors. *Mycoplasma* spp. (28/348; 8.05%) was detected predominantly in Madrid (18.92%) and Valencia (17.39%). Infectious bronchopneumonia was the least frequent LRTD (11/348; 3.16%). Inflammatory diseases associated with environmental irritants and allergens are the most frequent LRTD in cats while parasitic infections remain a significant concern. This study underscores the importance of raising awareness among veterinarians and pet owners about the prevention of parasitic and respiratory diseases in cats. Prophylactic care is crucial for all cats, regardless of habitat, to mitigate the risks of infection. Further research is recommended to better understand LRTD and improve diagnostic and treatment protocols.

Project funded by MSD Animal Health.

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***P-48. PRELIMINARY RESULTS OF RISK OF ATOPY IN OWNERS OF DOGS WITH HEARTWORM DISEASE.**

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Keywords: *Dirofilaria immitis*, humans, atopy, zoonosis, dogs.

The risk of zoonotic infection on the hyperendemic island of Gran Canaria (Canary Islands, Spain) is high for heartworm disease. Previous studies have shown the presence of specific IgE against *Dirofilaria immitis* in 17.2% of human seropositive samples. The percentage of positive and negative human samples with total IgEs were 34.5% and 8.1% respectively. The IgE response was mainly directed against two molecules: galectin and aldolase from *D. immitis*, which may be key in the survival mechanisms of the parasite and in the development of allergic reactions in individuals living in areas with heartworm disease. The aim of our study was to analyse the risk of having dogs infected with *D. immitis* and the associated atopy in their owners. A total of 648 samples from owned dogs were analysed. Dog serum samples were tested using the UranoVet *Dirofilaria* antigen test. Data were collected on breed, sex, age and habitat for the animals and age, sex and symptomatology related to atopic processes in the owners. Of all samples, 264 dogs were negative for *D. immitis* and their owners had no atopy (40.74%), 85 dogs were negative for *D. immitis* and their owners had atopy (13.11%), 104 dogs were positive for *D. immitis* and their owners had no atopy (10.05%). Finally, 199 dogs were positive for the test and their owners had atopy (30.7%). The ratio may be higher at older ages in both hosts. With these results, it is possible that owning heartworm-positive dogs may be a risk factor for the development of allergic diseases. Further studies are needed to elucidate the relationship between heartworm and allergies.

Project funded by Boehringer Ingelheim Animal Health.

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***P-49. INFLUENCE OF DIROFILARIA IMMITIS ON ATOPIC DEVELOPMENT IN A HYPERENDEMIC POPULATION.**

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Keywords: *Dirofilaria immitis*, *Toxocara canis*, humans, atopic patients

Dirofilaria immitis is a vector-borne parasitic nematode responsible for heartworm disease. It is now considered an emerging disease and a veterinary and public health problem. It is a zoonosis responsible for human pulmonary dirofilariasis leading to pulmonary nodules. In humans, there is a link between helminthic infections and the development of atopy, and elevated IgE levels against different filariae in allergy sufferers. The Canary Islands (Spain) have a significantly higher percentage of asthmatic individuals than the national average, being also a hyperendemic

area of dirofilariosis. Therefore, the aim of this study was to determine the presence of specific antibodies against *D. immitis* in the inhabitants of Gran Canaria (Canary Islands, Spain) in order to establish the degree of infection and exposure, and to evaluate whether continuous exposure to *D. immitis* could contribute to hypersensitivity in some inhabitants. For this purpose, anti-*D. immitis* and anti-*Wolbachia* (IgG) and anti-*D. immitis* (IgE) antibodies were tested with non-commercial ELISAs as well as anti-*Toxocara canis* IgG with a commercial kit (TECAN) in 125 atopic patients versus 34 healthy patients. Out of them, 23.2 % of atopic patients (29) tested positive for anti-*D. immitis* IgEs with ages between 4 and 59 years (7 males and 4 females) and with had rhinitis, asthma and egg allergy, and 6.4 % of atopic patients (11) were positive for anti-*D. immitis* and anti-*Wolbachia* IgG and anti-*D. immitis* IgE. They had rhinitis and asthma, with ages ranging from 7-28 years, being 3 males and 5 females with no significant differences between groups. Finally, 7.2% of atopic patients (9) were positive for anti-*Toxocara canis* IgGs aged 9-65 years, of which 4 were males and 5 females. Therefore, *D. immitis* influences the sensitivity of the immune system in humans in atopic processes in Gran Canary, such as hyperendemic area of heartworm disease.

Project funded by Boehringer Ingelheim Animal Health.

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*P-50. DISTRIBUTION PATTERN OF DIROFILARIA IMMITIS IN THE PROVINCE OF AVEIRO (PORTUGAL).

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Keywords: *Dirofilaria immitis*, dogs, cats, humans, Aveiro, Portugal.

Portugal's warm Mediterranean and temperate oceanic climates favour dirofilariosis transmission, by promoting the reproduction and spread of mosquito vectors, resulting in an overall canine prevalence of 5.9% [1]. Aveiro, a coastal region in northern Portugal with a temperate oceanic climate and the unique hydrographic system of the Ria de Aveiro, stands out as a significant hotspot, with a recorded canine prevalence of 15% [1]. This study focused on Aveiro to assess dirofilariosis prevalence in domestic dogs and cats, human exposure, and risk factors related to epidemiological and geo-environmental conditions. The study included 430 dogs, 426 cats, and 405 humans, with samples collected between 2021 and 2024. Dog serum samples were tested using a commercial antigen detection kit (UranoVet), while cat and human serum samples were analysed for anti-*D. immitis* and anti-*Wolbachia* IgG antibodies using a non-commercial ELISA [2,3]. The prevalence in dogs was 4.65%, whereas the seroprevalence in cats and humans was 8.92% (38 positive) and 2.96% (12 positive), respectively. Most positive animals and humans were geolocated in high-risk areas for infection, with only three positive dogs and one positive cat found in low-risk zones. The disease predominantly spreads along the coast and in population centres near cultivated areas and those irrigated by large rivers. The region bordering the Viseu district is considered a low-risk area for infection. The larger dog sample size in this study (430 vs. 80 in our previous study [1]) may explain the differences in canine prevalence, which, although lower than previously reported, aligns with the seroprevalence observed in cats and humans. These findings confirm

that dirofilariosis is well-established in Aveiro, affecting dogs as well as other species. Immediate control measures are necessary for both small animals and humans, given the disease's public health risk.

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*P-51. PREVALENCE OF DIROFILARIA SPP. AND A NOVEL WOLBACHIA SUPERGROUP B STRAIN IN AEDES VEXANS FROM UKRAINE.

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Keywords: Mosquitoes, Culicidae, *Dirofilaria immitis*, *Dirofilaria repens*, MLST, *Wolbachia* supergroup

More than 70 mosquito species transmit pathogenic *Dirofilaria* nematodes, including *D. immitis* and *D. repens*, which cause cardiac, pulmonary, and subcutaneous diseases. In Europe, 18 haplotypes of *D. repens* and nine haplotypes of *D. immitis* have been identified. *Dirofilaria* and mosquitoes harbor genetically distinct strains of the common endosymbiotic bacterium *Wolbachia*. Strains from the *Wolbachia* supergroup C, supporting nematode larval development, are found exclusively in filarial nematodes. In contrast, over 30% of mosquito species carry *Wolbachia* supergroup A and B strains, some of which can inhibit pathogens by activating the mosquito's immune response. However, the role of *Wolbachia* strains in *Dirofilaria* transmission remains unclear. This study aimed to assess the prevalence and genetic diversity of *Dirofilaria* and *Wolbachia* in mosquitoes from the Kharkiv region of Ukraine.

A total of 153 *Aedes vexans* females were collected from the villages Haidary and Yakovlivka in the Kharkiv region of Ukraine. Metabarcoding based on 12S and 16S rDNA markers was used to identify *Dirofilaria* and *Wolbachia*, respectively. The haplotypes of *Dirofilaria* were determined using mitochondrial markers (COI and NADH), while multilocus sequence typing (MLST) was used to classify *Wolbachia* strains based on *wsp*, *gatB*, *coxA*, *ftsZ*, *fbpA*, and *hcpA* genes.

Dirofilaria spp. were detected in nine *Ae. vexans* individuals: eight mosquitoes (5.23%) were positive for *D. immitis* (haplotype Di1), and one (0.65%) for *D. repens* (haplotype DR1). *Wolbachia* was detected in 44.45% (68/153) of mosquitoes: the *wDim* strain of supergroup C was found in four *D. immitis* positive individuals, while a novel supergroup B strain (designated *wAvexB*) was identified in both *D. repens* positive and nematode-free mosquitoes (62/153). Additionally, two co-infections with *wAvexB* and *wDim* were found in *D. immitis* positive mosquitoes. Among the identified *Wolbachia*, the *wsp* sequence was obtained for 47 individuals, revealing that in the Haidary population (n=41) it had the *wsp* 63 sequence type (HVR1:19, HVR2:17, HVR3:24, HVR4:33), while in the Yakovlivka population (n=6) it had the *wsp* 10 sequence type (HVR1:10, HVR2:8, HVR3:10, HVR4:8).

This study reveals a higher prevalence of *D. immitis* compared to *D. repens* in *Ae. vexans* from the study area and confirms that *D. immitis* haplotype Di1 is globally widespread. The identification of a novel *Wolbachia* supergroup B strain and its variability across different locations suggests a notable genetic diversity of *Wolbachia* in mosquito populations. Further research is required to determine whether *wAvexB* influences *Dirofilaria* transmission.

*P-52. VASCULAR ENDOTHELIAL CELL INVOLVEMENT IN THE PATHOGENESIS AND HOST-PARASITE INTERACTION OF CANID ANGIOSTRONGYLOSIS: A COMPARATIVE IN-VITRO STUDY IN RED FOXES (VULPES VULPES) AND DOMESTIC DOGS.

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Keywords: host-helminth interaction, immunobiology, canid host, differential host response

Angiostrongylosis caused by the nematode *Angiostrongylus vasorum* is a cardiopulmonary parasitic disease that predominantly affects domestic dogs and red foxes. While the disease in dogs often manifests as respiratory and cardiovascular dysfunctions accompanied by coagulopathies, red foxes largely remain asymptomatic. This study explored the responses of primary aortic endothelial cells from both species to *A. vasorum* antigens, including excretory-secretory products, adult worm antigens, and first-stage larval antigens. The results showed that ESP elicited only mild activation, whereas adult and L1 antigens induced more robust inflammatory responses. The expression of inflammatory markers was time-dependent, reflecting dynamic endothelial activation that evolved over the course of stimulation. Canine endothelial cells displayed a delayed yet pronounced and narrowly focused pro-inflammatory response, potentially linked to tissue damage during infection. In contrast, fox endothelial cells exhibited a rapid and diverse inflammatory profile, consistent with their role as reservoir hosts and the asymptomatic nature of their infections. These findings underscore species-specific host-parasite adaptations, revealing fundamental differences in immune responses between dogs and foxes. They provide valuable insights into the mechanisms driving differential pathogenesis in canine and vulpine angiostrongylosis, highlighting the intricate evolutionary interplay between host immunity and parasite survival strategies.

***P-53. CHARACTERISATION OF ANGIOSTRONGYLUS VASORUM EXCRETORY-SECRETORY PRODUCTS IDENTIFIES KEY COMPONENTS FOR HOST-PARASITE INTERACTION AND PROSPECTIVE THERAPEUTIC TARGETS CONSERVED ACROSS A WIDE RANGE OF HELMINTHS OF VETERINARY AND ZOOLOGICAL RELEVANCE.**

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Keywords: Helminth antigens, monoclonal antibodies, therapeutic targets, antigenic diversity, host parasite interaction

Parasite proteins, especially excretory-secretory products (ESP), are essential for various biological processes, host-parasite interactions, and immune evasion, making them key targets for studying parasitism and advancing therapeutic strategies. In this study, we combined monoclonal antibodies (mAbs) with proteomic characterisation, ELISA, immunohistochemistry, immunofluorescence, protein structure analyses, and therapeutic potential prediction. This enabled a detailed investigation of the antigenic landscape of helminths and the identification of potential targets for targeted diagnostics, vaccines, and therapeutics. Nine monoclonal antibodies produced against *Angiostrongylus vasorum* ESP were used to identify corresponding helminth antigens, their molecular diversity, conservation across parasite species, and potential suitability as drug targets. Both conserved and variable antigenic epitopes across a range of 74 different antigens from 48 parasite species were identified. Some mAbs bound antigens of the *Angiostrongylus* genus with stage-specific affinity, while others were species-specific for *A. vasorum*, or bound epitopes across species, genera, and classes. The *in situ* approaches found some mAbs to specifically target the reproductive and/or digestive organs, whereas others primarily associated with eggs or larvae. More specifically, mAb Av 56/1/2/1 exhibited affine binding specifically to the *Angiostrongylus* genus with varying stage-specific affinity, and mAbs Av 5/5, 70/1, and 33/2/2 were specific at the *A. vasorum* species level. mAbs Av 28/1, 1/1/2, 4/3/5, 7/2, and 8/5 appeared to bind epitopes across species, genera, and classes. Potential protein targets and their range of cellular functions such as protein synthesis, metabolic processes, and cytoskeletal organisation were identified. The majority of the 34 identified targets showed homology to known proteins in *Caenorhabditis elegans* and were associated with various drug-binding potentials. This study highlights specific antigens and their relevance for parasite survival within their hosts and their potential as drug targets. The latter suggests potential for future therapeutic exploration, though the specific functional roles of most targets remain to be characterised.

4.