XXXI CONGRESSO NAZIONALE DELLA SOCIETÀ ITALIANA DI PARASSITOLOGIA

I Parassiti Del Terzo Millennio

& 2021 **ESDA EVENT**

Dirofilariosis and Angiostrongylosis: from the past to the future

16-19 giugno 2021

SOIPA
2020

UNITE

Università degli Studi di Teramo

Facoltà di Medicina Veterinaria

SOIPA
Società Italiana di Parassitologia

SOIPA 2020

ESDA
European Society of Dirofilariosis and Angiostrongylosis

ISBN 9788894357530

Università degli Studi di Teramo

Facoltà di Medicina Veterinaria

COMITATO ORGANIZZATORE

Università degli Studi di Teramo: Donato Traversa (Presidente), Simone Morelli, Angela Di Cesare, Mariasole Colombo, Barbara Paoletti, Raffaella Iorio, Roberto Bartolini

Università degli Studi di Foggia: Annunziata Giangaspero

COMITATO SCIENTIFICO XXXI Congresso SoIPa

Università degli Studi di Teramo: Donato Traversa (Presidente), Angela Di Cesare, Simone Morelli, Mariasole Colombo, Barbara Paoletti
Università degli Studi di Foggia: Annunziata Giangaspero
Istituto Zooprofilattico Sperimentale "G. Caporale": Giovanni Savini
Parco Nazionale d'Abruzzo, Lazio e Molise: Leonardo Gentile
Parco Nazionale della Maiella: Simone Angelucci
Consiglio Direttivo SoIPa
Moderatori dei Simposi e delle Sessioni Scientifiche

COMITATO SCIENTIFICO 2021 ESDA Event

Università degli Studi di Teramo: Donato Traversa (Presidente), Angela Di Cesare
ESDA Board and Officers

CONSIGLIO DIRETTIVO SOIPA

PRESIDENTE: Fabrizio Bruschi (Università di Pisa)
VICE PRESIDENTE: Laura Rinaldi (Università di Napoli "Federico II")
CONSIGLIERI: Antonio Frangipane di Regalbono (Università di Padova), Ezio Ferroglio (Università di Torino), Simonetta Mattiucci (Università di Roma), Antonio Varcasia (Università di Sassari)
TESORIERE: Emanuele Brianti (Università di Messina)
SEGRETARIA: Valentina Mangano (Università di Pisa)

Cari Amici e Colleghi,

Il Congresso della Società Italiana di Parassitologia, organizzato dai parassitologi della Facoltà di Medicina Veterinaria dell'Università degli Studi di Teramo, si svolgerà, per la prima volta dalla costituzione della Società, in modalità telematica. La scelta di organizzare un Congresso virtuale è stata sofferta, ma necessaria, alla luce dell'attuale emergenza sanitaria da CoViD-19. In un periodo storico difficile, la cultura scientifica, proposta nel rispetto delle singole peculiarità e competenze, rappresenta una risorsa imprescindibile. La connotazione interdisciplinare della SoIPa è risultata fondamentale per un proficuo dibattito scientifico e un alto livello di adattamento a realtà in continuo mutamento.

Il XXXI Congresso Nazionale SoIPa "I Parassiti del Terzo Millennio" è incentrato su numerose parassitosi d'interesse medico e veterinario e affronta argomenti emergenti e di rilevanza nazionale e internazionale. Il 17 giugno si terrà infatti un evento congiunto con l'*European Society of Dirofilariosis and Angiostrongylosis*, il 2021 ESDA Event "*Dirofilariosis and angiostrongylosis: from the past to the future*".

Il Congresso si apre con una Sessione Plenaria dedicata al tema "*Parasites & Media*" e si articola, nel suo complesso, in una *Plenary Lecture* dal titolo "*Past, present and future of Parasite control in the One Health perspective*", nove Simposi, due Forum, cinque *Invited Talk Sessions*, cinque Sessioni Scientifiche e una Sessione dedicata alla Sezione Poster. Gli Atti raccolgono oltre 200 contributi scientifici, che offrono un'ampia panoramica dei temi affrontati e rappresentano un'occasione di conoscenza unica e la base per l'avvio di proficue collaborazioni.

Orientato allo stato dell'arte e alle prospettive della Parassitologia, il Congresso è caratterizzato dalla speciale attenzione rivolta ai partecipanti più giovani, il futuro della Società. Il Comitato Organizzatore e il Consiglio Direttivo della SoIPa hanno infatti riservato le sessioni di Comunicazioni Orali ai giovani ricercatori, e hanno offerto loro numerose opportunità di pubblicazione su riviste della Casa Editrice MDPI, e molti premi. Tra questi, ricordiamo il Premio intitolato alla memoria del Prof. Vezio Puccini, compianto e stimato Parassitologo e Mentore.

Assai preziosi per la realizzazione di questo Congresso, i contributi dell'Università degli Studi di Teramo, della Facoltà di Medicina Veterinaria di UniTe, delle Aziende Sponsor, dell'IZS dell'Abruzzo e Molise "G. Caporale", del Parco Nazionale d'Abruzzo, Lazio e Molise e del Parco Nazionale della Maiella. L'organizzazione del XXXI Congresso SoIPa / 2021 ESDA Event non sarebbe stata possibile senza l'impegno del Comitato Organizzatore, del Consiglio Direttivo della SoIPa e del Board ESDA, il supporto delle Istituzioni Pubbliche e delle Aziende Private, e la partecipazione attiva dei Moderatori delle diverse sessioni, dei Relatori Invitati e di tutti i partecipanti che, nonostante tutto, hanno avuto fiducia in noi.

A nome del Comitato Organizzatore
Il Presidente
Donato Traversa

Dear Friends and Colleagues,

The Conference of the Italian Society of Parasitology, organized by the parasitologists of the Faculty of Veterinary Medicine of the University of Teramo, will take place, for the first time since the establishment of the Society, virtually. The decision to organize a virtual conference was hard fought, but necessary, due to the current CoViD-19 sanitary emergency. In a difficult historical period, the scientific culture, along with individual peculiarities and skills, represents a crucial resource. The interdisciplinary connotation of SoIPa is fundamental for a fruitful scientific debate and a high level of adaptation to constantly changing reality.

The XXXI SoIPa National Conference "The Parasites of the Third Millennium" focuses on key parasitoses of medical and veterinary interest and addresses emerging topics of national and international relevance. Indeed, on June 17 there is a joint event with the European Society of Dirofilariosis and Angiostrongylosis, the 2021 ESDA Event "Dirofilariosis and angiostrongylosis: from the past to the future".

The Conference opens with a Plenary Session dedicated to the theme "Parasites & Media", followed by a Plenary Lecture entitled "Past, present and future of Parasite control in the One Health perspective", nine Symposia, two Forum, five Invited Talk Sessions, five Scientific Sessions and one Posters Session.

The Proceedings collect over 200 scientific contributions, offering a broad overview of the topics and representing a unique knowledge opportunity and a basis for fruitful and stimulating collaborations. The Congress is oriented towards the state of the art and future perspectives for parasitology and relies on a special attention paid to younger participants, the future of the Society. The Organizing Committee and the SoIPa Board have, in fact, reserved the sessions of Oral Communications for young researchers, who have been offered with numerous opportunities for publication in MDPI Publisher Journals, and many awards. Among these, we mention the Prize dedicated to the memory of Prof. Vezio Puccini, the late and esteemed Parasitologist and Mentor.

The organization of this Event has been supported by the invaluable contributions from the University of Teramo, the Faculty of Veterinary Medicine of UniTe, the several Sponsors, the IZS of Abruzzo and Molise "G. Caporale", the National Park of Abruzzo, Lazio and Molise and the National Park of Maiella. The organization of the XXXI SoIPa / 2021 ESDA Event Conference would not have been possible without the commitment of the Organizing Committee, the SoIPa and ESDA Boards, the support of the above Public Institutions and Private Companies, and the active participation of the Chairpersons, the Invited Speakers and of all the participants who, despite all, trusted us.

On behalf of the Organizing Committee
The President
Donato Traversa

XXXI CONFERENCE OF THE ITALIAN SOCIETY OF PARASITOLOGY & 2021 ESDA EVENT

INTRODUCTION

Dear Congress Participants, dear Colleagues and Friends,
I have the honour of introducing the XXXI Conference of the Italian Society of Parasitology. But I cannot start without thanking Donato Traversa, Chairman of the Organising Committee, for this invitation, which I have accepted also because of my connections with the University of Teramo, where I spent important years working to contribute to a situation that is even more lively now than when I left: allow me to say that I am both proud and pleased to see Donato continuing the tradition of activities, events (admittedly, a lot more limited!) and hospitality that was launched all those years and years ago.

There are aspects that make our Congress, in my opinion, very original and truly special.
It is inevitable that this Event is strongly influenced by the pandemic situation.
So, this **is** a different Congress. But we have attempted to capture the positive aspects of this old andsituation we are experiencing and convert the negative aspects into positive ones. The way this Convention is held puts distance between us. This was a difficult decision to make, but unavoidable in terms of protecting everyone's health. However, it allows even more people to participate, especially young people, without travel expenses. But of course, we miss so many other things: friendly hugs and smiles, handshakes and the time spent together. Above all, it is necessary to concentrate on the content of this Congress, analysing the organisational and cultural aspects underlying its organisation, based on decisions and ideas that I find fascinating, modern and far-sighted.

1.Public Parasitology

The Congress is open by a Plenary Session on "Parasites and the Media". I believe these themes have never been discussed at our previous meetings, which shows that there is now an urgent need to open up our discipline to a public of non-specialists by using a new kind of language, together with new methods and tools. We must pay more attention to communication, far beyond what is merely scientific and extremely technical, often characterised by an "esoteric" language that is only for the 'initiated'. We should embrace the principles of 'Public Science': so, we need to break down the barriers separating Parasitologists from present-day society. CoViD-19 has brought the role of scientists and their specialised skills back to the centre of attention from the media and public. But a great deal of this kind of progress is also being made in the field of Parasitology in Italy. For example, there is the MosquitoAlert App which belongs to what we call Citizen Science, and Padua IZS also has several didactic videos on YouTube.
So here is an opportunity not to miss: systematic actions aimed at spreading scientific culture and the scientific method must continue. I am convinced that true scientists can combine their specialised research and their ethical duty to communicate with everyone, in a way that is simple and clear (which does not mean simplistic and banal) to the general public, young people and also children. So, I think that Donato's idea to invite Daniel Riskin, a North American media star who is an academic and popular TV personality, is far-sighted, bold and in step with the times. Otherwise, we leave the field wide open to false scientists, to deniers and fake news, as shown in Massimo Polidoro's and Antonio Frangipane's lecture.

2. Together with the ESDA

This kind of partnership serves to make our (Italian) meetings less of a closed circuit, and increasingly international, providing a stimulus to our young people, especially those participating in our meetings for the first time. In fact, I am convinced that it is increasingly necessary to work towards creating a generation of researchers who are citizens of the world, also from the perspective of Next Generation EU: in this respect, our Congress delivers an important message. Specifically, deeper insights regarding topics like Dirofilariosis and Angiostrongylosis provide us with valuable enrichment in terms of our scientific culture. So, we can thank the President of the European Society of Dirofilariosis and Angiostrogylosis Elena Carretón and their Board for having chosen this Congress!

3. Next Generation EU

Regarding Next Generation EU, as you will have seen, also due to the inevitable difficulty of hosting a large number of contributions, this Congress is dedicated to Young People. They are the stars this year, the Parasitologists of the 3rd Millennium. I think there have never been as many initiatives organised to support them as there are at the present Congress, offering them so many opportunities, as you will have seen in the Programme. And I am happy to draw attention to the award entitled to Prof. Vezio Puccini, an initiative strongly supported by the many - including myself - who had the privilege of working alongside Prof. Puccini, who was always so focused on the success of young people.

4. Gender balance

Of the 9 Sessions reserved for Invited Speakers, 15 out of 34 involve women scientists; this positive situation is also confirmed by the number of Speakers in the Oral Talk Sessions, with 26 out of 34.

I would like this position not to be confused with old-style feminism because it demonstrates the revolution taking place in our society and shows us the need to overcome fractures and combat discrimination whether cultural, racial, sexual or gender-based.

I am saying this even if it may sound rhetorical.

Gender equality in science is not only just, but also useful: studies on gendered innovations have shown that neglecting gender equality in research can cause risks and missed growth opportunities.

Case studies by the Biomedical Research Foundation show that analysis of factors like gender represent a potential benefit for science and technology on the path towards excellence and innovation. In EU countries, women make up 46% of PhD students, but the percentages fall to 32% for those active in research and just 20% for professors.

This Congress confirms that women starting out in research are the majority, but we hope that the future will see these young women scientists increasingly present also in the top positions.

Science and society will be stronger and freer when these questions become distant memories of the past.

5. Our Sponsors

As you can see in the Scientific Program, many symposia have been supported by our Sponsors, which have been crucial for the whole organization. We must admit that we are not used to this kind of presence, which some people might consider “invasions”.

“We want support from Industry but prefer Industry to stay out of sight”.

Setting aside all hypocrisy, but at the same time never renouncing free and independent research, it is useful to make an alliance in the knowledge that scientific progress derives from our scientific activity and also from industry. I think that looking at things differently makes it possible also to understand the positive aspects of this decision.

So, I thank all Sponsors for their different contributions.

6. Social events

Our Congresses have always included trips in the areas where they are held, but these have been replaced this year with a solidarity event. Here is another novelty compared with our previous congresses. Some of us may have reservations about this, but once again I invite you to look at things in a different way: through the eyes of those who value personal and institutional solidarity, consisting of donations and simple concrete gestures.

I would like to thank all the Speakers, Moderators, the SOIPA Board and above all its President Fabrizio Bruschi all members of the Organising Committee, and once again, Donato Traversa. I think he has been the driving force for all of us in the Organising Committee, putting all his strength and energy into the organisation of this event, and I am grateful to him, as we all should be, for his commitment (which has been even greater in the present situation).

The Lecture entitled *"Past, present and future of Parasite control in the One Health Perspective"* by Dr. Lenaig Halos illustrates the many aspects of the importance of Control, focusing on the concept of One Health.

I think that the One Health concept should no longer considered a I would say "vision of the future", ending up as a kind of *leitmotiv* of our last 10 years. We can no longer allow this (and the Pandemic has shown us this). I think that we all want it to become a concrete 360° practice. There are excellent premises for this, considering that One Health has finally been included in school *curricula* after all these years. I would call this a Revolution! This will be a legacy (finally something positive) that we hand on to future generations.

The famous long-term perspective!

Annunziata Giangaspero
Department of Agriculture, Food, Natural Resources and Engineering (DAFNE)
University of Foggia, Italy

DIAMOND SPONSORS

Elanco™

zoetis

PLATINUM SPONSORS

Ceva

Boehringer Ingelheim

IDEXX

MSD Animal Health

GOLD SPONSORS

vetoquinol
ACHIEVE MORE TOGETHER

ARCOBLU

EUROIMMUN
a PerkinElmer company
ITALY

frontiers
in Veterinary Science
2.029 Impact Factor

SILVER SPONSORS

Virbac

KLIFOVET AG
ADDED VALUE TO ANIMAL HEALTH

MYLAV
Laboratorio La Vallonea

pathogens
an Open Access Journal by MDPI

PURINA
PRO PLAN

Hill's
Transforming Lives

Farmina
Pet Foods
Happy pet. Happy you.

affinity
ADVANCE
VETERINARY DIETS

PARTNERS

Corvino Travel
Tour Operator - Organizzazione di Eventi
Sede Legale Operativa: Viale Aldo Moro 4, 70043 Monopoli (BA)
Sede Operativa: Via Traiano 35, 20149 Milano
P.I. 08473690728, Tel: +39 328.6286913
www.corvinotravel.com
booking@corvinotravel.com

Cogito Ergo Vet s.r.l.s. – Formazione Veterinaria
Facebook: Cogito Ergo Vet – Formazione Veterinaria – Il Gruppo
http://www.cogitoergovet.com/
cogitoergovet@gmail.com

AT A GLANCE

16/06/2021 – SoIPa/ESDA
16.00-17.00
Opening Ceremony
17.00-20.00
Opening Plenary Session *Parasites & Media*

17/06/2021 – SoIPa/ESDA
8.45-9.00
SoIPa/ESDA Event - Introduction
9.00-10.00
Plenary Lecture
10.00-12.15
Forum ESDA/SoIPa *Dirofilariosis and Angiostrongylosis, from the past to the future*
13.45-14.45
Industry Forum ESDA/SoIPa *Dirofilariosis*
15.00-16.45
Industry Talks ESDA/SoIPa *Dirofilariosis and Angiostrongylosis*
17.00-17.30
Invited Talks ESDA/SoIPa *Dirofilariosis and Angiostrongylosis*
17.30-18.30
Oral Talks ESDA/SoIPa *Dirofilariosis and Angiostrongylosis*
18.30-20.00
Poster Discussion
19.00-21.00
Symposium *Old and New Parasites of Companion Animals*

17/06/2021 - SoIPa
15.00-16.50
Simposio *Striking news in Parassitologia umana*

18/06/2021 - SoIPa	
9.00-11.00	**9.45-10.45**
Sessione *Parassitologia Veterinaria*	Industry Invited Talks
11.00-13.00	**11.00-12.50**
Symposium *Modern Use of Parasiticides*	Simposio *Diagnosi delle zoonosi*
13.45-14.15	
Industry Invited Talks	
14.30-16.30	
Simposio *Ecto-endoparassiti del gatto: una panoramica italiana completa, da Nord a Sud*	Sessione *Parassitologia Umana*

17.00-19.00
Simposio *Vettori e Patogeni*
19.15-20.00
Invited talk
20.30
Assemblea dei Soci SoIPa

<table>
<tr><th colspan="4">19/06/2021 - SoIPa</th></tr>
<tr><td colspan="2">8.30-10.15</td><td colspan="2">8.30-10.30</td></tr>
<tr><td colspan="2">Simposio <i>Parassitosi degli animali da reddito</i></td><td colspan="2">Sessione <i>Zoonosi Parassitarie</i></td></tr>
<tr><td>10.45-12.15</td><td colspan="2">10.45-12.30</td><td>12.15-13.45</td></tr>
<tr><td>Simposio <i>Parassiti e Fauna selvatica</i></td><td colspan="2">Simposio <i>Malaria network</i></td><td>Sessione <i>Parassitologia Veterinaria e Umana</i></td></tr>
<tr><td colspan="4">14.30</td></tr>
<tr><td colspan="4">Evento Solidale e Chiusura</td></tr>
</table>

SESSIONI SPONSOR
17/06/2021
9.15-12.45 - Sessione Sponsor 1
15.00-19.15 - Sessione Sponsor 2
18/06/2021
11.15-14.30 - Sessione Sponsor 3
14.30-17.00 - Sessione Sponsor 4

PROGRAMMA SCIENTIFICO

16/06/2021

16.00-17.00 - Opening Cerimony
Dino Mastrocola (Rector, University of Teramo, Italy)
Augusto Carluccio (Dean, Faculty of Veterinary Medicine of Teramo, Italy)
Giovanni Savini (IZSAM "G. Caporale", Teramo, Italy)
Fabrizio Bruschi (President, Italian Society of Parasitology - SoIPa)
Elena Carretón (President, European Society of Dirofilariosis and Angiostrongylosis - ESDA)
Domenico Otranto (President, World Association of the Advancement of Veterinary Parasitology - WAAVP)

Presentation of the XXXI SoiPa Conference / 2021 ESDA EVENT
Donato Traversa (President, Organizing Committee)

17.00-20.00 - Opening Plenary Session *Parasites & Media* (Chairperson: Donato Traversa)

What's eating you: Parasites and media, **Daniel E. Riskin** (Evolutionary Biologist; Science and Technology Specialist for CTV Television, Canada)

Fake news and popular beliefs in Parasitology, **Massimo Polidoro** (CICAP, Italy); **Antonio Frangipane di Regalbono** (University of Padua, Italy)

17/06/2021

8.45-9.00 - SoIPa/ESDA Event–Introduction, **Annunziata Giangaspero** (University of Foggia, Italy)

9.00-10.00 - Plenary Lecture

Past, present and future of Parasite control in the One health Perspective, **Lénaïg Halos** (Senior Program Officer Animal Health chez Bill & Melinda Gates Foundation, USA)

Forum ESDA/SoIPa *Dirofilariosis and Angiostrongylosis, from the past to the future*
(Chairperson: Roland Schaper)

10.00-10.45 - Angiostrongylosis in animals and humans in Europe, **Eric Morgan** (Queen's University Belfast, United Kingdom)

10.45-11.00 - Human angiostrongylosis in Europe, present and future risk, **Claudia Paredes-Esquivel** (Universitat de les Illes Balears, Spain)

11.00-11.45 - Concern for *Dirofilaria immitis* and LOE/Resistance: current situation in USA and Europe, and future scenarios, **Anastasia Diakou** (University of Thessaloniki, Greece)

11.45-12.15 - Human dirofilariosis, the point of view of the physician, **Elisabetta Scoccia** (Maugeri Clinical-Scientific Institutes of Pavia, Italy)

Industry Forum ESDA/SoIPa *Dirofilariosis*

(Chairperson: Alessia Crippa)

13.45-14.15 - Importance to Map CVBD: an Italian survey on *Dirofilaria* spp., **Laura Kramer** (University of Parma, Italy)

14.15-14.45 - DOUBLE DEFENSE against Dirofilaria spp.: pratical recommendations, **Luigi Venco** (Veterinary Hospital Città di Pavia, Italy)

Industry Talks ESDA/SoIPa *Dirofilariosis and Angiostrongylosis*

(Chairperson: Luigi Venco)

15.00-16.45 - Dog relocation and rapidly changing parasite threats, **Jason Drake** (Elanco Animal Health)

15.45-16.15 - Unravelling diagnostic aspects in canine angiostrongylosis, **Manuela Schnyder** (University of Zurich, Switzerland)

16.15-16.45 - Experimental dog infection with *Dirofilaria repens*: new insights into biology, diagnosis and immunological response, **Lavinia Ciuca** (University of Life Sciences Iași, Romania)

Invited Talks ESDA/SoIPa *Dirofilariosis and Angiostrongylosis*

(Chairperson: Laura Kramer)

17.00-17.15 - *Dirofilaria* spp. and *Angiostrongylus vasorum*: current risk of spreading in Central and Northern Europe, **Hans-Peter Fuehrer** (University of Vienna, Austria)

17.15-17.30 - Exposure to *Angiostrongylus vasorum* in areas enzootic for VBDs, **Simone Morelli** (University of Teramo, Italy)

Oral Talks ESDA/SoIPa *Dirofilariosis and Angiostrongylosis*

(Chairperson: Laura Kramer)

17.30-17.40 - Highly variable clinical pictures in dogs naturally infected with *Angiostrongylus vasorum,* **Mariasole Colombo** (University of Teramo, Italy)

17.40-17.50 - Dirofilariosis: investigating the prevalence of the zoonotic parasitosis in dogs and humans from a hyper-enzootic area of Greece, **Dimitris Dimzas** (Aristotle University of Thessaloniki, Greece)

17.50-18.00 - Atypical intraocular migration of *Angiostrongylus vasorum*, **Iulia-Elena Filipescu** (Veterinary Clinic Cortonese, Italy)

18.00-18.10 - Cardio-pulmonary nematodes of the red fox in Sardinia island, Italy, **Francesca Nonnis** (University of Sassari, Italy)

18.10-18.20 - Selection of new diagnostic markers for *Dirofilaria repens* infections with the use of phage display technology, **Mateusz Pękacz** (Polish Academy of Sciences)

18.20-18.30 - First autochthonous infection of a cat with *Dirofilaria immitis* in Austria, **Maria Unterköfler** (University of Veterinary Medicine Vienna, Austria)

18.30-20.00 - Poster Discussion ESDA/SoIPa (Chairpersons: Alessandra Barlaam, Alessia Gazzonis)

Symposium *Old and New Parasites of Companion Animals*

(Chairperson: Ezio Ferroglio)

Elanco

19.00-19.45 - Old and new feline lungworms, **Angela Di Cesare** (University of Teramo, Italy)

19.45-20.15 - Parasitoses of puppies: risk assessment and control, **Fabrizia Veronesi** (University of Perugia, Italy)

20.15-21.00 - Veterinary aspects and zoonotic risks of dog and cat intestinal nematodes, **Paul Overgaauw** (University of Utrecht)

Simposio *Striking news in Parassitologia umana*

(Chairpersons: Fabrizio Bruschi, Pier Luigi Fiori)

15.00-15.30 - Microbial symbionts for the control of mosquito-borne diseases, **Guido Favia** (University of Camerino, Italy)

15.30-15.50 - The interactions between *Trichomonas vaginalis* and the vaginal microbiota, **Pier Luigi Fiori** (University of Sassari, Italy)

15.50-16.10 - Novel omics technologies in the study of anisakid nematodes, **Stefano D'Amelio** (Sapienza University of Rome, Italy)

16.10-16.30 - Parasitological diagnostics in fecal transplant procedures and in the characterization of human microbiota maps, **Lorenzo Putignani** (IRCCS, Rome, Italy)

16.30-16.50 - Public health challenges in the Bolivian Chaco: 35 years of experience, **Simona Gabrielli** (Sapienza University of Rome, Italy)

18/06/2021

Sessione *Parassitologia Veterinaria*

(Chairpersons: Mario Pietrobelli, Giovanni Garippa)

9.00-9.15 - *In vitro* evaluation of acute toxicity of five essential oils towards the parasitic mite *Varroa destructor,* **Roberto Bava** (Università di Catanzaro 'Magna Græcia', Italia)

9.15-9.30 - Immunological response of songbirds to tick infestation: tolerance or resistance?, **Beatrice Bisaglia** (Università di Antwerp, Belgio)

9.30-09.45 - Efficacy of condensed tannins against gastrointestinal nematodes of goats: *in vitro* evaluation of bioactive fodder and products, **Alessia Gazzonis** (Università di Milano, Italia)

09.45-10.00 - *Cytauxzoon* sp. and *Hepatozoon* spp. in cats in North-eastern Italy: Preliminary results, **Marika Grillini** (Università di Padova, Italia)

10.00-10.15 - Molecular detection of *Acarapis woodi* in honey bee colonies of Italy, Latium region, **Carmine Merola** (Università di Teramo, Italia)

10.15-10.30 - *Eucoleus garfiai* in wild boar (*Sus scrofa*) from Italy, **Laura Pacifico** (Università di Napoli 'Federico II', Italia)

10.30-10.45 - Summer sores secondary to a hoof crack in an Andalusian stallion, **Adriana Palozzo** (Università di Teramo, Italia)

10.45-11.00 - Updates on the distribution of *Eustrongylides excisus* Jägerskiöld, 1909 (Nematoda: Dioctophymatoidea) in lake fish from northern Italy, **Perla Tedesco** (Università di Bologna, Italia)

Industry Invited Talks

(Chairperson: Alessia Crippa)

9.45-10.05 - VECTRA 3D & CVBD: studies review, **Marie Varloud** (Ceva Santé Animale, Libourne, Francia)

10.05-10.45 - Vectra 3D against Leishmaniosis transmission, **Gaetano Oliva** (Università di Napoli 'Federico II', Italia)

Symposium *Modern Use of Parasiticides*

(Chairperson: Matthias Pollmeier)

11.00-11.45 - 3Rs and parasiticides in Companion Animals: Replacing models with patients, **Anja Joachim** (VedMedUni Vienna, Austria)

11.45-12.15 - Registration of novel parasiticides under the new 2022 EU regulation, **Klaus Hellmann** (Klifovet AG, Monaco, Germania)

12.15-13.00 - Clinical trials in veterinary medicine: the role of veterinarians, opportunities and challenges, **Fabrizio Solari Basano** (Arcoblu s.r.l., Milano, Italia)

Simposio *Diagnosi delle zoonosi*

(Chairperson: Giada Collovati)

11.00-11.45 - Laboratory diagnosis of the zoonotic parasitic diseases of the pets, **Simone Manzocchi** (IDEXX Laboratories, Novara, Italia)

11.45-12.30 - Epidemiological surveillance and One Health, **Vincenzo Veneziano** (Università di Napoli 'Federico II', Italia)

12.30-12.50 - Minimizing health risks in Animal Assisted Interventions, **Giulia Simonato** (Università di Padova, Italia)

Invited Talks

(Chairperson: Alessia Pede)

Boehringer Ingelheim

13.45-14.05 - The indoor cat: do the endoparasites knock at the door?, **Simone Morelli** (Università di Teramo, Italia)

14.05-14.15 - NexGard Combo: an innovative broad-spectrum antiparasitic formula, **Alessia Pede** (Boehringer Ingelheim Animal Health)

Simposio *Ecto-endoparassiti del gatto: una panoramica italiana completa, da Nord a Sud*

(Chairperson: Liliana Colombo)

MSD Animal Health

14.30-15.15 - New data on the prevalence of feline ecto-endoparasites in Italy, **Laura Kramer** (Università di Parma, Italia)

15.15-15.45 - Diagnosis, prevention and treatment of feline aelurostrongylosis, **Angela Di Cesare** (Università di Teramo, Italia)

15.45-16.30 - ESCCAP guidelines for the control of cat ecto-endo-parasitoses, **Ezio Ferroglio** (Università di Torino, Italia)

Sessione *Parassitologia Umana*

(Chairpersons: Federica Berrilli, David Di Cave)

14.30-14.45 - Inflammatory mechanisms in Caco-2 cells stimulated with *Anisakis*-derived messengers of pathogenicity**, Ilaria Bellini** (Università di Roma 'La Sapienza', Italia)

14.45-15.00 - Small-RNA analysis from third-stage larvae and exosomes provides the first miRNAs catalogue from anisakid nematodes, **Serena Cavallero** (Università di Roma 'La Sapienza', Italia)

15.00-15.15 - *Leishmania* promastigotes and endothelial cells: production of CXCL8 and neutrophils recruitment, **Sarah D'Alessandro** (Università di Milano, Italia)

15.15-15.30 - Prevalence and genotyping of *Pneumocystis jirovecii* pneumonia in patients with previously untreated acute myeloid leukemia and during induction therapy, **Valentina Del Prete** (Università di Roma 'Tor Vergata', Italia)

15.30-15.45 - Genetic variability of human *Giardia duodenalis* in Italy: what's news from a neglected parasite?, **Isabel Guadano Procesi** (Università di Roma 'Tor Vergata', Italia)

15.45-16.00 - Interactions between *Acanthamoeba* and Shiga toxin-producing *Escherichia coli* (STEC): a possible relevant aspect of public health importance?, **Margherita Montalbano Di Filippo** (Istituto Superiore di Sanità, Roma, Italia)

16.00-16.15 - Development of a sensitive molecular assay for *Plasmodium* spp. for low parasitaemic samples, **Marco Prato** (IRCCS Ospedale Sacro Cuore Don Calabria, Verona, Italia)

16.15-16.30 - The response of *Anisakis simplex* s.s. to the anthelmintic drugs - specific changes in the metabolism of invasive larvae, **Robert Stryiński** (Università di Warmia e Mazury, Olsztyn, Polonia)

Simposio *Vettori e Patogeni*

(Chairperson: Domenico Otranto)

17.00-17.30 - Best known and less known hemoprotozoa of pets, **Laia Solano-Gallego** (Università Autonoma di Barcellona, Bellaterra, Spagna)

17.30-18.15 - Monitoring and detection of new endemic foci of canine leishmaniosis in northern continental Italy: an update from a study involving five regions (2018-2019), **Gaetano Oliva** (Università di Napoli 'Federico II', Italia); **Luigi Gradoni** (Istituto Superiore di Sanità, Roma, Italia)

18.15-19.00 - Anti-vectorial prophylaxis and vector-borne diseases in dogs and cats, **Emanuele Brianti** (Università di Messina, Italia)

Invited Talk

(Chairperson: Fabrizia Veronesi)

19.15-20.00 - Anthelmintic resistance in horses: where is it a problem and what are the guidelines to prevent/postpone its development? **Georg von Samson-Himmelstjerna** (Università di Berlino, Germania)

20.30 **Assemblea dei soci SoIPa**

19/06/2021

Simposio *Parassitosi degli animali da reddito*

(Chairperson: Giuseppe Cringoli)

8.30-9.15 - Parasites of ruminants, an even-present reality, **Antonio Scala** (Università di Sassari, Italia)

9.15-9.45 - European strategies to control helminth infections in ruminant livestock, **Laura Rinaldi** (Università di Napoli 'Federico II', Italia)

9.45-10.15 - Parasite monitoring and control: ecto *vs*. endo, **Rudi Cassini** (Università di Padova, Italia)

Sessione *Zoonosi Parassitarie*

(Chairpersons: Gioia Capelli, Francesca Mancianti)

8.30-8.45 - Characterization of the bacterial flora in Swedish ticks using 16S amplicon sequencing, **Giulia Chiappa** (National Veterinary Institute, Uppsala, Svezia)

8.45-9.00 - Modified BG sentinel trap as an alternative to CDC for Flavivirus surveillance in Italy, **Sara Manzi** (Università di Roma 'La Sapienza', Italia)

9.00-9.15 - Detection of *Toxocara* larvae in ostrich meat intended for "carpaccio" preparation, **Alice Michelutti** (IZSVe, Padova, Italia)

9.15-9.30 - First evidence of pyrethroid resistance in West Nile Virus vector, *Culex pipiens*, across Italy, **Verena Pichler** (Università di Roma 'La Sapienza', Italia)

9.30-9.45 - The zoonotic nematode *Anisakis pegreffii* releases extracellular vesicles: microscopic and proteomic characterization, **Marialetizia Palomba** (Università di Roma 'La Sapienza', Italia)

9.45-10.00 - The drug's efficacy on zoonotic nematodes of Family Anisakidae - new data about metabolic status, **Iwona Polak** (Università di Warmia e Mazury, Olsztyn, Poland)

10.00-10.30 - Invited Talk

Parasitology & One Health – Perspectives from Africa and Beyond, **Vincenzo Lorusso** (Vetoquinol, Francia)

Simposio *Parassiti e Fauna selvatica*

(Chairpersons: Luca Rossi, Leonardo Gentile)

10.45-11.15 - Wildlife Disease Surveillance and Monitoring experiences in Apennine National Parks, **Simone Angelucci** (PNM, Abruzzo, Italia)**; Leonardo Gentile** (PNALM, Abruzzo, Italia)

11.15-11.35 - *Trichinella britovi* larval biomass in wild canids in Abruzzi region, Italy, **Pietro Badagliacca** (IZSAM, Teramo, Italia)

11.35-11.55 - The role of migratory birds in emerging pathogens spread, **Federica Iapaolo** (IZSAM, Teramo, Italia)

11.55-12.15 - The role of insects in spreading emerging pathogens, **Maria Goffredo** (IZSAM, Teramo, Italia)

Simposio *Malaria network*

(Chairpersons: Donatella Taramelli, Guido Favia)

10.45-11.00 - The Italian Malaria Network, **Donatella Taramelli** (CIRM. IMN; Università di Milano, Italia)

11.00-11.15 - Symbiotic yeasts: a promising tool for the control of vector-borne diseases, **Irene Ricci** (Università di Camerino, Italia)

11.15-11.30 - *Wolbachia*-induced immune activation and the control of vector-borne diseases, **Claudio Bandi** (Università di Milano, Italia)

11.30-11.45 - Malaria mosquito behavior in semi-field condition, **Roberta Spaccapelo** (Università di Perugia, Italia)

11.45-12.00 - Immune factors in the salivary glands of the malaria mosquito *Anopheles coluzzii*, **Fabrizio Lombardo** (Università di Roma 'La Sapienza', Italia)

12.00-12.15 - Entomological evidence of low community protection of LLINs in Burkina Faso, **Marco Pombi** (Istituto Superiore di Sanità, Roma, Italia)

12.15-12.30 - *Plasmodium vivax* malaria resurgence risk in the European Region, **Carlo Severini** (Istituto Superiore di Sanità, Roma, Italia)

Sessione *Parassitologia Veterinaria e Umana*

(Chairpersons: Roberta Galuppi, Simonetta Mattiucci)

12.15-12.30 - Fresh produce contaminated by pathogenic parasites: what is new in Italy?, **Alessandra Barlaam** (Università di Foggia, Italia)

12.30-12.45 - Genome investigations on neglected representatives provide novel perspectives on *Rickettsiales* evolution, **Michele Castelli** (Università di Pavia, Italia)

12.45-13.00 - Feeding behaviour of *Anopheles messeae/daciae* and *Anopheles maculipennis* s.s. in North-eastern Italy**, Francesco Gradoni** (IZSVe, Padova, Italia)

13.00-13.15 - *Aspergillus fumigatus* pneumonia and oxalate nephropathy in a foal, **Jasmine Hattab** (Università di Teramo, Italia)

13.15-13.30 - Comparative genomics and phylogeny of *Coxiella* symbionts of ticks, and their relation to *Coxiella burnetii*: acquired or ancestral pathogenicity?, **Tiago Nardi** (Università di Pavia, Italia)

13.30-13.45 - Entomological surveillance in former malaria-endemic areas of Southern Italy, **Ilaria Vasco** (IZSPB, Foggia, Italia)

14.30 - Evento Solidale e Chiusura

SESSIONI SPONSOR

17/06/2021

Session 1

(Chairpersons: Stefania Perrucci, Paola Beraldo)

9.15-10.00 - Impiego della miltefosina nella terapia della leishmaniosi: risultati preliminari di un follow-up a lungo termine, **Gaetano Oliva** (Università di Napoli "Federico II")

10.00-11.00 - Leishmaniosi canina: metodi diagnostici a confronto, **Walter Bertazzolo** (Laboratorio Clinico, Ospedale Veterinario Città di Pavia)

11.00-11.45 - La tosse nel gatto: ricordiamoci dei parassiti, **Eva Spada** (Università di Milano)

11.45-12.45 - Diarrea cronica in un gatto...e se fosse un parassita?, **Veronica Giordano** (Università di Teramo)

Tecnologia ActivBiome + Hill's Pet Nutrition, **Francesca Scarani** (Hill's Pet Nutrition)

Sessione 2

(Chairpersons: Stefania Zanet, Chiara Bazzocchi)

15.00-15.45 - Join the fight against CVBD: MyVBDMap, Video: Il progetto MyVBDMap

15.45-16.30 - I supplementi a supporto dell'idratazione e dell'equilibrio intestinale, **Franca Vaccari Simonini** (Purina)

16.30-17.15 - Shock e diarrea emorragica in un cane.... l'importanza dell'esame copromicroscopico, **Luca Corna** (Endovet Group)**;** Tecnologia ActivBiome + Hill's Pet Nutrition, **Cristiana Zocchi** (Hill's Pet Nutrition)

17.15-18.15 - Le attività del Parco Nazionale d'Abruzzo, Lazio e Molise

18.15-19.15 - Diagnosi e terapia utilizzando la topografia lesionale del prurito da ectoparassiti… Si… può… fareee!, **Luigi Venco** (Ospedale Veterinario Città di Pavia, Italia)**; Luisa Cornegliani** (Clinica Veterinaria Centrale, Milano)

18/06/2021

Sessione 3

(Chairpersons: Riccardo Lia, Marco Genchi)

11.15-12.15 - Filariosi cardiopolmonare dal 1626 al 2021. Cosa abbiamo imparato?, **Luigi Venco** (Ospedale Veterinario Città di Pavia)

12.15-13.00 - Allevamenti bovini intensivi e parassiti: un problema inesistente o…?, **Ezio Ferroglio** (Università di Torino)

13.00-13.45 - EUROIMMUN AG: un nuovo partner per la diagnostica delle malattie da vettori e delle zoonosi, **Fabio Tummolo** (EUROIMMUN IT)

13.45-14.30 - Geodiagnostica delle malattie trasmesse da vettori nel cane, gatto e cavallo, **Giulia Simonato** (Università di Padova)

Sessione 4

(Chairpersons: Antonio Varcasia, Manuela Diaferia)

14.30-15.30 - Getting to know Frontiers in Veterinary Science, **Shania Khan** and **Rui Fernandes** (Journal Managers, Frontiers, Switzerland)

15.30-16.15 - Coccidiosi nei cuccioli, cosa c'è da sapere?, **Fabrizia Veronesi** (Università di Perugia, Italia)

16.15-17.00 - Pro Plan® LiveClear®, Un approccio nutrizionale innovativo nella gestione degli allergeni del gatto, **Guido Poncini** (Purina)

ELENCO POSTER

POSTER ESDA - *DIROFILARIOSIS AND ANGIOSTRONGYLOSIS*

B ALBERIGI, A BENDAS, D SOARES, N LABARTHE. Cardiopulmonary evaluations in *Dirofilaria immitis* naturally infected cats

B ALBERIGI, N LEMOS, M ALBUQUERQUE, N LABARTHE. Canine heartworm in a cardio-respiratory unit routine

C BECSKEI, P DOHERTY, SP MAHABIR. Efficacy of Simparica Trio™ against L5 stages of *Angiostrongylus vasorum* in experimentally infected dogs

C BECSKEI, JL WILLESEN, M SCHNYDER, N MIROSHNIKOVA, SP MAHABIR. Efficacy of orally administered combination of moxidectin, sarolaner and pyrantel (Simparica Trio™) for the prevention of *Angiostrongylus vasorum* infection in dogs

LJ CURCIN, N MILOJKOVIĆ, M SCHNYDER, R SCHAPER, LJ SPASOJEVIC KOSIC, M ZEKIC STOSIC, S SAVIC. Seroprevalence of *Angiostrongylus vasorum* in dogs in Serbia

Y FALCÓN, JA MONTOYA-ALONSO, N COSTA, S MARTÍNEZ, L FRANCO, A CARO, SN GARCÍA RODRÍGUEZ; JI MATOS-RIVERO, E CARRETÓN. Serum acute phase proteins in dogs with heartworm disease (*Dirofilaria immitis*) before and after adulticide treatment

S GIUBEGA, M IMRE, MS ILIE, K IMRE, I LUCA, T FLOREA, GH DARABUS, S MORARIU. Canine *Dirofilaria* infection– from species to disease in the last decade

A MELIÁN HENRÍQUEZ, JI MATOS-RIVERO, JA MONTOYA-ALONSO, SN GARCÍA RODRÍGUEZ, N COSTA-RODRÍGUEZ, E CARRETÓN. Echocardiographic evaluation of pulmonary hypertension in dogs with heartworm disease using the pulmonary vein to pulmonary artery ratio

N MILOJKOVIĆ, D MILOSAVLJEVIĆ. Usage of rigid alligator forceps in surgical treatment of caval syndrome due to heartworm disease

R MORCHÓN, JA MONTOYA-ALONSO, E CARRETÓN, J VICENTE-BENGOCHEA, I RODRÍGUEZ-ESCOLAR, X MURCIA-MARTÍNEZ, P PÉREZ, JL ZUMAQUERO. *Dirofilaria immitis* in stray dogs in the municipality of Puebla, Mexico

R MORCHÓN, JA MONTOYA-ALONSO, P PÉREZ-PÉREZ, JA SÁNCHEZ AGUDO, I ESCOLAR-RODRÍGUEZ, X MURCIA-MARTÍNEZ, M RUIZ-SOMACARRERA, E CARRETÓN. Epidemiological study of distribution of *Dirofilaria immitis* in dogs from Castilla y León (Spain)

R MORCHÓN, JA MONTOYA-ALONSO, JA SÁNCHEZ AGUDO, J VICENTE-BENGOCHEA, I ESCOLAR-RODRÍGUEZ, X MURCIA-MARTÍNEZ, E CARRETÓN. Presence of *Angiostrongylus vasorum* in dogs in one of the largest regions of the Iberian Peninsula and Europe

E NAPOLI, R PANARESE, G DE BENEDETTO, G GAGLIO, MA BEZERRA-SANTOS, JA MENDOZA-ROLDAN, D OTRANTO, E BRIANTI. *Dirofilaria immitis* infection in the Pelagie archipelago: the southernmost hyperendemic focus in Europe

R PANARESE, R IATTA, JA MENDOZA-ROLDAN, A ZATELLI, F BEUGNET, D OTRANTO. Insecticidal efficacy of afoxolaner (NexGard®) in the prevention of *Leishmania infantum* and *Dirofilaria immitis* transmission to sheltered dogs in a high endemic area

L SEGERITZ, A CARDONA, A TAUBERT, C HERMOSILLA, A RUIZ. Autochthonous *Angiostrongylus cantonensis*, *Angiostrongylus vasorum* and *Aelurostrongylus abstrusus* infections in terrestrial gastropods from the Macaronesian Archipelago, Spain

LJ SPASOJEVIĆ KOSIĆ, V LALOŠEVIĆ, V VRAČAR, G KOZODEROVIĆ, A POTKONJAK, S SIMIN. *Wolbachia* detection in dogs naturally infected with *Dirofilaria* spp. and treated for the heartworm disease with an alternative therapy

EE TIERI, G GIOVANNINI, D SANTOLERI, M PRIMAVERA, G C TELERA, D MALATESTA, S DEFOURNY, M DE ASCENTIS, S D'ALESSIO, A COCCO, P BADAGLIACCA, MS LATROFA, D OTRANTO, A PETRINI. *Dirofilaria repens* from the subcutaneous tissue of a dog native to the Abruzzo region, Italy

POSTER SOIPA - *PARASSITOLOGIA VETERINARIA*

K BERGVALL, M ISAKSSON, I SKAAR, G GRÖNDAHL, E OSTERMAN-LIND, S BORNSTEIN, G GRANDI. Characterisation of equine pastern dermatitis (greasy heel, mud fever) in Nordic breeds Døla and North Swedish coldblooded horse

MA BEZERRA-SANTOS, JA MENDOZA-ROLDAN, G SGROI, A CAMARDA, R IATTA, D OTRANTO. Flea mite phoresy and high prevalence of *Rickettsia asembonensis* in *Archaeopsylla erinacei* fleas from European hedgehogs

V BLANDA, A CARUANA, S BONACCORSO, F LA RUSSA, R D'AGOSTINO, S VILLARI, G CAMMILLERI, A TORINA, V FERRANTELLI. Development of a Reverse Line Blot for simultaneous detection of Tick-Borne Pathogens in equines

F BUONO, L PACIFICO, C RONCORONI, D PIANTEDOSI, E CASTALDO, G CRINGOLI, V VENEZIANO. A national survey on equine intestinal strongyle infections in Italy and Parasitological Assistance Program in Equids (PAPE)

F CASTAGNA, E PALMA, C PIRAS, C CRINGOLI, L RINALDI, A BOSCO, V MUSOLINO, D BRITTI, V. MUSELLA. Ethnoveterinary pharmacology to control gastrointestinal nematodes in sheep: evaluation of three aqueous plant macerates

M CHAOUADI, F SCARPA, I AZZENA, P COSSU, KH HARHOURA, M AISSI, F TAZEROUTI, G GARIPPA, P MERELLA, M CASU, D SANNA. Characterization of mitochondrial DNA of *Fasciola hepatica* from Algerian and Sardinian isolates and relationships with other areas of the world

L CIUCA, G MARTINESCU, L MIRON, C ROMAN, D ACATRINEI, G CRINGOLI, L RINALDI, MP MAURELLI. Occurrence of *Babesia* species in symptomatic dogs and co-infection with *Hepatozoon canis* in eastern Romania

M COLOMBO, G CAPITANI, S MORELLI, A DI CESARE. Life-threatening strongyloidosis in a puppy

M COLTRARO, V C NEVE, A STAMILLA, G CASCONE, F ANTOCI, F SALINA, M BARBAGALLO, F SPADOLA, R PULEIO, SA MIGNACCA. Bovine besnoitiosis: a case report in Sicily

PE CRISI, B PAOLETTI, S MORELLI, G SIMONATO, M COLOMBO, PG TISCAR, A BOARI. Molecular detection of *Tritrichomonas foetus* in cats from Abruzzo region (central Italy)

A DE BONIS, M COLOMBO, A GHIRALDELLI, R BACCI, S MORELLI, M VIGNOLI. Potential role of *Hepatozoon canis* in a fatal systemic disease in a puppy

G DESSÌ, C TAMPONI, G SANNA, AP PIPIA, A VARCASIA, L MELONI, S KNOLL, L CAVALLO, F PORCU, C PASINI, MF SINI, F NONNIS, A SCALA. Prevalence and molecular identification of *Cryptosporidium* spp. in sheep farms from Sardinia

M DIAFERIA, C FRANCHETTO, M CAPASSO, L FERRETTI, G MORGANTI, L RINALDI. Preliminary epidemiological investigation on *Leishmania infantum* infection in captive ring-tailed lemurs (*Lemur catta*)

F DIANA, C TAMPONI, S PINNA, F NONNIS, L CAVALLO, S CARTA, S CAFIERO, G DESSI', F PUDDA, A VARCASIA, A SCALA. Epidemiological survey on parasites of terrestrial tortoises from Sardinia

FM DINI, G POGLAYEN, C BENAZZI, A GENTILE, B MORANDI, R GALUPPI. Microscopic and histological investigations show that *Onchocerca dukei* is widespread in Tanzanian cattle

AL GAZZONIS, S A ZANZANI, L VILLA, G ALOISIO, E MIGLIORATI, MT MANFREDI. Influence of management measures on gastrointestinal parasites in dairy cattle herds in northern Italy

A GRECO, S MINIERI, S MANCINI, I ALTOMONTE, G ROCCHIGIANI, D GATTA, G PACI, S PERRUCCI. Coccidiostatic effects of two condensed and hydrolysable tannin mixes used as feed additives in rabbits

M GRILLINI, G SIMONATO, G DOTTO, C TESSARIN, A FRANGIPANE DI REGALBONO. Molecular approach for contemporary detection of *Hepatozoon* spp. and *Cytauxzoon* spp. in felids: preliminary data

S ILLIANO, L CIUCA, S PENNACCHIO, A BOSCO, MP MAURELLI, G CRINGOLI, L RINALDI. A retrospective analysis of hookworm infection in dogs from southern Italy

M ISAKSSON, G GRANDI, G GRÖNDAHL, S BORNSTEIN, I SKAAR, E OSTERMAN-LIND, K BERGVALL. Development of a qPCR for the detection of *Chorioptes bovis* in equine skin scrapings

PD JIMENEZ CASTRO, A VENKATESAN, E REDMAN, R CHEN, D A ZULUAGA SALAZAR, R AVRAMENKO, A MALATESTA, H HUFF, JS GILLEARD, RM KAPLAN. Multiple drug resistance in hookworms infecting greyhounds in the USA

L LANZONI, M CHINCARINI, J DI PASQUALE, S MORELLI, G VIGNOLA, B PAOLETTI, A DI CESARE. Animal welfare and parasite infections in organic and conventional dairy farms in central Italy: a pilot study

F MACCHIONI, G ROMEO, V TROCCHI, F CECCHI, E ROMI, V SERGI, A COLI, C LENZI, G MONNI, L STANCAMPIANO. Intestinal parasites of *Lepus corsicanus* (DE WINTON, 1898) in Sicily

M MAESTRINI, G POLITANO, S MANCINI, M B. MOLENTO, M LONGINOTTI, S PERRUCCI. Prevalence and risk factors of gastrointestinal parasite infections in show jumping horses in the district of Pisa, Italy

R MAGGI, AG TADDEO, M COLOMBO. Serological and molecular detection of *Babesia caballi* and *Theileria equi* in horses from Latium, between July 2018 and April 2021: a retrospective study

A MAGRI, R GALUPPI, ML FIORAVANTI, M CAFFARA. Survey on the presence of *Leishmania infantum* in peridomestic rodents from the Emilia-Romagna region

A MAURIZIO, C TESSARIN, L STANCAMPIANO, C ASTI, E MARCHIORI, R CASSINI. Comparing pooled with individual faecal samples for the assessment of gastrointestinal strongyles infection burden in goats

P MERELLA, C BURREDDU, FE MONTERO ROYO, G GARIPPA. Trials of oral treatments against gill monogenean parasites of *Sparus aurata* L.

G MESZAROS, R RIZZI, J BASSI, M DI GIANCAMILLO, M MORTARINO. Acaricidal treatment with formic acid shortens honey bee pupal lenght: a Computed Tomography-based study

SA MIGNACCA, C TAMPONI, L MELONI, A VARCASIA, S KNOLL, F NONNIS, L CAVALLO, F PORCU, C PASINI, MF SINI, G DESSÌ, L MANUNTA, A SCALA. *Taenia multiceps* coenurosis in adult sheep with neurological disorders

B MORANDI, G ORLANDI, G POGLAYEN, M PIETRA, R GALUPPI. Retrospective study on the parasites frequency of dogs referred to the Veterinary Teaching Hospital (VTH)-University of Bologna, combined to haematological results

S MORELLI, M COLOMBO, A DIAKOU, D TRAVERSA, M GRILLINI, A DI CESARE. Development of *Aelurostrongylus abstrusus* in hibernated *vs* non-hibernated *Cornu aspersum* snails

G MORGANTI, AL GAZZONIS, P ROCCABIANCA, G AVALLONE, G RIGAMONTI, C BRACHELENTE, M DIAFERIA, F VERONESI. Molecular detection of *Leishmania infantum* in cutaneous lesions of horses from endemic CanL areas of northern and central Italy: preliminary results

M NOCERINO, A BOSCO, M SANTANIELLO, G CRINGOLI, L RINALDI. Mapping the spatial distribution of *Calicophoron daubneyi* infection in a Mediterranean area

B PAOLETTI, S ANGELUCCI, S MORELLI, C SMOGLICA, A BARLAAM, A DI CESARE. Strongylid nematodes shared between domestic and wild ruminants in the Maiella National Park (Abruzzo region, central Italy)

S PAVONE, A BAZZUCCHI, M GOBBI, A FRATTO, B CAPONI, R RINOLDO, T MARCHETTI, F VERONESI. Preliminary study on proventricular parasites of birds of prey in Italy: anatomo-histopathological findings

F PORCU, F FIORE, C TAMPONI, L MELONI, C PASINI, S KNOLL, MF SINI, A VARCASIA, L CAVALLO, G DESSÌ, A SCALA. Endoparasites in goat breedings of Sardinia, Italy

V RINALDI, PE CRISI, A BOARI, G MARRUCHELLA. Concurrent cutaneous mast cell tumor and *Leishmania* sp. infection in a dog

L SALVATI, S MORELLI, A DI CESARE, E DI GIULIO, M COLOMBO, D TRAVERSA. Absence of *Leishmania infantum* in cats with dermatological signs in an endemic area

A SCALA, A SPEZZIGU, C TAMPONI, G DESSÌ, L MELONI, S KNOLL, F NONNIS, G COSSO, S NAITANA, V CARCANGIU. The influence of melatonin on the Faecal Egg Count of gastrointestinal nematodes in sheep

C TAMPONI, L MELONI, A VARCASIA, G DESSÌ, G SANNA, SEDDA G, S KNOLL, L CAVALLO, F PORCU, C PASINI, MF SINI, G PREDIERI, A SCALA. Large-scale epidemiological survey on *Parascaris* spp. egg shedding in horses of Italy and conditioning factors

P TEDESCO, M KOLEGA, S ČOLAK, A GUSTINELLI, D MEJDANDŽIĆ, M CAFFARA, R BARIC, ML FIORAVANTI. First report of *Ktariella polyorchis* Vala & Euzet, 1977 (Monogenea: Calceostomatidae) infection in farmed meagre *Argyrosomus regius* (Asso, 1801) (Actinopterygii: Sciaenidae)

FV TINOCO, S MORELLI, MF BRITO, GO PEREIRA, MC OLIVEIRA, D TRAVERSA, M COLOMBO, M GRILLINI, A DI CESARE. Acute hemorrhagic meningoencephalomyelitis due to erratic migration of *Aelurostrongylus abstrusus* in a kitten

M TOMASONI, V MENCONI, P PASTORINO, D MUGETTI, G ESPOSITO, M PREARO. Importance of prophylaxis on parasitic diseases in fish used for experimental trials

F TONIOLO, S RAVAGNAN, E PORCELLATO, S SGUBIN, A MIGLIO, A NATALE, A CARMINATO, M VASCELLARI, G CAPELLI, MT ANTOGNONI. Looking for dog blood donors in low and high endemic areas for vector-borne infections of North and Central Italy

I VAROTTO-BOCCAZZI, I ARNOLDI, P GABRIELI, R NODARI, B BISAGLIA, A NEGRI, M GRAMICCIA, L GRADONI, V TRANQUILLO, C BANDI, S EPIS. Enhance the immunomodulatory capability of the bacterium *Asaia* with the expression of a *Wolbachia* protein: implication for the control of leishmaniases

L VILLA, AL GAZZONIS, D CARTAGENA, M MORTARINO, E BONACINA, D GUADAGNINI, C ALLIEVI, MT MANFREDI, SA ZANZANI. Parasitological monitoring of mammals endoparasites in an Italian faunistic park: effect of two prophylactic treatments with ivermectin on nematodes infections

SA ZANZANI, AL GAZZONIS, L VILLA, M VILLA, G ZANATTA, MT MANFREDI. Condensed tannins in adult grazing goats: effect on gastrointestinal nematodes

POSTER SOIPA - *PARASSITOLOGIA UMANA*

A BARLAAM, L PUTIGNANI, S PANE, RA PAPINI, A GIANGASPERO. What's in a child's ear? The first case of otomyiasis by *Sarcophaga argyrostoma* (Diptera, Sarcophagidae) and review of the literature

G BATISTI BIFFIGNANDI, F TAMAROZZI, A VOLA, M MARICONTI, E OLIVIERI, L PETRONE, D SASSERA. Bioinformatically informed peptide microarray for the development of a specific and sensitive test for the diagnosis of hepatic cystic echinococcosis

G BEVIVINO, P AVELLINO, F SANTOLAMAZZA, F LOMBARDO, D MODIANO. Development of a new molecular tool to study the transmission of *Plasmodium falciparum* parasite inside its main vector, the mosquito *Anopheles coluzzii*

S BUEZO MONTERO, P GABRIELI, A POINSIGNON, BZ HUBERT ZAMBLE, F REMOUE, B ARCÀ. Analysis of IgG responses to the *Aedes albopictus* 34k2 salivary protein in individuals from Dengue and Chikungunya endemic areas

E CALVO-ALVAREZ, F PEREGO, D TARAMELLI, N BASILICO. *Leishmania infantum* dampens neurotoxic NO release by infected macrophages in the presence of amyloid β

B CAPUTO, CM DE MARCO, A MILES, A DELLA TORRE. Whole-genome sequencing analysis of populations with an uncertain species status at the Far-West of *Anopheles gambiae* range

D CROTTI, R GARGIULO, A RAGLIO, L PETRULLO, AMCLI-COSP WORKING GROUP. Intestinal parasitosis in Italy: results of the third AMCLI-CoSP national survey

T DI MUCCIO, D TONANZI, G LA ROSA, M GRAMICCIA. Hsp-70 polymorphism characterizes *Leishmania donovani* complex strains from Mediterranean area

R FONNESU, R FAIS, G TRILLOCCO, E BALESTRI, A CARA, M PRATO, A LUPETTI, V MANGANO. Evaluation of a multiplex Real-Time PCR assay for the diagnosis of human intestinal protozoa in Azienda Ospedaliero Universitaria Pisana

S GABRIELLI, M SPINICCI, F MACCHIONI, M STROHMEYER, M ROSELLI, A BARTOLONI. Seroprevalence of *Trypanosoma cruzi* infection among school-age children in the Bolivian Chaco

SS LONGONI, C PIUBELLI, F PERANDIN, E RIZZI, N LUCHETTA, M DEGANI, S TAIS, A MORI, Z BISOFFI. Preliminary evaluation of a new Schistosoma Immunochromatographic Test

P MASINI, S ZAMPETTI, F BIANCOLINI, G MIÑÓN LLERA. Microscopic examination of indoor dust: a useful survey tool for diagnosis and verify of environmental contamination of human scabies mite *Sarcoptes scabiei*

P MASINI, S ZAMPETTI, F BIANCOLINI, G MIÑÓN LLERA. Outbreak of occupational dermatitis in a cereal and legume trading company caused by mite *Pyemotes ventricosus*

P MASINI, S ZAMPETTI, F BIANCOLINI, R ROMANI, G MIÑÓN LLERA. A case of aetiological diagnosis of ectoparasitosis by crab louse *Pthirus pubis* through a microscopic examination of the indoor dust

P MASINI, S ZAMPETTI, F BIANCOLINI, R ROMANI, G MIÑÓN LLERA. The pathogenic potential of *Sclerodermus domesticus* and *Cephalonomia gallicola*, two flat wasps that sting man in indoor environments

I MORETTA, L STINGENI, MA PRINCIPATO. Outbreaks of occupational dermatitis caused by *Pyemotes ventricosus*

M OUEDRAOGO, I NEBIE, Y KABORE, SB SIRIMA, F BRUSCHI, D MODIANO, VD MANGANO. Investigating the impact of *Schistosoma haematobium* infection on immunity to *Plasmodium falciparum* malaria in populations from Burkina Faso

S PARAPINI, S PAONE, M POURSHABAN, E ERBA, A CONTINI, S D'ALESSANDRO, A OLIVIERI. In vitro antimalarial activity of inhibitors of human Rac1: a possible new way to fight *Plasmodium*?

L PETRULLO, A FIORETTI, M ASCIERTO, L ATRIPALDI, MG COPPOLA. Neglected parasitic infections in Campania, South Italy region, from 2015 to 2019

A PIZZARELLI, S CAVALLERO, I BELLINI, S D'AMELIO. Gene expression profiles and temperature in *Anisakis simplex* ss, *Anisakis pegreffii* and *Hysterothylacium aduncum*

V RODRIGUEZ FERNANDEZ, MG ROSSINO, R AMATO, S PIAGGI, B PINTO, G CASINI, F BRUSCHI. An organotypic model for the study of retinal *Toxoplasma gondii* infection

N TIBERTI, D BUONFRATE, C CARBONE, G PIRO, Z BISOFFI, C PIUBELLI. Systemic profile of immune factors in an elderly Italian population affected by chronic strongyloidiasis

POSTER SOIPA – *ZOONOSI PARASSITARIE*

A AGRESTI, F BERRILLI, M MAESTRINI, I GUADANO PROCESI, E LORETTI, N VONCI, S PERRUCCI. *Giardia duodenalis* and other intestinal parasites in kennels of Tuscany (central Italy)

F AHMED, A VARCASIA, M SANNA, L CAVALLO, MF SINI, G DESSÌ, F NONNIS, S KNOLL, C TAMPONI, C PASINI, F PORCU, L MELONI, A SCALA, MG CAPPAI, S CARTA, F BERLINGUER. How can vultures contribute to Cystic Echinococcosis control?

M BERTOLA, G DA ROLD, S CARLIN, F OBBER, E PORCELLATO, C FALCARO, S ORMELLI, F MONTARSI, S RAVAGNAN. Occurrence and identification of *Ixodes ricinus* - borne pathogens in north-eastern Italy

G BONGIORNO, R BIANCHI, T DI MUCCIO, C DE LIBERATO, M CALZOLARI, A MICHELUTTI, F MONTARSI, F CIPRIANO, M DOTTORI, G SATTA, L GRADONI, P ANGELINI. Update of sand fly species distribution in six Italian Regions as a preliminary information for risk-map construction of phlebotomine-borne diseases

A BOSCO, P PEPE, MP MAURELLI, P QUARANTA, L BALDI, P CIARAMELLA, F CAPUANO, L MORENA, R PINTO, P SARNELLI, L RINALDI, G CRINGOLI. EchinoCamp: an integrated approach to control of *Echinococcus granulosus* infection in a hyperendemic area of southern Italy

G CHIAPPA, A CAFISO, M PERINI, F COMANDATORE, M MASSIMINO, G GRANDI, C BAZZOCCHI. Taking aim at *Borrelia burgdorferi* s.l. complex: a new molecular tool for *B. garini*, *B. afzelii* and *B. burgdorferi* s.s. detection in *Ixodes Ricinus*

A COSTA, G CAMMILLERI, ML RIZZUTO, L PANTANO, F G GALLUZZO, A BRUNONE, E GIANGROSSO, MD BUSCEMI, V FERRANTELLI. A study on the possible use of *Anisakis* as biological indicators of heavy metals contamination in marine environments

L GUARDONE, E POLSINELLI, F SUSINI, F MANCIANTI, A ARMANI. A systematic review on *Eustrongylides* spp. in fish species: preliminary results

G JOANNY, G DESSÌ, C TAMPONI, F NONNIS, F AHMED, C HOSRI, A SCALA, A VARCASIA. Cystic echinococcosis of livestock and humans in Lebanon

E MARCHIORI, G DOTTO, G FRANZO, DA RAELE, G PENNUZZI, R KUCHTA, A GUSTINELLI, L DI RENZO, F MARCER. Tapeworm infection in a Mediterranean Monk Seal (*Monachus monachus*) pup

JA MENDOZA-ROLDAN, RP LIA, G ANNOSCIA, R MANOJ, D OTRANTO. *Raillietiella hemidactyli* (Pentastomida) in *Tarentola mauritanica* geckoes from Linosa island, Italy: a new zoonotic parasite for Europe?

B MORONI, M DUTTO, M BARRASETAS, A MOLINAR MIN, L ROSSI. Red foxes as a source of zoonotic scabies in a rural area of Piedmont, Italy

MOSQUITOALERT ITALIA TASK FORCE (B CAPUTO). Mosquito Alert: a Citizen Science project to monitor mosquitoes in Italy

J NOWICKA, M GRZYBEK, B BIERNAT, D ANTONOVÁ, K TOŁKACZ, A BAJER, M ALSARRAF, J BEHNKE-BOROWCZYK, J PALEOLOG, JM BEHNKE. Seroprevalence of *Toxoplasma gondii* among sylvatic rodents in Poland

C PASINI, C TAMPONI, S MIGNACCA, E CONTU, G DESSÌ, A VARCASIA, L MELONI, S KNOLL, L CAVALLO, F PORCU, MF SINI, F NONNIS, A SCALA. Clustered cases of human ophtalmomyiasis in tourists caused by *Oestrus ovis*

P PEPE, A BOSCO, G MANGIERI, F CAPUANO, L BALDI, A GIORDANO, A MANCUSI, M BUONANNO, L MORENA, R PINTO, P SARNELLI, G CRINGOLI, L RINALDI. Towards an integrated approach for monitoring toxoplasmosis in southern Italy

G ROMANO, P PEPE, S CAVALLERO, P COCIANCIC, L DI LIBERO, G GRANDE, G CRINGOLI, S D'AMELIO, L RINALDI. Small bowel volvulus by *Ascaris suum* in a 75-year-old man: a case report

S RONDÓN, S CAVALLERO, A LINK, C GONZÁLEZ, S D'AMELIO. Prevalence of intestinal parasites infecting free-ranging non-human primates in Colombia

A RUSCONI, P PRATI, R BRAGONI, M CASTELLI, U POSTIGLIONE, D SASSERA, E OLIVIERI. Occurrence of *Eustrongylides excisus* (Nematoda: Dioctophymatidae) in European perch (*Perca fluviatilis*) and Great cormorant (*Phalacrocorax carbo*) in Northern Italy, Lake Annone

S SGUBIN, V CAGNIN, G DA ROLD, E QUARANTA, G SIMONATO, S PASQUALOTTO, A ZOROASTER, E PORCELLATO, S RAVAGNAN. Identification of cestodes in veterinary diagnostics: evaluation of two PCR methods

C VIRGILLITO, M MANICA, G MARINI, B CAPUTO, R ROSÀ, A DELLA TORRE. Bayesian statistical models to evaluate the efficacy of traditional and innovative mosquito control interventions

POSTER SOIPA – *PARASSITOLOGIA VETERINARIA E UMANA*

R ACO-ALBURQUEQUE, S GABRIELLI, M PALOMBA, F MARCER, E MARCHIORI, M SANTORO, S MATTIUCCI. *Blastocystis* in the marine environment: new insights into the genetic diversity and distribution in cetaceans and marine turtles from the Mediterranean Sea

I ARNOLDI, A NEGRI, B BISAGLIA, I VAROTTO-BOCCAZZI, R NODARI, S EPIS, P GABRIELI. Unravelling the development of the female mosquito proboscis using X-ray Tomography

I BERNARDINI, M DI LUCA, F SEVERINI. Identification of mosquitoes: new simplified keys for morphological analysis

I BERNARDINI, RP LIA, S MANZI, C POGGI, V PETRARCA, F BEUGNET, J MACA, MS LATROFA, D OTRANTO, M POMBI. Questionnaire survey of *Thelazia callipaeda* prevalence in Italy and vector samplings indicate Lazio region as a hot-spot of thelaziosis

G BONGIORNO, T DI MUCCIO, L GRADONI, A GIACOMI, M POMBI, S GABRIELLI, M GRAMICCIA. Natural infections of *Sergentomyia minuta* with kinetoplastid flagellates detected by gold standard methods in Rome province

MP MAURELLI, A AMADESI, C SANSONE, S MARRONE, M GRAVINA, A BOSCO, B CELANO, L RINALDI, G CRINGOLI. The Kubic FLOTAC Microscope: a new tool for helminth eggs diagnosis in veterinary and human field

M MENEGON, F SEVERINI, D BOCCOLINI, L TOMA, I VASCO, MA CAFIERO, M DI LUCA, DA RAELE. Morphological identification and molecular characterization of *Anopheles algeriensis* Theobald, 1903 (Diptera: Culicidae) from Apulia region

I MORETTA, E MEARINI, MA PRINCIPATO. Cheese infesting mites: experience in a small dairy in Umbria (Italy)

A NEGRI, I ARNOLDI, R NODARI, I VAROTTO-BOCCAZZI, B BISAGLIA, G CATTANEO, P GABRIELI, S EPIS. *Aedes koreicus* as an emerging invader in the Lombardy region

M PALOMBA, E BELLO, R ACO-ALBURQUEQUE, P CIPRIANI, G NASCETTI, S MATTIUCCI. Novel nuclear markers for the identification of the sibling species of the *Anisakis simplex* (s.l.) complex in a multilocus genotyping approach

V PICHLER, E MANCINI, M MICOCCI, M CALZETTA, D ARNOLDI, A RIZZOLI, V LENCIONI, F PAOLI, R BELLINI, R VERONESI, S MARTINI, A DRAGO, C DE LIBERATO, A ERMENEGILDI, RP LIA, D OTRANTO, J PINTO, A DELLA TORRE, B CAPUTO. Novel PCR-based genotyping approach allowed to detect spreading of pyrethroid resistance in *Aedes albopictus* across Italy

G SGROI, R IATTA, RP LIA, V VENEZIANO, G ANNOSCIA, D OTRANTO. Monitoring tick exposure and Rickettsiales in hunters and hunting dogs: a citizen science approach

PLENARY SESSION

PARASITES & MEDIA

What’s Eating You: Parasites and Media

D RISKIN

Evolutionary Biologist; Host of *Monsters Inside Me* (*“Io e I miei parassiti”*) on Animal Planet; Science and Technology Specialist for CTV Television (Canada); Author of *Mother Nature is Trying to Kill You*.

Keywords: Science communication, Popular audiences, Television

The communication of scientific and medical information to popular audiences is critically important, as it can help guide people to make decisions that benefit society. After all, getting people to wash their hands to prevent the spread of COVID-19, or to reduce their carbon footprint in response to the climate emergency, depends on clear and effective communication of those guidelines by experts. In no field is this moretrue than in parasitology, where the prevention of disease relies on the ability of scientists to clearly and effectively share their findings with the public. Unfortunately, however, scientists are rarely trained in science communication, and are instead left to figure it out by trail and error. In this keynote, I will try to make that process a little less painful. I will explore why *Monsters Inside Me* was a surprisehit for Animal Planet, discuss how we put our stories together to engage audiences, and link that to the way we communicate as experts. I will also offer concrete lessons for academics who want to share their work more broadly, whether in a television interview, a public lecture, or a video for your lab website.

Fake news and popular beliefs in Parasitology

M POLIDORO[1], A FRANGIPANE DI REGALBONO[2]

[1] Comitato Italiano per il Controllo delle Affermazioni sulle Pseudoscienze, Via Pascoli 1/A, Padova;

[2]Department of Animal Medicine, Production and Health, University of Padua, Viale dell'Università 16, Legnaro (Padua).

Keywords: Parasitology, Fake news, Delusion, Science communication

INTRODUCTION. In the last decades, internet and social media have provided unquestionable benefits to modern society but, on the other hand, a rapid and global growth of the dissemination of "fake news" in different areas of life. The main objective of this study is to focus on misleading information and erroneous popular beliefs circulating in the media concerning parasites, and to stress the essential role of the scientific community and practitioners (both physicians and veterinarians) in contrasting disinformation and helping disseminate proper scientific knowledge and public engagement.

MATERIALS AND METHODS. In 2020, internet was surfed using Google's search engine and typing in Italian language the combination of two terms: "parasite+human" and "zoonosis+dog+cat", consulting for each combination the first 50 web sites available n the rank pages. Each web site was deeply evaluated, and classified as a font of Misinformation=M (involuntary false information), Disinformation=D (voluntary dissemination of false information), or Correct information=C. The reliability of each URL (presence of malware and signs of phishing) was tested by TrendMicro (https://global.sitesafety.trendmicro.com/), and the popularity of each link was estimated by means of the Alexa rank=AR (the lower rank, the better, and *vice versa*), and the daily average time=T spent by a visitor (https://www.alexa.com/siteinfo). Case studies attributable to "delusional parasitoses" and popular beliefs on parasites have also been investigated.

RESULTS AND CONCLUSIONS. Investigated web sites were classified as D (8% of the cases), M (40%), and C (52%). All URLs were assessed as "safe", except one ("untested") in category M. Similar T values (min, sec) were detected for websites D (2:58), M (2:42), and C (2:52), while the ratios between AR values of web sites C/M and C/D were 1.7 and 2.6, respectively. The AR ratio M/D was 1.5. These results highlight that diving into web sources not strictly related to science and academia lead people to misleading information in about 50% of the cases, and in 8% of the cases to be victims of information purpose fully created and shared to induce harm and make illegal profit. Even with the limits of virtual assistant Alexa in estimating web sites' traffic, it appears that web sites M and D result as more popular, and are visited, comparing to C web site, 1.7 and 2.6 times more, respectively. These results, associated with the high diffusion of erroneous popular beliefs and unfounded fears, presented in the Session "Parasites & Media" of the XXXI Congress of the Italian Society of Parasitology, highlight the need toimprovecritical thinking among the public, and to strongly counter the spread of fake news in parasitology.

PLENARY LECTURE

Past, Present, and Future of Parasite control in a One-Health perspective

L HALOS

Senior Program Officer Animal Health, Livestock Team - Agriculture Development, Bill & Melinda Gates Foundation.

The One Health concept, which is a driver of the current global health strategies, states the interconnection between Human, Animal and Environmental health. This interconnexion is fundamental in Parasitology, probably more than in any other field of medicine, in relation with the complex life cycles of parasites. Evidence of parasites in humans and livestock date from several thousand years BC and parasites continue to exert their toll, particularly in the developing world. The control of parasites has seen tremendous change over the past 100 years. Prior to the 1960s the use of chemotherapeutic agents was virtually nonexistent. Human and animals simply co-existed with their parasite populations. Malaria was for centuries one of the determining factors of the evolution of a large part of Southern-Europe. Livestock producers were essentially dependent on the genetic resistance or tolerance of the host and the implementation of pasture management practices to sustain body growth and reproduction, often at a high cost to the welfare and production efficiency of the animal. Modern chemotherapy in parasitology was developed during the 20st century and culminated with the discovery of artemisinin and ivermectin in the 70s. It demonstrated to be an unequalled method of controlling parasites and has been used across parasite phyla and host species. Thanks to the development of several classes of insecticide, acaricide, anthelmintic and protozoocidal compounds, parasite infections were treated and prevented with effective drugs on a routine basis. In addition, the broad spectrum of several classes such as macrocyclic lactones or benzimidazoles offered an increased ease of use especially in veterinary medicine. However, as for the entire filed of infectious diseases, the optimism of the 70s has been restrained at the end of the twentieth century. If some parasite diseases were effectively controlled, the surge of resistance jeopardized most of the control programs. Drug resistance has arisen across a wide range of hosts in many environments around the world and has been reported in many parasite species and against most drug classes. At the start of the twenty-first century, malaria has been eliminated from most of Europe and from the Unites States, but the global burden of the disease remained significant, particularly in sub-Saharan Africa mainly because of the spread of resistance to first-line treatments in *Plasmodium falciparum*. Among parasites of veterinary interest, there are cases where the cost of control failure is so high that industries are threatened such as small ruminants breeding due to multidrug resistance in gastro-intestinal nematodes. In parallel, the environmental burden of parasiticide treatments, especially those for livestock, and their potential impact on wild micro-fauna are also increasingly pointed out by regulation authorities worldwide. The challenge that the world will face in the coming century will make the role of parasite control even more significant. The 60% increased need for animal proteins predicted by 2050 will require highly productive agriculture, especially in developing countries where the increase in population will be more significant. In the meantime, environmental constraints request that the human population pays more attention to the use of the land and the greenhouse gases emissions. An improved animal health and the control of parasites would permit to increase the productivity with an equal or reduced number of animals. Climate change is expected to exert an overwhelming negative effect on the human and animal-health interface. The effect of climate on infectious diseases, combined with the rise of drug, insecticide and acaricide resistance is likely to increase the burden of diseases, particularly those that are vector-borne and will require continuous adaptation to changing patterns. Future integrated approaches for parasite control must be systematic, combining innovative technologies and taking the biology of the target parasites in consideration. Parasite vaccines will eventually be accorded their due significance in the field as they are regarded the most promising, environmentally friendly, and sustainable strategy.

FORUM ESDA/SOIPA

DIROFILARIOSIS AND ANGIOSTRONGYLOSIS, FROM THE PAST TO THE FUTURE

SOIPA
Società Italiana di Parassitologia

ESDA
European Society of Dirofilariosis and Angiostrongylosis

Angiostrongylosis in animals and humans in Europe

E MORGAN[1], **D MODRY**[2], **C PAREDES-ESQUIVEL**[3], **P FORONDA**[4], **D TRAVERSA**[5]

[1]Queen's University Belfast, School of Biological Sciences, Belfast, United Kingdom; [2]University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Veterinary Medicine, Brno, Czech Republic; [3]University of the Balearic Islands, Applied Zoology and Animal Conservation Group, Palma de Mallorca, Spain; [4]University La Laguna, University Institute of Tropical Diseases and Public Health, Tenerife, Canary Islands, Spain; [5]University of Teramo, Faculty of Veterinary Medicine, Teramo, Italy.

Keywords: *Angiostrongylus vasorum*, *Angiostrongylus cantonensis*, Epidemiology, Climate change

Members of the genus *Angiostrongylus* are associated with significant disease in domestic and wild animals globally, and a sub-set of zoonotic species also infects humans. Here we introduce the key members of the genus and their impacts on health and review the current epidemiological situation in Europe. We then use data on parasite distribution alongside increasing knowledge of life cycles and ecology to ask what is driving their emergence, and what might be expected in terms of future spread in Europe.

From a veterinary perspective, *Angiostrongylus vasorum* is the most important species. It has risen to prominence in Europe in recent decades as a result of geographic spread beyond previously identified endemic foci, increasingly recognized disease in dogs, and associated development of improved methods for diagnosis and prevention. Infection in dogs most commonly causes respiratory disease, but can also inhibit bleeding, provoking severe and highly variable clinical outcomes. Increased clinical awareness and high-throughput serological diagnostic tools have contributed to apparent spread in Europe, but epidemiological surveys also indicate rapid spread in fox populations. Urbanization of foxes, invasion of exotic snail species, and climate change have variously been proposed to explain emergence, but evidence remains inconclusive. *Angiostrongylus vasorum* and *A. chabaudi* have also been recorded in cats and are pathogenic, although their overall clinical significance is so far limited.

Zoonotic disease caused by *A. cantonensis* is of enormous global significance and mainly reported in tropical regions, but insular foci of infection in Europe must stimulate concern for potential future spread. The situation on Mallorca is explained in detail by Paredes-Esquivel (this meeting). As well as eosinophilic meningitis in humans, *A. cantonensis* can cause neurological disease in accidental hosts, e.g. in zoo and wild animals. For both *A. vasorum* and *A. cantonensis*, experimental data allow parametrization of computer models to predict potential distribution under current and future climate, although modifying ecological factors are less understood and add significant uncertainty to model outputs.

Human angiostrongylosis in Europe, present and future risk

S DELGADO-SERRA[1], J SOLA[2], N NEGRE[2]; D MODRÝ[3,4], L HAINES[5], C PAREDES-ESQUIVEL[1]

[1]Applied Zoology and Animal Conservation Research Group, Universitat de les Illes Balears, Spain; [2]Mallorca Wildlife Hospital; [3,4]Department of Botany and Zoolog, Masaryk University & Institute of Parasitology & CINeZ, Czech University of Life Sciences; [5]Liverpool School of Tropical Medicine, Liverpool, United Kingdom.

Keywords: Rat lungworm, Europe, *Cantonensis*, Eosinophilic meningitis

INTRODUCTION. Neural angiostrongyliasis is an emerging zoonosis caused by the gastropod-borne nematode *Angiostrongylus cantonensis,* the rat lungworm. Since its first description in China in 1935, this parasite has expanded westward. To date, around 3000 human infections have been reported in 30 countries worldwide. Human angiostrongyliasis is often severe. Symptoms include debilitating migraines, skin sensitivity, and in severe cases: paralysis, bladder dysfunction and coma. Long term neurological damage has been reported and the death rate can be up to 10% in children. Rats and other vertebrates acquire the disease by ingesting nematode-infected gastropods, but only in rats can the parasite complete its life cycle.

MATERIALS AND METHODS. Indigenous North African hedgehogs were admitted to the local wildlife hospital in 2018 displaying symptoms of an acute neurological disease. We have since established a surveillance strategy in symptomatic and asymptomatic hedgehogs that arrive to the local wildlife hospital. Necropsies are followed by enzymatic digestion of brain tissue to screen for *A. cantonensis*.

RESULTS AND CONCLUSIONS. Europe was considered non-endemic for angiostrongyliasis but predictions indicated that global warming could favour the establishment of *A. cantonensis* in the continent later in this century. However, in October 2018 we discovered hedgehogs infected with *A. cantonensis* in Mallorca. In the last three years, we have consecutively found eight positive animals, which demonstrate that *A. cantonensis* is well-established in the island. We have also reported the unexpected presence of a gravid adult female in the hedgehog brain. It remains unknown whether larvae can be released in the faeces of these animals. Understanding this would shed light on the life cycle and reveal potentially a new epidemiological risk in the continent. Furthermore, in a region where snails are an important part of the island cuisine and a large tourism industry exists, the arrival of the rat lungworm to Mallorca opens not only important scientific questions, but also presents social challenges. Following our published findings, our research fuelledinternational media distortion on the risks associated to the disease. Our goal is to use the Balearic scenario as an example of early disease detection and response using a *One Health* approach. Such a strategy would help develop other effective and cost-effective practices to detect emerging zoonoses in other European countries.

Concern for *Dirofilaria immitis* and LOE/Resistance: current situation in the USA and Europe, and future scenarios

A DIAKOU

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

Keywords: *Dirofilaria immitis*, Macrocyclic lactones, Resistance, Prevention

Dirofilaria immitis infection (heartworm disease) is one of the most severe parasitic diseases of dogs. Prevention is achieved bythe administration of drugs containing macrocyclic lactones (MLs). These products are very safe and highly effective, targeting the third and fourth larval stages (L3, L4) of the parasite. Until 2011, claims of ineffectiveness of MLs, reported as “Lack of Efficacy” (LOE), were generally attributed to owners’ non-compliance, or other reason for inadequate preventative coverage (Hampshire 2005 Vet Parasitol. 133: 191-5). There was solid argumentation that a resistance problem is not likely to occur because of i) the great extent of refugia, ii) the complexity of resistant development to MLs, and iii) the big number of genes involved in resistance selection (Prichard 2005 Vet Parasitol. 133: 243-53). Nevertheless, today it is unequivocally proven that MLs resistant *D. immitis* strains exist, at least around the Mississippi Delta region, USA. Accordingly, tools have been developed, to evaluate and confirm the susceptibility status of *D. immitis* strains. A simple, in-clinic, microfilariae suppression test, 7 days after ML administration (Geary et al., 2011 Top Companion Anim Med. 26: 186-92), and a “decision tree” (algorithm), including compliance and preventatives’ purchase history, and testing gaps, may be applied for assessing any resistant nature of the parasite (Moorhead et al., 2017 Parasit Vectors. 10: 479). On the molecular level, specific SNPs may be used as markers of ML resistance, offering a basis for validation of clinically suspected resistant strains (Ballesteros et al., 2018 Int J Parasitol Drugs Drug Resist. 8: 596-606). In Europe, no LOE/resistance claims have been reported so far, and the existent conditions (stray dogs, rich wildlife, majority of owned dogs not on preventive MLs treatment) do not favour selection pressure on the parasites. Considering the genetic basis of resistance and the epizootiological characteristics of *D. immitis*, ML resistance neither establishes easily nor spreads quickly, a fact confirmed by the current known dispersion of the problem, which is limited. Nevertheless, ML resistance may propagate from an initial geographical point, via animal and vector mobility, to other regions, while it can also emerge as an independent evolutionary process in a remote area. For this reason and taking into account the augmenting usage of endectoparasiticides with MLs as a potential selection pressure, it is important to remain vigilant for timely detection of any ML LOE/resistance, in all continents where *D. immitis* is enzootic.

Human dirofilariosis, the point of view of the physician

E SCOCCIA

Senology Dept. Istituti Clinici Scientifici Maugeri Pavia S.p.a., Italy.

Keywords: *Dirofilaria repens*, Neoplasia, Diagnosis

Dirofilariosis is a vector-borne parasitic disease caused by *Dirofilaria repens*, found mainly in domestic and wild carnivores that act as “reservoir”, which is endemic in many countries of the Old World, and *Dirofilaria immitis*, which has a worldwide distribution. In recent years, an increasing number of human cases has been reported, suggesting that dirofilariosis is an emergent zoonosis.

Humans are accidental dead-end hosts of *Dirofilaria* spp. as in most cases, the infective larvae die before reaching worm maturity causing a focal granuloma. Patients show different clinical manifestations depending on the localization of the worms. Patients with ocular and subcutaneous nodules have been reported as, less frequently, with pulmonary nodules.

While in ocular and some subcutaneous lesions redness, swelling and itch are common and the nematode may be visible under the conjunctiva, lung e deep subcutaneous nodules in some region as breast are misidentified as malignant neoplasm, thus requiring invasive investigations before the correct diagnosis.

Italy is probably the country with the highest number of human clinical cases in Europe but a general lack of awareness of this parasitic disease still persists in physicians. As diagnostic imaging cannot rule out a lung or breast malignant neoplasm and no indirect diagnostic test are available most cases of human dirofilariosis are mainly diagnosed based on histopathological findings and molecular diagnosis, based on direct sequencing of the parasites’ DNA.

Human dirofilariasis should always indeed be considered when examining solitary nodules and more and closer collaboration between physicians and veterinarians would be advisable, considering that veterinarians have greater skills and a more appropriate training path as regards zoonotic diseases.

INDUSTRY FORUM ESDA/SOIPA

DIROFILARIOSIS

Importance to Map CVBD: an Italian survey on *Dirofilaria* spp.

L KRAMER

Department of Veterinary Science, University of Parma, Parma Italy.

Keywords: *Dirofilaria* spp., Epidemiology, Prevention, Mapping

INTRODUCTION. It has been reported that numerous factors are leading to the spread in many canine vector-borne diseases, including heartworm disease (*Dirofilaria immitis*) and subcutaneous dirofilariosis (*D. repens;* Morchón et al., 2012Front Physiol. 3:196-206). These includeclimate change, modifications of landscapes and habitats due to human activities, as well as an increase in the movement of reservoirs and new species of competent vectors. Initiatives like "MyVBDmap" (myvbdmap.com/it) will help to understand not only not only where these parasites are, but where they are going.

MATERIALS AND METHODS. Results from a previous study carried out several years ago on the prevalence of *Dirofilaria* species in the different Italian regions will be presented. Our veterinary practitioners working in the field are an essential source of reliable information and together, we will identify what data we need in order to update the current epidemiological situation in Italy.

RESULTS AND CONCLUSIONS. There are many challenges in *Dirofilaria* prevention at the moment. Perhaps the greatest is the belief that *Dirofilaria* infections are no longer a problem. A recent study (Panarese et al., 2020 Int J Parasitol 50(8):555-559) reported a prevalence of 44% for heartworm infection in shelter dogs that did not receive routine preventive measures. The European Society for Dirofilariosis and Angiostrongilosis (ESDA) have developed clear and user-friendly guidelines for the diagnosis, treatment and prevention of dirofilariosis, along with many other parasites of concern in our pets. We will be going over them, with particular attention to diagnosis. If we want to monitor the spread of infection, we need to know how to correctly diagnose it. Screen for infection, report your cases and map!

Double Defense against *Dirofilaria* spp: practical recommendations

L VENCO

Ospedale Veterinario Città di Pavia, Pavia, Italy.

Keywords: Heartworm disease, Permethrin, Anti-feeding, Mosquitoes

It is well known that Heartworm preventives effectively protect against heartworm disease by preventing the development of L3 infective larvae to adult stage into the pulmonary arteries. In human medicine for the prevention of vector-borne diseases a primary prevention that seeks to avoid contact with the pathogen's vectors or with the pathogen itself and a secondary prevention that blocks or interferes with the action of the pathogen are usually associated. For the prevention of Malaria, in addition to the intake of antimalarial drugs, the use of repellents is recommended and for the recent SARS-CoV-2 the use of face masks is put besides the administration of vaccines. Discovering that a Permethrin based drug as topical parasiticide, do repel and kill mosquitoes (with overall 98.5 percent anti-feeding efficacy) a new "Double-Defense" protocol allowing for a second layer of defense by preventing the mosquito from biting in the first place is considered the most safe and effective prevention.

The advantages are evident and are mainly related to remedying client's lack of compliance in correctly administering the macrocyclic lactones, to the possible appearance of *Dirofilaria immitis* strains resistant to their action, and to protect dogs against *Dirofilaria repens,* a zoonotic parasite*,* against which few macrocyclic lactones are effective.

In the same areas where both Heartworm disease and Leishmaniasis are present furthermore the Double-Defense Protocol provides effective protection against two deadly diseases difficult to be treated if present in the same subject.

INDUSTRY TALKS ESDA/SOIPA

DIROFILARIOSIS AND ANGIOSTRONGYLOSIS

Dog relocation and rapidly changing parasite threats

J DRAKE

Elanco Animal Health.

Keywords: Parasite prevalence, Europe, United States, Canine importation

A variety of dog rescue networks have evolved which move hundreds of thousands of dogs and puppies across and between countries. The originating sites often also have relatively high parasite prevalence. Individuals relocating dogs are often unaware or unconcerned with the risk of parasites being moved with dogs, as evidenced by a high percentage of organizations which do not test or treat dogs for parasites prior to transport (Simmons et al., 2016 Animals. 6: 11).

Intra-European trade of approximately 46,000 dogs/month occurred in 2014 alone, with 68% of registered trade originating in Hungary, Slovakia, or Spain, and 71% of registered trade destinations listed as France, Germany or the United Kingdom (IBF International Consulting et al., 2015. Specific Contract SANCO 2013/12364: Final Report, European Commission). Approximately 300,000 dogs enter the United Kingdom annually via the Pet Travel Scheme (Norman et al., 2020 Vet Record. 186: 248). The state of Colorado, in the United States of America, received approximately 114,000 dogs over a four-year period from 2014-2017, with the majority of these dogs originating in the southern parts of the USA where heartworm and many gastrointestinal nematodes are high in prevalence (Drake et al., 2017 Parasit Vectors. 12: 1-10; Drake et al., 2020 Parasit Vectors. 13: 1-6).

Examples of changing parasite prevalence or parasitic disease outbreaks which may be related to dog relocation will be presented. Relocation of thousands of dogs from areas of relatively high parasite prevalence may be changing the prevalence of parasites at the destinations where the dogs are adopted, rapidly changing the local risk, and possibly spreading resistant parasites. Veterinarians and dog owners should consider greater precautions related to the control of parasitic diseases than may have been standard of care in the past for relocated dogs as well as dogs living in communities receiving relocated dogs.

A consensus statement on the topic of rehomed dogs, parasites and vector borne diseases from the Companion Vector-Borne Diseases (CVBD) World Forum was recently published (Wright et al., 2020 Parasit Vectors. 13: 546). Veterinarians should encourage pet owner adherence to these guidelines whenever feasible.

Unravelling diagnostic aspects in canine angiostrongylosis

M SCHNYDER

Institute of Parasitology, Vetsuisse, Faculty, University of Zurich, Zurich, Switzerland.

Keywords: Coproscopy, Serology, PCR, Variable temporal dynamics

INTRODUCTION. The clinical picture of canine angiostrongylosis is highly variable, and slow progressive or acute manifestations make the diagnosis challenging. Lung tissue damages are possible at a very early stage of infection. Therefore, timely diagnosis is fundamental. Premising disease awareness, different procedures are suitable for the confirmation of clinical suspect cases. It is important to understand their comparative properties (Table 1, Schnyder et al., 2015 Parasitol. 142: 1270-77; 2014 Paras Vect. 7:72):

Test	Material	First positive, weeks p.i. Mean (range)	Proportion pos., within dogs Mean (range)	Total proportion positive	Last positive, weeks after trm Mean (range)
Baermann	Faeces	7.4 (7-8)	0.95 (0.4-1)	0.97	2.2 (2-3)
AG ELISA (IPZ)	Serum, plasma	8.7 (7-11)	1	1	4.0 (3-7)
AG rapid assay (IDEXX)		10.7 (9-14)	1	1	4.6 (3-7)
Ab ELISA (IPZ)		4.6 (3-6)	1	1	5.7 (3-9)
PCR	Blood	6.3 (2-10)	0.78 (0.45-1)	0.70	1.0 (1-5)
	Faeces	9.1 (8-11)	0.91 (0.5-1)	0.84	2.0
	Swab	12.9 (10-15)	0.71 (0.5-1)	0.69	2.7 (2-3)

CONCLUSIONS. The Baermann-funnel method faeces and also the serological antigen rapid detection assay are easy and immediate methods that can be recommended in practice, premising correct sample storage and knowledge about their test characteristics. Further diagnostic procedures performed with faeces (smears, FLOTAC) may have their justification. Also PCR performed with different materials are indicated in particular cases, e.g. with histological material for species confirmation, or with bronchial mucus in dogs under anaesthesia. Serological antibody combined with circulating antigen detection are valuable for mass-screening in epidemiological inquiries and for individual challenging cases. Examples related to studies and to individual cases, including the detection of *A. vasorum* in newly detected hosts, will illustrate advantages and disadvantages of the different test methods.

New insights into the biology, diagnosis and immune response to *Dirofilaria repens* in the canine host

L CIUCA[1,2], A VISMARRA[3], W LEBON[4], F BEUGNET[4], R MORCHON[5], L RINALDI[2], G CRINGOLI[2], L KRAMER[3], M GENCHI[3]

[1]Department of Parasitic Diseases, University of Life Sciences "Ion Ionescu de la Brad" Iași, Romania; [2]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, CREMOPAR, Naples, Italy; [3]Dipartimento di Scienze Medico-Veterinarie, Università di Parma, Italy; [4]Boehringer Ingelheim Animal (BI-AH) Health Research Centre, in France; [5]Group of animal and human dirofilariosis, Parasitology area, Faculty of Pharmacy, University of Salamanca, Spain.

Keywords: *Dirofilaria repens* antibodies, *Wolbachia* antibodies, Non-commercial IgG-ELISA, Amicrofilaraemic dogs

INTRODUCTION. The lack of a commercially available test for serological diagnosis of *D. repens* infection in dogs is likely one of the most important limitation (Simón et al., Clin Microbiol Rev. 25: 507-44; Capelli et al., 2018 Review Parasit Vectors. 11: 663). Thus, the aim of the present study was to provide new insights from experimental infections of dogs with *D. repens*, focusing on the evaluation of: 1) the pre-patent period and 2) the antibody response against *D. repens* somatic antigens and against the *Wolbachia* endosymbiont.

MATERIAL AND METHODS. Twenty purpose-bred Beagle dogs were experimentally infected with 50 infective larvae (L3) of *D. repens* on Day 0. Starting from Day 58 until the last day of the study (Day 281), blood samples were collected on a monthly basis for detection of antibodies against *D. repens* (Dr) and recombinant *Wolbachia* surface protein (rWSP) by non-commercial IgG-ELISAs. Additional samples were collected on Days 220, 245 and 281 for the detection of mff using the modified Knott's test and biomolecular analysis, following two PCR protocols: protocol A (Gioia et al., 2010 Vet Parasitol. 172: 160-63; protocol B (Rishniw et al., 2006 Vet Parasitol. 135: 303-14).

RESULTS AND CONCLUSIONS. Overall, the outcome of the study revealed that out of the 20 dogs experimentally infected with *D. repens*, 16 (80%) were microfilaraemic, 17 (85%) were positive at DNA detection in the blood, 18 (90%) had *D. repens* antibodies and 16 (80%) had *Wolbachia* antibodies on the last day of the study. The overall k agreement between Knott's and PCR protocol B was 0.442 (P=0.0001) and increased throughout the study, reaching 0.828 (P=0.0001) on Day 281. To the authors knowledge, this is only the second study reporting antibody response to *D. repens* somatic antigen in experimentally infected dogs. ELISA results showed that an antibody response develops before the onset of patency, and steadily increases with time. Results would suggest that the development of an immunological response to infection could lead to application in epidemiological studies, risk assessment and as an aid in the diagnostic approach in dogs, in particular for early infections without mff.

SYMPOSIUM

OLD AND NEW PARASITES OF COMPANION ANIMALS

Elanco™

Old and new feline lungworms

A DI CESARE

Faculty of Veterinary Medicine, University of Teramo, Italy.

Keywords: *Aelurostrongylus abstrusus*, *Troglostrongylus brevior*, *Capillaria aerophila*

Feline lungworms have become of growing interest in feline medicine because of their expanding distribution, the unexpected findings in cats and their high pathogenic role (Morelli et al., 2021 Pathogens 10: 454). *Aelurostrongylus abstrusus*is known as the prevailing respiratory nematode of cats and, until a few years ago, it was considered The Cat Lungworm. *Capillaria aerophila* was also traditionally considered able to infect cats though its role in feline clinical medicine has been neglected for a long time. This "old" scenario has been reshaped in the last 10 years, due to the report of "new" species (i.e. *Troglostrongylus brevior*, *Troglostrongylus subcrenatus*, *Oslerus rostratus* and *Angiostrongylus chabaudi*) in domestic cats. The findings of these practically unknown respiratory nematodes have spurred a high number of laboratory and field studies which have led to new genetic, epizootiological and biological acquisitions and to a new awareness on the role of lungworms in causing feline respiratory infections. Epizootiological surveys and clinical or post-mortem cases published in the last 10 years have shown that i) *A. abstrusus*, *T. brevior* and *C. aerophila* are a primary cause of respiratory diseases in cats and ii) the negligible importance of *T. subcrenatus*, *O. rostratus* and *A. chabaudi* in cat parasitology (Traversa et al., 2021 Pathogens 10: 30). In terms of geographic distribution, *A. abstrusus*is apparently spreading and there is the evidence that *T. brevior* is now enzootic in domestic cats of Southern and Eastern Europe and in the Middle East. It has been demonstrated that the wildcat, i.e. the natural host of *T. brevior*, has played a crucial role in fostering this establishment. Nevertheless, the risk of introduction and spreading of *T. brevior* in non-enzootic areas and in regions where the wildcat is absent through the movement of infected animals from endemic to free regions cannot be ruled out. Changes in intermediate hosts seasonal population dynamics along with the high adaptability of intermediate hosts to new areasmight indeed nurture the current dispersal and spread of these nematodes. A constant epizootiological surveillance is therefore crucial, along with implemented diagnostic, therapeutic and control measures. Constant monitoring is also essential to understand if minor species will gain importance in cat parasitology.

Parasitoses in puppies, risk assessment and control

F VERONESI, G RIGAMONTI

Department of Veterinary Medicine, University of Perugia.

Keywords: Puppy, Endoparasites, Ectoparasites, Control, Risk assessment

Puppy represents one of the most vulnerable stages over the life of pets. The immune system not fully developed, the inefficient intestinal, cutaneous and airway microbiota, the environmental stressors and changes may increase the exposure and the sensibility to a wide range of pathogens including internal and external parasites. The most common and dangerous parasites of puppy's life consisted on geohelminths (e.g. *Toxocara* and *Ancylostoma*), tapeworms as *Dipylidium caninum*, protozoan agents of enteritis (e.g. *Cystoisospora* and *Giardia*) and also ectoparasites as *Ctenocephalis canis*, *Demodex* spp., *Otodectes cynotis* and *Phlebotomus* spp.. Besides to the illness directly related to the parasites, they may impact on a range of different aspects, e.g. onset of virus and bacteria infections and their level of morbility and mortality, interference with the active immunization and may have long-term effects on the growth of the animals. Considering these premises, puppies should undergo strict parasite control, however no single parasitic control plan will fit all puppies and a wide range of antiparasitic medications are commercially available, having different caracteristics and spectrum of action. A correct risk group allocation of puppies was required, taking into account lifestyle, cohabiting animals (mostly dams) and their parasitic infection/infestation history, location and general health. Risk assessments are useful to establish if preventative antiparasitic treatment are required as well as the frequencies. For instance due to climate changes and the use of central heating in owner's house the flea *C. felis*, can persist over all the year and thus puppies born and hold in confined conditions have a increased hazard to became early infested if routine prophylaxis is not applied (Halos et al., 2014 Trends Parasitol. 30: 228-33). Again, since all puppies and kittens show to be infected by roundworms at or after birth, due to the transplacental and/or transmammary routes, the preventive treatment of the bitch is of primary concern to prevent a massive environmental contamination with eggs and a large parasitic burden developing with clinical effects (Lloyd et al., 1991 J Comp Pathol. 105: 93-104; Payne and Ridley 1999 Vet Parasitol. 85: 305-12). Further considerations for parasite control are the human health impacts that puppies might have, considering the zoonotic potential of some parasites (i.e. *Giardia, T. canis, D. caninum, A. caninum, C. felis*) (Baneth et al., 2016 J Comp Pathol. 155: S54-74).

Veterinary aspects and zoonotic risks of dog and cat intestinal nematodes

P OVERGAAUW

Institute for Risk Assessment Sciences (IRAS), Department of Population Health Sciences, Division Veterinary Public Health, Faculty of Veterinary Medicine, Utrecht University.

Keywords: Dog, Cat, Nematodes, Zoonosis

INTRODUCTION

Dogs have been by man's side for tens of thousands of years, but cats entered around 7,500 years ago. Worms in dogs and cats have always been apparent. Medical and veterinary professionals still have difficulty recognising typical human or typical dog parasites, which have their own life cycles, from zoonoses. The ubiquitous problem among stray dogs in urban areas of the world emphasises the need to diagnose, treat and prevent zoonoses, including parasitic nematodes.

Ascarids. Ascarid infections occur across Europe, whilst the distribution of other infections is geographically related. The most important ascarids are *Toxocara* spp. which are zoonotic and may cause several syndromes in humans such as visceral larva migrans, ocular larva migrans, covert toxocarosis and neurotoxocarosis. *Toxascaris leonina* is generally less pathogenic and the zoonotic potential is absent or very limited.

Hookworms. After ascarids, hookworms are the most found nematodes in carnivores. Dogs may harbour two genera of importance: *Ancylostoma* and *Uncinaria*. Only two zoonotic hookworms are known to use humans as definitive hosts: *A. ceylanicum,* often successfully and *A. caninum,* only occasionally without developing to full maturity. *A. braziliense* is mainly responsible for cutaneous larva migrans or 'creeping eruption' in humans. The feline hookworm *A. tubaeforme* is not capable of invading human skin. Natural human infection with *U. stenocephala* have not been reported.

Whipworms. *Trichuris vulpis*, also known as kennel worm, is the canine whipworm. In the cat there are two species *T. campanula* and *T. serrata*, neither of which occurs in Western Europe. The infections are not zoonotic.

Threadworms. *Strongyloides stercoralis* is an intestinal threadworm of dogs, primates and humans. The disease is usually mild and self-limiting, but in some cases bronchopneumonia and diarrhoea can be seen. Penetration of the infective larvae into the skin may produce an intense local pruritis and erythema. Dogs are considered reservoirs for the zoonotic transmission of *S. stercoralis* to humans.

***Trichinella spiralis*.** Dogs may acquire trichinae mostly by eating rats and other rodents. In Europe, only a few cases have been reported. As dogs are not eaten in Western Europe the worm is not considered a zoonosis.

SIMPOSIO

STRIKING NEWS IN PARASSITOLOGIA UMANA

Microbial symbionts for the control of mosquito-borne diseases

G FAVIA

School of Biosciences and Veterinary Medicine, University of Camerino, Italy.

Keywords: Mosquito, Symbiosis, *Asaia*, *Wolbachia*

INTRODUCTION. Mosquitoes host diverse bacterial communities influenced by the sex of the mosquito, tissue, and developmental stages, among other factors. Consequently, the mosquito microbiota may impact on several aspects of the host biology, including nutrition, development and reproduction. I report some advances in the study of microbial symbiosis in mosquitoes with particular reference to the bacteria *Asaia* and the *Wolbachia*, the best characterized mosquito symbionts, for which some conditioning on the biology of the host are proposed, including for example a possible impact on insecticide-resistance mechanisms developed by mosquitoes.

MATERIALS AND METHODS. Several strains of mosquitoes have been analyzed. Metagenomics analysis have characterized the microbiota associated to all the mosquito strains. Microbial and functional studies have characterized some mosquito bacterial symbionts.

RESULTS AND CONCLUSIONS. We have recently revealed a shared core microbiota among different mosquito species, although interesting inter- and intra-species differences were detected. Additionally, we showed deep divergences between genera, underlining microbiota specificity and adaptation to their host suggesting that the holobiont of different mosquito species may significantly vary. Moreover, mosquito species are characterized by distinctive microbiota in different organs, likely reflecting different functions and/or adaptation processes. Understanding of the microbiota of mosquitoes in relation to sex, developmental stage and tissue, may ultimately provide crucial insights and novel targets for possible application of symbionts in innovative strategies for the control of vector borne diseases, globally named Symbiotic Control (SC).

The interactions between *Trichomonas vaginalis* and the vaginal microbiota

V MARGARITA[1], P RAPPELLI[1,2], N DIAZ[1], D DESSÌ[1,2], N BAILEY[3], RP HIRT[3], JM FETTWEIS[4,5,6], PL FIORI[1,2]

[1]Dipartimento di Scienze Biomediche, Università degli Studi di Sassari, Sassari, Italy; [2]Mediterranean Center for Disease Control (MCDC), Sassari, Italy; [3]Biosciences Institute, Faculty of Medical Sciences, Newcastle University, Newcastle uponTyne, United Kingdom; [4]Department of Microbiology and Immunology, School of Medicine, Virginia Commonwealth University, Richmond, VA, USA; [5]Department of Obstetrics and Gynecology, School of Medicine, Virginia Commonwealth University, Richmond, VA, USA; [6]Center for Microbiome Engineering and Data Analysis, Virginia Commonwealth University, Richmond, VA,USA.

Keywords: *Trichomonas vaginalis*, Endosymbiosis, Vaginal microbiota, *Mycoplasma* spp.

The anaerobic protist *Trichomonas vaginalis* is the etiologic agent of trichomoniasis, the most prevalent non-viral sexually transmitted infection in humans, responsible for nearly 250,000 cases each year worldwide. Trichomoniasis is associated with vaginal dysbiosis, increased risk of HIV infection, adverse pregnancy outcomes (pre-term delivery), and with cervical and prostate cancer. Resident vaginal microbiota is represented by different population of aerobic and anaerobic microorganisms, that establish a physiologically dynamic system dominated by bacteria of the genera Lactobacillus: *T. vaginalis* infection profoundly alters local microbial ecology, causing a microbial vaginal imbalance. One of the most intriguing aspects of *T. vaginalis* is the ability to establish relationship with intracellular microbial symbionts: a couple of eubacteria belonging to the Mycoplasma genus, and a group of four dsRNA viruses belonging to family of Totiviridae (*T. vaginalis* virus, TVV). The intracellular symbionts exclusively associated with *T. vaginalis* not only reside in the cytoplasm but are also able to replicate synchronously with the protozoan cells.

The presence of Mycoplasma and viruses modulates the protozoan pathophysiology, and both endosymbionts synergistically upregulate the host proinflammatory response. The symbiotic relationships influence the clinical presentation of trichomoniasis and its morbid sequelae, including cervical and prostate cancer and adverse reproductive outcome, contributing to the broad diversity of trichomoniasis symptomology.

Novel omics technologies in the study of anisakid nematodes

S D'AMELIO, A PIZZARELLI, I BELLINI, S CAVALLERO

Department of Public Health and Infectious Diseases, Sapienza University of Rome.

Keywords: *Anisakis*, Genomic, Transcriptomic, Proteomic

INTRODUCTION. Anisakids (Superfamily Ascaridoidea) are a family of aquatic parasitic nematodes that are causative agents of anisakiasis or anisakidosis, a relatively poorly known fish-borne zoonosis which results from accidental ingestion of raw or undercooked marine products harbouring L3 infective larvae belonging mainly to *Anisakis* and *Pseudoterranova* genera. The pathology shows mild to severe gastrointestinal and/or allergic symptoms and clinical signs, as rhinitis, urticaria and anaphylactic shock, and increasing evidence report the occurrence of tumours in the same localization of L3. Given the large occurrence of L3 in fishes and their simple maintenance in basic culture media, Anisakids represents a suitable model to study pathogenesis and host-manipulation strategies in helminthic parasitic diseases, that are largely neglected despite their public health and socioeconomic relevance. Recent advancements in NGS technologies are supporting studies aimed at describing the molecular profiles of the studied organisms.

GENOMICS. The diversity within the *Anisakis simplex* complex may complicate the fine definition of genomes, transcripts and proteins and the precise assignment of genes or gene products to a given species. Among anisakids, the draft genome is so far available for the species *Anisakis simplex* s.s. but not for *A. pegreffii*, thus complicating precise mapping and assembly of outputs from transcriptomic and proteomic data. A project aimed to define the genome of this species is ongoing with the most accurate technology available to date.

TRANSCRIPTOMICS. Comparative studies on fundamental adaptive processes as molecular pathways related to parasitic survival, pathogenesis, allergenic potential, life-cycle stage, species, temperature and tissues are contributing to understand physiology and pathogenic potential of Anisakids.

PROTEOMICS. Exploring the immunobiology of anisakid infections relies mainly on protein components of the ES parasitic products. MALDI-TOF/TOF and Tandem Mass Tag-based quantitative proteomics analyses allowed to describe differentially expressed proteins in members of the *A. simplex* complex. Recently, the first global proteome of *A. simplex s.l.* allowed to detect sets of modulated proteins providing stage-specific proteomic signatures. Moreover, with the advent of omics approaches, confirmatory and explorative studies on *Anisakis* allergens started to become available. Sequencing and bioinformatics comparison of predicted peptides with the AllergenOnline database allowed to identify novel putative allergens among all the molecules of the *A. simplex* and *A. pegreffii* transcriptomes.

Parasitological diagnostics in fecal transplant procedures and in the characterization of human microbiota maps

S PANE[1], A RUSSO[1], F DEL CHIERICO[2], A ONETTI MUDA[3], CF PERNO[4], L PUTIGNANI[1,2]

[1]Department of Diagnostic and Laboratory Medicine, Unit of Parasitology; [2]Multimodal Laboratory Medicine Research Area, Unit of Human Microbiome; [3]Department of Diagnostic and Laboratory Medicine; [4]Unit of Microbiology and Immunology, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy.

Keywords: Microbiota profiling, Diagnostic parasitology, Faecal microbiota transplantation, Donors' screening

INTRODUCTION. A growing body of evidences is showing that dysbiotic gut microbiota may correlate with a wide range of disorders; hence, the clinical use of microbiota maps and fecal microbiota transplantation (FMT) can be exploited to treat some infectious diseases. Through direct or indirect ecological and functional competition, FMT may stimulate decolonization of pathogens or opportunistic pathogens, modulating immune response and colonic inflammation, and restoring intestinal homeostasis, with reduction of host damage. Herein, we discuss how diagnostic parasitology may contribute in appropriate donors' screening programs boosting safety standard in FMT programs, especially in pediatric subjects.

MATERIALS AND METHODS. Specific molecular assays, morphological and morphometric evaluation of protozoan parasites and significance of microbiota maps are discussed in the framework of FMT donors' selection, especially in pediatrics.

RESULTS AND CONCLUSIONS. The consequences of a more specialized diagnostics in the context of gut microbiota communities may improve the clinical parasitology and extend its applications to the prevention and treatment of several communicable and even non-communicable disorders.

Public health challenges in the Bolivian Chaco: 35 years of experience

S GABRIELLI[1], F MACCHIONI[2], M SPINICCI[3], M STROHMEYER[3], M ROSELLI[3], C TRIGOSO[4], H GAMBOA[5], AL VILLAGRAN[6], F COSMI[7], J MONASTERIO[8], A NICOLETTI[9], G CANCRINI[1], A BARTOLONI[3]

[1]Dipartimento di Sanità Pubblica e Malattie Infettive, Università di Roma Sapienza, Rome, Italy; [2]Dipartimento di Scienze Veterinarie, Università degli Studi di Pisa, Pisa, Italy; [3]Dipartimento di Medicina Sperimentale e Clinica, Università degli Studi di Firenze, Florence, Italy; [4]Instituto Nacional de Laboratorios de Salud (INLASA), Ministerio de Salud, La Paz, Bolivia; [5]Facultad Integral del Chaco, Universidad Autónoma Gabriel René Moreno, Camiri, Plurinational State of Bolivia; [6]Hospital Básico de Villa Montes, Villa Montes, Plurinational State of Bolivia; [7]Escuela de Salud del Chaco Tekove Katu, Gutierrez, Plurinational State of Bolivia; [8]Servicio Departamental de Salud de Santa Cruz, Santa Cruz, Plurinational State of Bolivia; [9]Department of Medical and Surgical Sciences and Advanced Technologies "G.F. Ingrassia", Neuroscience Section, University of Catania, Catania, Italy.

Keywords: Bolivia, Infectious diseases, Sanitation, Epilepsy, Antibiotic resistance

The Bolivian Chaco is a semi-arid and sparsely-populated region, located in the south-east of the Plurinational State of Bolivia. Here, extreme poverty remained high in rural areas (36.1%), where social vulnerability is widespread. Local economy is based on agriculture and animal farming. People are mainly of the Guaraní ethnic group, living in isolated communities with low standard of sanitation and medical services. In this context, several research activities have been carried out since the late 1980s, promoted by an Italian Franciscan monk, Tarcisio Ciabatti, who lived there since 1976, supporting the Guaraní population, within an official agreement between the Bolivian Ministry of Health (MoH) and the Convenio de Salud, with the aim to develop basic health services, to promote sanitation, prevention, control and treatment of infectious diseases. In 1987, an official agreement was signed between the University of Florence and the Bolivian MoH, and up to now it has been regularly renewed. The research activity has been conducted without interruption until today with the progressive inclusion of different institutions such as Un. of Rome Sapienza, of Siena, of Catania, of Pisa, London School of Hygiene and Tropical Medicine, Karolinska Institutet, Centro Salute Globale of Tuscany Region and Universities from other Latin-American countries. The magnitude of research areas included the epilepsy and parasitic diseases, epidemiology of parasitic diseases and the control of STHs. Further projects have been dedicated on the surveillance of antimicrobial resistance, prevalence of West Nile and Dengue virus, vaccination programs, WASH education, community epidemiology, non-communicable diseases (support to national programs of cancer prevention). To date, the longstanding collaboration led to the publication of approximately one hundred scientific articles on international peer-reviewed journals. Results of these programs allowed to describe the epidemiological scenarios of several infectious diseases in this neglected area, to promote healthy behaviors and access to health facilities in the rural population, and to train local sanitary personnel on the diagnosis and management of parasitic diseases.

SYMPOSIUM

MODERN USE OF PARASITICIDES

ARCOBLU

KLIFOVET AG
ADDED VALUE TO ANIMAL HEALTH

3Rs and Parasiticides in Companion Animals: Replacing models with patients

A JOACHIM[1], D TRAVERSA[2], H GRIMM[3]

[1]Institute of Parasitology, Department of Pathobiology, University of Veterinary Medicine Vienna, Austria; [2]Faculty of Veterinary Medicine, University of Teramo, Italy; [3]Institute for Ethics and Human-Animal Studies, Messerli Research Institute, University of Veterinary Medicine Vienna, Austria.

Keywords: Reduction, Replacement, Ethics, Antiparasitic efficacy studies

The authorization of and use of veterinary medicines such as antiparasitic drugs in Europe is regulated by the European Medicines Agency. Current guidelines foresee intentional infections of animal models in experiments to evaluate efficacy of drugs and drug application schemes. While undoubtedly such experimental infections have provided valuable information on treatment efficacy in the past, it is questionable whether they are still mandatory or could be replaced by field trials with unintentionally infected animals, i.e. patients.

We hereby review the current literature on alternative protocols for efficacy testing for parasiticides in small animals and address ethical reassessments of the use of animal models versus client-owned patients. Several novel methods for refined evaluation of the parasite (helminth) burden of small animals before and after treatment with parasiticides have been developed and published that could be applied in field trials on animal patients, provided that suitable animals can be identified and are available for studies. Necropsy to determine worm burdens could, for example, be replaced by worm determination *in vivo* with imaging techniques or quantitative worm antigen detection, and dose confirmatory experimental trials by field trials.

From an ethical point of view, the shift from gaining knowledge with animal *models* to gaining it from animal *patients* can meliorate a core problem of animal research, which is the unfair distribution of harms and benefits: Animal models never benefit themselves in experiments. In fact, typically all harmsare on the animal models' side and the benefits go to someone else, like humans, other animals or the environment (Art. 38 Directive 2010/63/EU).

The presented research aims at overcoming the unfair distribution of harms and benefits by replacing the animal models with patients who themselves can benefit from procedures without losing insights on drug efficacy.

Registration of novel parasiticides under the new 2022 EU regulation

S RICHTER, J HUNTE, K HELLMANN

Klifovet AG, Munich, Germany.

Keywords: Antiparastics, Novel therapy, Registration

Antiparasitics (AP) are indispensable for the routine treatment of livestock companion animals. Two important points to consider on this class of medications are the development of resistance and their impact on the environment. These issues are also addressed in the regulation on veterinary medicinal products (VMPs), Regulation (EC) 2019/6, thatapplies from 28 January 2022. There is a stronger focus on the assessment of the development of resistance of AP and their influence on the environment.

The latter is a logical consequence of the policy from the European Union. Applicants will have to demonstrate to a greater extent the impact of their AP on soil-dwelling and aquatic organisms. AP may be persistent, bioaccumulative or toxic substances. Unless it can be proven that it is essential to control or prevent a serious risk to the health of food-producing animals, an authorisation will not be granted. As well known, resistance of AP can develop. Future applications for AP will need to contain information on current and potential prospective resistance in the target parasites. The mechanisms of resistance should be discussed as well as the potential transfer rate of resistance determinants. The co- and cross-resistance to other AP has to be examined andmeasures to limit the development of resistance have to be included. If the risk for public health by the development of resistance to a new AP outweighsthe benefit of the product, no marketing authorisation is granted. However, for a variety of legal requirements, the scientific means have not been established yet: especially the measurement of antiparasitic resistance is considered to remain a challenge for a variety of parasites. In the absence of validated and simple methods, it will be interesting to see, how the requirements will be fulfilled in future registration procedures. On the other hand, Regulation (EU) 2019/6 facilitates the authorisation of AP as novel therapies. APs intended for use in limited markets may also benefit. As the development of novel therapies are generally supported, this may also increase the technologies targeting parasites and facilitating AP registration.

It can be concluded that with the Regulation (EU) 2019/6 challenges arise for companies wishing to develop AP. This is especially true for environmental safety establishing diagnostic measures for the development of antiparasitic resistance. Nevertheless, the new regulation provides opportunities to develop novel therapies for the treatment of parasites in animals.

Clinical trials in veterinary medicine: the role of veterinarians, opportunities and challenges

F SOLARI BASANO[1], R NAZZARI[1], G VENEGONI[1], S MACRI'[2]

[1] Arcoblu s.r.l. Via A. Milesi 5, 20133 Milan, Italy; [2] Ministry of Health, Department of Veterinary Public Health, Food Safety and Collegial Bodies for Health Protection, Directorate General of Animal Health and Veterinary Drugs, Via Giorgio Ribotta, 5, 00144 Rome, Italy.

Keywords: Clinical, Studies, GCP, Veterinary

Clinical trials are planned experiments to evaluate the performances of a treatment, a procedure, or the use of a device, by comparing the outcomes in groups of patients receiving the test factor against a comparable group receiving a control treatment under the same conditions and in the same timeframe. The vast majority of veterinary clinical trials are performed to collect data on the efficacy and safety of new veterinary products or to enlarge the claims of products that are already licensed. With the adoption of the Good Clinical Practices (GCP) in 1998 and the implementation of the guideline concepts in local regulations, we have an international ethical and scientific quality standard for designing, conducting, monitoring, recording, auditing, analysing and reporting clinical studies evaluating veterinary investigational products. This guidance, together with a wide range of subject specific guidelines, provides regulatory authorities public assurance on the integrity and accuracy of the data, respect of animal welfare and safety for personnel, environment and for the consumers of animal products. Veterinarians investigators have the responsibility of all the aspects of study conduction at study sites, including animal welfare and adherence to study protocol but the role of the veterinarian in the clinical trial process is not limited to the clinical activity performed during the in vivo phase but covers most of the steps of the research workflow, including protocol design (Sponsor or Contract Research Organisation), evaluation of the dossiers for the regulatory approval for study conduction (National Authorities), study monitoring, reporting and the public surveillance on the correct conduction of study procedures. Clinical research is a fascinating activity based on the cooperation of a multitude of professional figures, a *trait d'union* between animal health industry, clinicians, academy, animal production sector, animal owners, and regulatory agencies. The participation of practitioners to clinical studies provides their patients the access to innovative interventions/treatments, allows the possibility to experience in advance the characteristics of new investigational products and impose a systematic and accurate approach that may contribute to a deeper professional knowledge in the direction of the evidence based medicine.

SIMPOSIO

DIAGNOSI DELLE ZOONOSI

IDEXX

Laboratory diagnosis of the zoonotic parasitic diseases of the pets

S MANZOCCHI

IDEXX LABORATORIES, Novara Day Lab, Granozzo con Monticello (NO), Italy.

Keywords: Laboratory, Pets, Zoonoses, Diagnostic

INTRODUCTION. Pets can harbor a wide range of parasites, some of which are transmissible to men. The risks associated with some zoonotic parasites of the pets are often overstated while with some other risks are largely ignored, and the range of illness can be extremely variable from limited to fatal (Baneth et al, 2016, *J Comp Pathol*. 155: S54-74). For most of the companion animals zoonoses and effective and quick diagnosis is of paramount importance for preventing the human transmission. Laboratory tests are commonly the most important tools for diagnosis of the zoonotic parasitic diseases of the pets.

MATERIALS AND METHODS. The major zoonotic parasites of the companion animals in Europe and their relative laboratory diagnostic approach are reviewed. Special focus is given to emerging parasitic zoonoses with relevant medical impact as *D. repens* (Genchi & Kramer, 2017, *Parasit Vector*. 10:517), to technological novelties in the laboratory diagnostic like coproantigen tests for intestinal nematodes (Elsemore et al 2014, *J Vet Diagn Invest*. 26:404-411) and to technical compliance with correct diagnostic procedures (Ballweber et al, 2014, *Vet Parasitol*. 204:73-80).

RESULTS AND CONCLUSIONS. Limiting zoonotic parasites transmission from pets to human requires an integrated approach involving veterinary and human medical research and collaboration between clinical professional figures. The role of the veterinary practician and her/his knowledge of the diagnostic tools available on the market for the parasites of the pets and their strength and limitations is central in the one-health holistic vision.

Epidemiological surveillance and One Health

V VENEZIANO

Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Naples, Italy.

Keywords: One medicine, Zoonosis, Dog

In the early '80s an American veterinarian, Calvin Schwabe, for first coined the term “One Medicine”; he starting from the theory of the German physician Rudolf Virchow, who coined the term zoonosis, elaborated the original “One Medicine concept”. Virchow affirmed: “*between animal and human medicine there are no dividing lines - nor should there be*”. Today, “One Medicine” is commonly referred to as “One Health” worldwide. This terminology change occurred during the first decade of the 21st century, representing the evolution of the earlier used term “One Medicine” (Saunders, 2000 Vet Phatol. 37: 199-207). During the last decade, in research and health systems there has been a growing interest on the One Health approach based on multidisciplinary strategies to achieve better stakeholder engagement and sustainable disease prevention. The one health approach is better well-established in the field of farm animals, based on “herd health” concept and, in everything associated with foodstuffs, while it is often incomplete in the field of companion animals that should include a strong crossing over between veterinarians and physicians for the control of zoonoses (Moore and Lund, 2009 Vet Clin North Am Small Anim Pract. 39: 225-40). Herein we want to focus on some of the main parasitic zoonoses that can be transmitted by dogs to humans and, that should be monitored by veterinarians in collaboration with physicians. In dogs several intestinal parasites, helminths and protozoa, are zoonotic and are considered important for public health, such as *Toxocara canis*, *Ancylostoma caninum*, *Uncinaria stenocephala*, *Strongyloides stercoralis*, *Trichuris vulpis*, *Echinococcus* spp. and *Giardia duodenalis* (Traversa, 2012 Parasit Vectors. 5: 91). Although the veterinary profession has always been based on an indirect contribution to human health, the activities and monitoring programs on human and animal health very often still remain in separate boxes (Grant and Olsen, 1999 Emerg Infect Dis. 5: 159-63).

Now more than ever with the COVID-19 pandemic, the transdisciplinary alliance between physicians and veterinarians became crucial according to a one health vision. A comprehensive public health perspective takes the animal-environment-human interface into consideration through disease surveillance and reporting in animals, including pets.

Minimizing health risks in Animal Assisted Interventions

G SIMONATO

Department of Animal Medicine, Production and Health, University of Padova, Legnaro, Italy.

Keywords: Animal assisted interventions, Public health, Health risks, Parasites

INTRODUCTION. Recently, Animal Assisted Interventions (AAIs) are spreading on national territory and their positive effects on human health are greatly recognised. AAIs are based on the establishment of a good human/animal relationship in order to help people to gain a better quality of life. Given the general suspicion that AAIs could expose stakeholders to potential risk of infections, through the close contact between animals and people or environmental contamination, there is the need to investigate on this topic.

MATERIALS AND METHODS. A review of the Italian literature was performed in order to select and examine parasitological investigations on ecto- and endo-parasites (including zoonotic ones) in different animal species involved in AAIs (equids, dogs, cats, birds, rabbits, rodents, and goats).

RESULTS AND CONCLUSIONS. The most representative publications on this topic concern the last three years (Gerardi et al., 2018Ital J Anim Sci. 1:269-72; Maurelli et al., 2019 Animals (Basel), 9: 827; Simonato et al., 2020 Int J Environ Res Public Health. 17: 7914). The available scientific literature highlighted the potential risk for stakeholders to be exposed to infective stages of zoonotic helminths (i.e. *Toxocara* spp., *Eucoleus* spp., ancylostomatids) and protozoa (e.g. *Giardia* spp.) and of geophylic dermatophytes (e.g. *Nannizziagypsea* and *Paraphytonmirabile*), potential agents of cutaneous mycosis, by contact with contaminated fur and environment. Since the collected data reveal heterogeneous results, the need to plan effective control programs is strongly recommended to guarantee animal and human health. Italian national guidelines for AAIs (2015, https://www.salute.gov.it/imgs/C_17_opuscoliPoster_276_allegato.pdf) provide general suggestions to correctly and evenly applied interventions in the national territory. On the other hand, no structured protocols to check animal health and the identification of potential zoonoses have been provided. Recently, the National Health Institute (2019, Report ISTISAN 19/4) elaborated some generic schedules for the evaluation of animal health status and wellbeing but they represent only the starting point for the improvement of standardized specific protocols to be applied in different kinds of interventions according to the risk of exposure. Since AAIs involve patients and/or users potentially susceptible, it is very important to appropriately manage recruited animals to minimize zoonotic risks. Planned protocols, properly applied, have the potential to reduce the risk factors, and simultaneously to maintain the benefits, making this therapy more accessible and sustainable for patients.

SIMPOSIO

ECTO-ENDOPARASSITI DEL GATTO: UNA PANORAMICA ITALIANA COMPLETA, DA NORD A SUD

MSD
Animal Health

New data on the prevalence of feline ecto-endoparasites in Italy

L KRAMER

Department of Veterinary Science, University of Parma, Parma, Italy.

Keywords: Cats, Parasites, Epidemiology, Mapping

INTRODUCTION. Parasites infecting cats include protozoa, helminths and arthropods. Many are also transmissible to humans. Effective control relies on knowledge of parasite distribution and risk factors for infection. The present study was aimed at evaluating prevalence of major feline parasites in Italy and risk factors associated with their occurrence.

MATERIALS AND METHODS. Over a 12-month study period, thirteen study centers across Italy analyzed feces, hair and ectoparasites from naturally infected cats coming from feral colonies, shelters and private households. Samples from cats (n=987) were analyzed by all centers using the same diagnostic methods. Prevalence values and risk factors were evaluated statistically for identification of predictors of risk.

RESULTS AND CONCLUSIONS. The overall prevalence of gastro-intestinal (GI) and bronco-pulmonary (BP) nematodes was 35.9% (354/987). *T. cati* was the most prevalent species (253/987; 25.6%), followed by hookworms (98/987; 9.9%). Among BP nematodes, *A. abstrusus* was the most common (76/987; 7.7%). Approximately 35.7% (352/987) of the study population was infested by ectoparasites. The most common were fleas 29.4% (290/987), followed by ear mites *O. cynotis* 9.8% (97/987). Predictors of risk for parasite infection included age, a predominantly or exclusively outdoor lifestyle, geographic area and lack of anti-parasitic treatment.

Both ecto- and endoparasites are still common in cats throughout Italy, many of them being of zoonotic concern and vectors of pathogens to humans. Given the presence of parasites throughout the entire study period, year-round treatment should be considered. Furthermore, data confirm the need to protect the human-animal bond using proper endo- and ecto-parasiticides to reduce the risk of human infection, in application of the One-Health concept.

Diagnosis, prevention and treatment of feline aelurostrongylosis

A DI CESARE

Faculty of Veterinary Medicine, University of Teramo, Italy.

Keywords: *Aelurostrongylus abstrusus*, Aelurostrongylosis, Prevention, Treatment

The cat lungworm *Aelurostrongylus abstrusus* is presently spreading and emerging in many countries. This parasite causes a verminous pneumonia with clinical presentation varying from subclinical or self-limiting to severe and fatal signs. Respiratory and generalized signs such as coughing, sneezing, nasal discharge, panting, respiratory sounds, dyspnea, lethargy, weight loss, anorexia are frequently detected in affected cats. Reversible and irreversible secondary pulmonary hypertension have also been described. Despite the widespread distribution and its clinical importance, the disease is often underestimated in clinical settings, likely for a paucity of information on the epidemiology of *A. abstrusus* and for the drawbacks inherent to the diagnosis. Clinical presentations overlap those of other common feline respiratory diseases, e.g. bronchial infectious disease or feline asthma. Non-specific alterations, i.e. bronchial, nodular, and unstructured interstitial patterns, may be found on thoracic radiographs (rev in Morelli et a., 2021 Pathogens 10: 454). Though the clinical diagnosis is almost impossible, the Baermann's method, i.e. the *gold standard* technique for the etiological diagnosis, is not commonly performed in clinical practice. Moreover, this method has some limitations, such as difficulties in differentiating the larvae of *A. abstrusus* from those of other feline lungworms, e.g. *Troglostrongylus brevior*, that may require a different treatment. As aelurostrongylosis may be severe and sometimes life-threatening, awareness on appropriate and timely diagnosis and prompt and efficacious treatment options are of primary importance. At the same time, chemopreventative treatments in cats with outdoor access and living in endemic areas should be implemented to limit the geographical spreading of *A. abstrusus* and safeguard the health of at-risk cats. To date, spot-on 1% moxidectin, 0.4% eprinomectin and 2.1% emodepside are licensed to treat aelurostrongylosis. Of them, the products containing 1% moxidectin and 0.4% eprinomectin are also labeled to kill larval stages with monthly administrations. Recently, the efficacy of a topical solution containing 2% moxidectin against larval stages of *A. abstrusus* has been proved under laboratory conditions. In particular, a single administration of this solution was safe and reliable in inhibiting the establishment of adult *A. abstrusus* and in preventing visible lung damages and larval excretion for at least 3 months after treatment (Raue et al., 2021 Parasit Vectors. 14: 110). New avenues are thus open for the long-term prevention of cat aelurostrongylosis in endemic regions.

The control of ect-endoparasitoses of cats: ESCCAP guidelines

E FERROGLIO

Department of Veterinary Science, University of Turin, Turin, Italy.

Keywords: ESCCAP, Guidelines, Parasite, Control

ESCCAP's aim is to provide veterinary professionals with free expert information in the form of guidelines. These guidelines are derived from independent and soundly-based investigation and research and are continually revised and updated. Our group of leading European veterinary parasitologists and public health professionals are committed to raising awareness and to the recommendation of appropriate and proven control measures to protect companion animals. It is the aim of ESCCAP to produce a guideline which delivers comprehensive information and support to assist both veterinarians and pet owners to successfully control worm infection in dogs and cats. The GL1 concentrates on the most important groups of companion animal worms, both intestinal and non-intestinal, while other canine and feline parasites are addressed in other guidelines. For more information on the control of ectoparasites, superficial mycoses, vector-borne diseases and intestinal protozoa see ESCCAP guidelines at www.esccap.org/guidelines/.

Parasite infections should be controlled through endoparasite and ectoparasite management and treatment. Few parasite infections are strictly age-related; the risk continues as the animal ages and so consideration should be given to provide each dog and cat with appropriate worm control throughout its lifetime. The routine treatment and prevention of all worms depends upon legislation in individual countries, veterinaryprofessionals taking local epidemiological circumstances into account, owner perception and individual risk assessments i.e. hunting pets, previous lungworm exposure, raw meat diets etc. Deworming practices should therefore always be on the advice of a veterinary professional

SIMPOSIO

VETTORI E PATOGENI

Elanco

Best known and less known hemoprotozoa of pets

L SOLANO-GALLEGO

Departament de Medicina i Cirurgia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, Bellaterra, Spain.

Keywords: *Babesia*, Cytauxozoon, *Hepatozoon* and *Leishmania*

Parasites are responsible for some of the most important and widespread diseases of domestic dogs and cats. Some of these parasitic infections are also zoonotic and can infect humans. Furthermore, the vast majority of these parasitic infections such as those caused by hemoprotozoa are transmitted by several competent vectors including ticks and sandflies. The main hemoprotozoans infecting dogs includes pathogens of several genera such as *Babesia*, *Hepatozoon* and *Leishmania*. The most important hemoprotozoans infecting cats are *Babesia*, *Cytauxzoon*, *Hepatozoon* and *Leishmania*. Furthermore, different species of these protozoans exist making the diagnosis and treatment protocols more difficult. *Babesia* are tick-borne protozoan parasites of erythrocytes that infect dogs and cats. Canine infections caused by different tick-borne *Babesia* species have a worldwide distribution and global significance. In Europe, large form *Babesia* spp. include *B. canis* and *B. vogeli* while the small *Babesia* spp. include *B. gibsoni* and *B. vulpes*. Interestingly, feline *Babesia* infections are rare in Europe and the Middle East with sporadic descriptions of *B. canis* and *B. canis* subsp. *presentii*. So far, only one species of *Hepatozoo*n (*H. canis*) transmitted by oral ingestion of *Rhipicephalus sanguineus* ticks has been documented in dogs in Europe while three species of *Hepatozoon*, namely, *H. felis*, *H. canis* and *H. silvestris* may affect domestic and/or wild felids. *Cytauxzoon* spp. has been described in Europe in domestic cats and wild felids but limited information is available on this species which is genetically different and of decreased pathogenicity when compared with *Cytauxzoon felis* distributed in America. Recently, three new species of *Cytauxzoon* have been documented in Europe in wild felids. The tick vector for *Cytauxzoon* spp. infection in felids in Europe remains unknown as well as other possible modes of transmission. *Leishmania infantum* is the best-known protozoan infecting dogs, cats as well as other domestic and wild animals. A thorough understanding of the pathogenesis and epidemiology of these parasitic diseases is required in order to treat infected pets and prevent the spread of disease efficiently. This talk focuses on important hemoprotozoal diseases with European distribution in dogs and cats with emphasis on their etiology, pathophysiology, clinical manifestations, diagnosis, treatment and prevention.

Monitoring and detection of new endemic foci of canine leishmaniosis in northern continental Italy: an update from a study involving five regions (2018-2019)

L GRADONI[1], E FERROGLIO[2], S ZANET[2], W MIGNONE[3], L VENCO[4], G BONGIORNO[1], E FIORENTINO[1], R CASSINI[5], M GRILLINI[5], G SIMONATO[5], A MICHELUTTI[5], F MONTARSI[6], A NATALE[6], M GIZZARELLI[7], V FOGLIA MANZILLO[7], F SOLARI BASANO[8], R NAZZARI[8], O MELIDEO[9], D GATTI[9], G OLIVA[7]

[1]Reparto di Malattie trasmesse da vettori, Istituto superiore di Sanità, Roma; [2]Dipartimento di Scienze Veterinarie dell'Università degli Studi di Torino, Grugliasco, Torino; [3]Istituto Zooprofilattico sperimentale del Piemonte, Liguria e Valle d'Aosta, Sezione di Imperia; [4]Veterinariolibero professionista, Santa Giuletta, Pavia; [5]Dipartimento di Medicina Animale, Produzioni e Salute, Università degli Studi di Padova, Legnaro, Padova; [6]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova; [7]Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli Federico II, Napoli; [8]Arcoblu s.r.l., Milano; [9]Elanco Italia S.p.A., Sesto Fiorentino, Firenze, Italy.

Keywords: Northern Italy, Canine leishmaniosis, Phlebotomine sand flies

INTRODUCTION. Canine leishmaniosis (CanL) is an emerging zoonosis caused by *Leishmania infantum* and transmitted in southern Europe by *Phlebotomus* (*Larroussius*) sp. sand flies. Endemic foci have been recorded in northern continental Italy since early 1990s and attributed to the northward expansion of vectors associated with travelling/relocated infected dogs from the Mediterranean littoral. In this study, the presence of new endemic CanL foci was monitored during 2018-2019 in five regions (Aosta Valley, Piedmont, Lombardy, Veneto and Friuli-Venezia Giulia) with focus to territories where investigations have not been performed in the past.

MATERIALS AND METHODS. Clinical cases identified by veterinary practitioners and confirmed by reference centres were regarded as autochthonous if their origin from, or travel to, areas endemic for CanL were excluded in the previous ≥2 years. Around these index cases, i) serosurveys for *L. infantum* were carried out where indicated, ii) sampling from eligible dogs was intensified by collaborating veterinary practitioners, and iii) suitable sites were investigated for the presence of phlebotomine vectors.

RESULTS AND CONCLUSIONS. Fifty-seven municipalities whose enzootic status of CanL was unreported before 2018, were identified as endemic. The stability of 27 foci recorded over the past decade, was also confirmed. Competent phlebotomine vectors, mainly *P. perniciosus*, were collected for the first time in 23 municipalities. The newly recorded endemic municipalities were distributed over a west-to-east decreasing gradient: 30 in Piedmont, 21 in Lombardy, 4 in Veneto and 2 in Friuli-Venezia Giulia. Note that a restricted territory of Veneto was investigated as several municipalities had already been detected as endemic for CanL in the past. Cold climate conditions of the easternmost region of Friuli-Venezia Giulia bordering non-endemic territories of Slovenia, are probably less favourable to *L. infantum* transmission.

Anti-vectorial prophylaxis and vector-borne diseases in dogs and cats

E BRIANTI[1], **D OTRANTO**[2]

[1]Department of Veterinary Sciences, University of Messina, Messina Italy; [2]Department of Veterinary Medicine, University of Bari, Bari, Italy.

Keywords: Vector-borne diseases, Dog, Cat, Prophylaxis

Vector-borne diseases (VBDs) threaten the health and welfare of dogs and cats globally, with some pathogens (*e.g., Leishmania* spp., *Rickettsia* spp., *Bartonella* spp. and *Dirofilaria* spp.) being of zoonotic concern. A variety of arthropod vectors transmit pathogens while feeding on their host according to their biology and ecology.

The transmission of VBD causing pathogens may be prevented by blocking arthropod feeding or by causing a fast killing before pathogens' transmission occurs. A number of pyrethroids (e.g., permethrin, deltamethrin and flumethrin) with repellent and anti-feeding effects are efficacious to reduce the risk of transmission of VBPs in both laboratory and field studies. Other chemical compounds, by virtue of their fast-killing effect, may also reduce the risk of VBP transmission in dogs and cats. For example, isoxazolines (e.g., afoxolaner, fluralaner, lotilaner and sarolaner) might reduce the risk of transmission of several tick- and flea-borne pathogens.

The use of insecticides and acaricides with repellent and/or killing effects are currently regarded as the most efficient strategy to prevent transmission of VBDs in dogs and cats. While laboratory studies are the primary route to assess the efficacy of a product in preventing arthropods and reducing the risk of VBP transmission, field studies are beneficial to confirm the data obtained under experimental conditions and to gain more information on large number of animals of diverse breed and age, wild vectors, through long study period and under natural conditions where co-infections with multiple VBD causing pathogens may occur. In this presentation, evidence-based data yielded from clinical field trials aimed at assessing the efficacy of veterinary products against transmission of the main VBDs and to control their incidence in highly exposed dog and cat populations will be reviewed. Also, the new edges of prevention by combining products having a different mode of action for a broader and multi-level protection against VBD transmission will be presented.

SIMPOSIO

PARASSITOSI DEGLI ANIMALI DA REDDITO

zoetis

Parasites of ruminants, an even-present reality

A SCALA

Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Italy.

Keywords: Ruminants, Parasitosis, Control

Parasites of ruminants are frequently underestimated, but they result in severe consequences such as mortality (babesiosis and theileriosis), decrease in fertility (gastro-intestinal nematodes- GIN), abortions (toxoplasmosis and neosporosis), neonatal diseases (e.g. cryptosporidiosis), decrease in weight gain and productions (e.g. gastrointestinal nematodes, hypodermosis, dystomatosis, ectoparasitosis) and public health risks (e.g. toxoplasmosis, cryptosporidiosis, cystic echinococcosis). However, the list of parasites existing at ruminant farms is by no means exhaustive, it further includes bronchopulmonary nematodes, cerebro-spinal coenurosis, myiasis from *Hypoderma* spp. and by *Oestrus ovis*. Moreover, the existing legislations pertaining animal welfare sparsely considered parasitic challenge in the various decrees, represent a noxa, posing serious health implications.

Despite this, the control measures regarding parasites of ruminants are often not considered and implemented in a rational and effective way, as they remain unknown or seldom applied in practice and/or considered economically disadvantageous. For example, the NGI infestations are often controlled through the sole administration of anthelmintics with inappropriate procedures, without a qualitative-quantitative diagnostic assessment lacking rational evaluation of the chronology of the intervention, the choice of the molecule, the dosage, and its modalities of administration along with the risk of emergence of anthelmintic resistance (AR) which is a growing concern.

So, what to do? Empirically, at first implement necessary AR control and prevention measures, reassess the biological parameters of parasitic agents along with pursuance of control measures according to parasitic ecology and epidemiology for example, the rotation of pastures and the alternative use of available drugs and/or of pesticides with the aim of optimal and economically sustainable productivity levels, at the same time low environmental impact as far as possible. Taking this into account, it would be extremely desirable to encourage research aimed at genetic selection schemes of animal lines that are naturally more resistant to infestations, even until now the data available do not guarantee their successful application in the field in the near future. Unfortunately, the same problem seems to involve studies concerning the preparation of possible vaccine products. The slaughterhouse should be used / re-evaluated as a privileged epidemiological observer for the real-time and cost-free monitoring of important parasites such as metacestodosis and dystomatosis.

European strategies to control helminth infections in ruminant livestock

L RINALDI[1], S SOTIRAKI[2], E CLAEREBOUT[3], E MORGAN[4], G VON SAMSON-HIMMELSTJERNA[5], F KENYON[6], H HOSTE[7] , A BOSCO[1], G CRINGOLI[1], J CHARLIER[8]

[1]University of Naples Federico II, Department of Veterinary Medicine and AnimalProduction, Napoli, Italy; [2]Laboratory of Parasitology, VetResInst, HAO-DEMETER, Thessaloniki, Greece; [3]Laboratory of Parasitology, Faculty of Veterinary Medicine, Ghent University, Belgium; [4]Queen's University Belfast, Institute for Global Food Security, United Kingdom; [5]Freie Universität Berlin, Institute for Parasitology and Tropical Veterinary Medicine, Berlin, Germany; [6]Moredun Research Institute, Pentlands Science Park, Edinburgh Scotland, UK; [7]UMR 1225 IHAP INRA/ENVT, Toulouse, France; [8]KREAVET, Kruibeke, Belgium.

Keywords: COMBAR, Ruminants, Helminths, Anthelmintic resistance

INTRODUCTION. The worldwide increased difficulty to combat helminth infection in ruminant livestock, due to progressing anthelmintic resistance (AR), calls for an enhanced and standardized strategies at national and transnational level. The COST Action COMBAR (Combatting Anthelmintic Resistance in Ruminants) aims at coordinating research at the European level to advance knowledge in this area, and to develop and promote new solutions for parasite control and disseminate current knowledge among all relevant stakeholders.

MATERIALS AND METHODS. The COMBAR Network (2018-2022) has been organised around three Working Groups (WG): WG1 "Improving Diagnosis" which aims to prioritise, evaluate and implement cost-effective methods for the diagnosis of helminth infections and AR; WG2 "Understanding the socio-economic aspects" which aims to develop, disseminate and apply methods to study the economics and human behaviour in the field of helminth control in ruminants and WG3 "Innovative, sustainable control methods" which aims to develop practical and sustainable helminth control strategies that integrate current insights from diagnostics, targeted (selective) treatment approaches, epidemiology, anti-parasitic forages, vaccinology, farm economics and human behaviour.

RESULTS AND CONCLUSIONS. Through joint collaboration, COMBAR has paved the way towards a transnational, multi-actor initiative involving researchers, farmers, veterinarians, industry to promote the uptake of sustainable helminth control approaches and develop new solutions (Charlier et al., 2020 Prev Vet Med. 182: 105103; Maurelli et al., 2020 Front Vet Sci. 7: 580649; Rinaldi et al., 2019 Parasit Vectors. 12: 353; Vineer et al., 2020 Parasite. 27: 69).

Parasite monitoring and control: ecto *vs*. endo

R CASSINI

Department of Animal Medicine, Productions and Health, University of Padova.

Keywords: Endo-parasites, Ecto-parasites, Quantitative estimation, Sampling size

INTRODUCTION. Endo- and ecto-parasites are an important health problem in farm animals, generally managed at population level. The diagnostic approach should be cost-effective in providing sound data for an evidence-based decision about treatment. However, some aspects (i.e. sample size determination, pooled *vs*. individual samples, quantitative estimation methodology, thresholds definition) are still lacking a general agreement.

MATERIALS AND METHODS. The characteristics of the different approaches proposed for quantitative estimation of endo- and ecto-parasites (lice and mange mites) in alive animals were summarised based on author experience and literature review. Only parasitological methods, based on the direct identification and counts of adult, immature or reproductive stages of parasites, were included. The study considered only domestic ruminants (cattle, goats, sheep).

RESULTS AND CONCLUSIONS. Literature research is summarised in Table 1.

	Parasite group	Sampling size determination	Pools	Quantitative estimation method	Thresholds
Endo	Nematoda	Generic indications (e.g., 10-12 animals within a group or 10% of the group); Farm-tailored sampling size (based on prevalence and abundance values)	3-20 samples per pool; correlation between pools and individual samples varies according to emission and aggregation levels	FEC (McMaster, FLOTAC, …); Quantitative biomolecular and Ag-based techniques	Individual: overall (200-1000 EPG) and genus-specific (e.g. *Haemoncus* 500-2000 EPG); Genus-specific degree of infection proposed; No threshold indication for pool samples
	Trematoda	No specific indications	10 samples per pool; comparable sensitivity with individual samples	FEC (McMaster, FLOTAC, sedimentation or flotation techniques, …); Quantitative biomolecular and Ag-based techniques	Genus-specific (*Fasciola* spp. 100-200 EPG)
	Cestoda	No specific indications	None	FEC (McMaster, FLOTAC, …)	None
	Coccidia	Generic indications (e.g., 6-16 young animals; 10 kids)	No specific indications	FEC (McMaster, FLOTAC, …)	Individual: 500 OPG; Difficult to determine (differences among species in pathogenicity and emission levels)
Ecto	Lice	Generic indications (e.g., 10 adults +10 young animals, within a group); Optimal sampling size influenced by prevalence and intensity values	n.a.	On-site counts in 3-80 hair partings of 4-15 cm or in 3-20 target areas (overall 32.5-150 cm^2); Counts in laboratory after 3 hair cuttings of 50 cm^2; Lamp test; Table locks test;	None; Rating scale or density scores proposed
	Mange mites	None	n.a.	Counts in laboratory after 3-6 scrapings of 4-9 cm^2 (or 10% area of lesions);	None; Rating scale or density score proposed

Table 1 – characteristics of the different approaches for endo- and ecto-parasites quantitative estimation

Endo-parasites have been widely investigated in a quantitative way thanks to the standardised approach of the faecal egg count (FEC). However, the sampling approach (including the use of pooled samples) and the threshold interpretation need further research and discussion to provide sound evidence for decision on treatment. Ecto-parasite burden, instead, was sparingly studied quantitatively, through highly variable methods in terms of surveyed skin areas. The use of more sensitive diagnostic approach for their detection may support a timely intervention.

SIMPOSIO

PARASSITI E FAUNA SELVATICA

Wildlife Disease Surveillance and Monitoring experiences in Apennine National Parks

S ANGELUCCI[1], L GENTILE[2]

[1]Maiella National Park, Wildlife Research Center, Veterinary Office; [2]Abruzzo Lazio and Molise National Park, Veterinary Services.

Keywords: Wildlife surveillance, National parks, Parasites, Conservation medicine

The territory of a National Park is subject to "a special protection and management regime, with the aim of pursuing [...] the conservation of animal species [...] of biotopes, of biological communities [...] of ecological balances" (Italian Law of 6 December 1991, n. 394). For at least 20 years, in these protected areas where neither hunting nor any significant harvesting of wild animals has been carried out, health monitoring activities have been carried out on wild animals, as first experience of setting up a surveillance system on free-ranging populations.

The activities of park-vets are developed in the context of eco-epidemiological monitoring, in the planning and implementation of wildlife captures and animal welfare protection, in conservation programmes, in forensic diagnostics, as part of the study of the interface between anthropic activities/wild animals (pastures, livestock damage caused by predators, coexistence management), in the field of recovery and rehabilitation of rescued animals and finally in educational/informative field.

Among the monitoring activities carried out, those on parasites and host/parasite relationships are of particular importance, especially when are directed on endangered species (Martella et al. 2003 J Mt Ecol. 7: 251-6; Stancampiano et al., 2008 Hystrix It J Mamm. (n.s.) Supp. 2008; Paoletti et al. 2017 Ann Parasitol. 63: 205-12) or, more recently, can be related to the ecological study of animal populations, their territories, animal movement behavior, or spatial organization of resources (White et al. 2018, PNAS: 115: 7374-79).

National Parks have become fields of application of Conservation Medicine (Aguirre, 2002 Oxford University Press) as especially suitable areas for the study of complex relationships and interactions between animal health, human health and ecosystem health, with a more holistic and interdisciplinary approach to the conservation of biodiversity. This approach may be regarded as the application of One Health to the conservation of Biodiversity.

Trichinella britovi larval biomass in wild canids in Abruzzi region, Italy

P BADAGLIACCA, D DI SABATINO, S SALUCCI, F DE MASSIS, A COCCO, EE TIERI, G ROMEO, N DE DOMINICIS, L RICCI, A PETRINI

Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo.

Keywords: *Canis lupus*, Larval biomass, *Vulpes vulpes*, Wildlife

INTRODUCTION. In Italy, wild canids play an epidemiological role in maintaining the sylvatic cycles of *Trichinella britovi* infection (Badagliacca et al., 2016 Vet Parasitol. 231: 124-27). Currently, trend of *Trichinella* infection in wildlife is based on the proportion of positive on total tested samples (apparent prevalence – AP). More recently the estimation of *Trichinella* larval biomass (TLB), at the host population level, has been used as a different way to assess *Trichinella* infection in wildlife (Kärssin et al., 2017 Parasit Vectors. 10: 609).

MATERIALS AND METHODS. In the 2015-2020, 213 wolves (*Canis lupus*) and 418 red foxes (*Vulpes vulpes*) carcasses recovered from Abruzzi region were investigated for *Trichinella* larvae per gram (LPG) enumeration by muscle digestion methods and a set of data for each carcass including weight was recorded. The per cent of total muscle mass (MM) and the LPG coefficient for muscle sites were estimated by carrying out a preliminary study on the proportion of muscle mass in wolf and red fox and on the distribution of LPGs in 20 muscle sites in 7 *Trichinella*-positive wolves and 5 foxes, as described by Kirjušina et al., (2016 Vet Parasitol. 231: 110-14). Finally, the individual TLB of carcasses was estimated by the formula: $MM*(LPG*LPG_{coefficient})$.

RESULTS AND CONCLUSIONS. The AP (Fig. 1) in wolf carcasses ranged from 16.6% to 35.3% with a linear trend from 2017 to 2020 and is on average 4 times higher than in the red fox. The TLB showed a variable trend (Fig. 2) both in the wolf and in red fox, with peaks in 2015 and 2019. As the TLB trend correlates with LPG and number of positive carcasses, it could be a better indicator than the AP, that correlates with only positive carcasses, to assess *Trichinella* infection in wild canids.

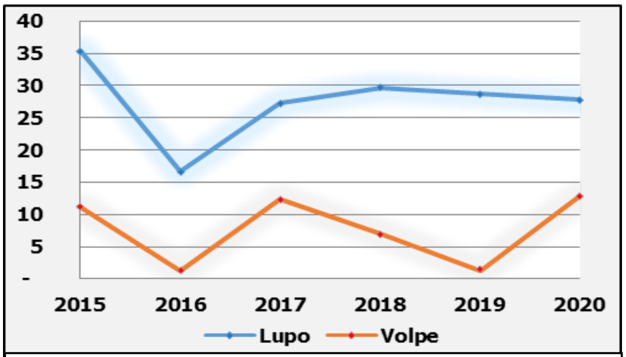

Figure 1. Trend of apparent prevalence of *Trichinella britovi* infection in wild canids in Abruzzi region.

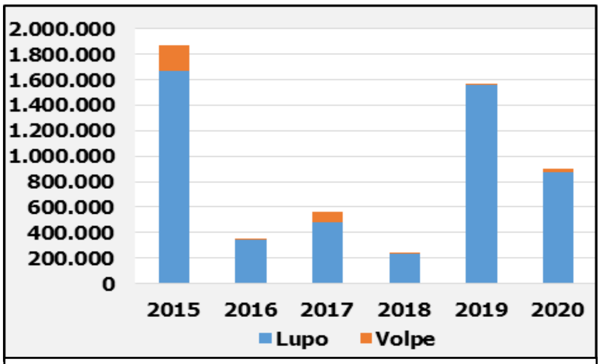

Figure 2. *Trichinella britovi* larval biomass in wild canids in Abruzzi region.

The role of migratory birds in emerging pathogens spread

F IAPAOLO, E MANCUSO

Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise G. Caporale, Teramo.

Emerging infectious diseases are a significant burden on global economies and public health. Among them, zoonoses represent the 75% and they principally originate from wildlife. Twice a year, billions of migratory birds flyfor thousand kilometers across national and international borders overflying geographical barriers and creating an "ecological bridge" between continents. In particular, many European migrants leave their breeding grounds during the late summer/fall to spend the winter in Africa and return the following spring. Since birds carry pathogens either as a reservoir host or by dispersing infected arthropod vectors, especially migratory species have been often indicated as an important source for novel incursions of pathogens in new areas and closely related with the introduction and the spread of emerging diseases of public health concern in Europe. Italy hosts an intense passage of birds migrating along major routes connecting winter quarters in Africa and breeding areas in Europe, thus, the country is particularly exposed to the risk for introduction of new pathogens. The occurrence of West Nile virus (WNV) and Usutu virus (USUV) in Italy is one of the most significant examples of zoonotic emerging diseases strictly dependent by birds, in particular introduced through migratory species and locally maintained by resident and synanthropic ones. USUV and WNV are mosquito-borne Flavivirus emerged in Tuscany region respectively in 1996 and 1998, and reappeared 10 years later involving new areas. Since 2008, annual USUV andWNV outbreaks of varying intensities have been reported in Italy sustained by genetically divergent isolates and to date both the viruses have been detected at least once in 16 of the 20 Italian regions. Migratory birds have also an important role in spreading exotic ticks during migration from Africa as confirmed by the high prevalence of typical sub-Saharan ticks found on birds arriving in Italy during the spring migration and by several findings of adults of the same tick species around Europe as possible result of immature stages seeded by avian hosts. Simultaneously, ticks parasitizing birds were often found infected by tick-borne zoonotic pathogens like bacteria of genus *Rickettsia*, *Coxiella* and *Anaplasma*, but also by emerging zoonotic viruses representing a threat for human health like the Crimean-Congo hemorrhagic fever virus. Currently, the role of migratory birds in introducing and spreading some emerging infectious diseases in Italy is still unclear and the study of bird-vector-pathogen interaction in relation to climatic and environmental changes represents a priority task to early detect possible diseases emergence.

The role of insects in spreading emerging pathogens

M GOFFREDO

Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale".

Keywords: Insects, Arboviruses

Nowadays, as a result of globalization and climate changes, emerging pathogens transmitted by vectors should be taken into account, particularly those represented by arboviruses.

A pivotal role as biological vectors of arboviruses is played by *Culicoides* biting midges and mosquitoes. The former are terrible pests of livestock transmitting arboviruses of relevant impact for animal welfare and trade, whereas the latter are able to transmit a huge number of viruses to animals and\or humans.

Usually, *Culicoides*-borne arboviruses follow a "simple route", meaning that the pathogen circulates among hosts (i.e. horses, cattle, sheep and goats) and vector populations strictly linked to livestock. Neverthless, midges can take the infection from wild asymptomatic hosts and then transmit the pathogen to domestic animals, which could show a severe disease (i.e. African Horse Sickness, from zebras to horses).

Conversely, for mosquito-borne diseases, more complicated spreading routes should be considered, usually including both a maintenance cycle and an epidemic cycle. Typically, the virus circulates silently in a wild environment, where mosquitoes allow the continuous infection of their wild hosts (i.e. birds rodents). Due to some particular events, the virus can leave his maintenance cycle ("spill over") and start an epidemic cycle elsewhere.

As examples of the diverse roles that insects can play in spreading emerging pathogens, different scenarios of zoonotic arboviruses are described, such as West Nile, Usutu, Equine Encephalitis, Rift Valley Fever, Japanese Encephalitis, and Yellow Fever viruses.

The knowledge of the transmission routes, and specifically the role of insects, may lead the strategies for surveillance and control actions.

In Italy, entomological surveillance plans are in place, at national level, for both *Culicoides* (within Bluetongue surveillance) and mosquitoes (within West Nile and Usutu viruses surveillance). The implementation of this extensive entomological surveillance, will be a valuable resource in case of occurrence of further emerging vector borne diseases.

SIMPOSIO

MALARIA NETWORK

The Italian Malaria Network-Centro Interuniversitario di Ricerca sulla Malaria (CIRM.IMN): a Virtual Institute for Malaria Research in Italy

CIRM. IMN[1]

[1]Donatella Taramelli, Director, University of Milan; Guido Favia, Vice-Director, University of Camerino.

http://www.disfeb.unimi.it/ecm/home/ricerca/centri-di-ricerca/centro-interuniversitario-di-ricerca-sulla-malaria

Keywords: Malaria, Drug discovery, Vector control, Immuno-pathogenesis

From 2000 to 2015, thanks to the political commitment of the Millennium Goals and the increased funding from the Global Fund andPrivate-Public Partnerships,a 37% and 60% decrease in malaria incidence and mortality, respectively, were observed worldwide (WHO Malaria Report 2020).Those achievements became possible because research on malaria was highly encouraged:new tools were delivered to tackle drug- and insecticide-resistance of Plasmodium parasites and vectors, respectively, as well asnew diagnostics and new treatments.

At the same time, Italian malariologists, mostly small groups scattered in different Universities and the Istituto Superiore di Sanità, combined their interdisciplinary expertise and efforts and participated jointly to research projects financed by Italian and European Institutions.Prof. Paolo Arese, from the University of Torino, was pivotal in proposing and sustaining such an initiative and finding the initial economic support.

The Italian Malaria Network (CIRM-IMN) was legally established in 2010 among the Universities of Torino, Piemonte Orientale "Amedeo Avogadro", Milano, Brescia, Siena, Camerino, Perugia, Roma “La Sapienza”, Napoli “Federico II” and the Istituto Superiore di Sanità in Rome, with the lateraddition ofthe Universities of Bologna and Pisa. Since then, more than 100 researchers in different cities have been working as a large “virtual” Institute on Malaria, producing hundreds of peer reviewed publications, and training several young scientists both from Italy and malaria endemic countries. Research interests range from studies on host-parasite relationships to the discovery of antimalarial drugs; from the identification of new molecules or natural products to the screening and characterization of new compounds; from studies on vector insects, the Anopheles mosquitoes, to identify tools for inhibiting the transmission of malaria to interactions between mosquitoes and their microbial communities.The main objectives of CIRM-IMN, research, training and networking between Italian and foreign institutions, remain particularly valid, but new projects and funding are needed to reverse the flattening of the positive trend of incidence and mortality of malaria observed in the last four years.

Symbiotic yeasts: a promising tool for the control of vector-borne diseases

I RICCI

School of Biosciences and Veterinary Medicine, Laboratory of Parasitology and Molecular Entomology, University of Camerino, Camerino (Mc), Italy.

Keywords: *Wickerhamomyces anomalus*, Mosquito, Malaria, Biocide

INTRODUCTION. The yeast *Wickerhamomyces anomalus* (Ascomycota, Saccharomycetales) is a mutualistic symbiont of different insects including mosquitoes (Ricci et al., 2011 Environ Microbiol. 13: 911-21). The symbiotic strain displays a killer phenotype mediated by protein toxins with broad-spectrum antimicrobial activities (Cappelli et al., 2014 PlosOne. 9: e95988a). A killer toxin (KT) secreted from a *W. anomalus* strain associated to the malaria vector *Anopheles stephensi* (WaF17.12), has shown a strong anti-plasmodial activity i) against early sporogonic stages of the murine malaria parasite *Plasmodium berghei* and ii) in mice. Interestingly, iii) the killer yeast can be administered as food to mosquito larvae and be still present in adults, thus acting as an antimalarial biocide in mosquitoes.

MATERIALS AND METHODS. i) Sugar solution added with WaF17.12 expressing KT was provided for a few days to *An. stephensi* adults, before the blood meal on malaria infected mice. Twenty-four hours after the infected blood meal, the number of early sporogonic stages of *Pb*GFP$_{\mathrm{CON}}$ in the mosquito midguts were analysed. ii) Recipient mice received infected blood after incubation with KT and five days after the inoculation the parasitemia was evaluated. iii) Larval food added with WaF17.12 was provided to larvae, then the yeast amount during the whole mosquito life cycle was assessed by qPCR and immunofluorescence with monoclonal antibody targeting KT.

RESULTS AND CONCLUSIONS. i) A dietary supplementation to adult females with WaF17.12 cells, strongly interferes with the ookinete development, significantly reducing the parasite number in mosquitoes up to 65,2%; WaF17.12 affects *P. berghei* early sporogonic stages in the midgut by causing the damage of the parasite membrane. ii) Parasitemia in mice is significantly reduced up to 55,4% through a KT-mediated mechanism. iii) *W. anomalus* follows a trans-stadial route, spreading from larvae to adult females. Overall, the results i) and iii) demonstrate that WaF17.12 can be released in the larval breeding sites as an innovative biocide based on killer yeasts for blocking the malaria transmission, and the results iii) motivate the investigation of novel anti-parasitic drugs based on KT.

Wolbachia-induced immune activation and the control of vector-borne diseases

C BANDI[1], **I VAROTTO-BOCCAZZI**[1], **R SPACCAPELO**[2], **A CRISANTI**[3], **G FAVIA**[4], **S EPIS**[1]

[1]Department of Biosciences and Pediatric Clinical Research Center "Romeo and Enrica Invernizzi", University of Milan, Milan, Italy; [2]Department of Experimental Medicine, University of Perugia, Perugia, Italy; [3]Department of Microbiology, University of Padova, Padova, Italy; [4]School of Biosciences & Veterinary Medicine, University of Camerino, Camerino, Italy.

Keywords: *Wolbachia*, Control strategies, Immunity

INTRODUCTION. *Wolbachia* is an obligate intracellular bacterium associated with several arthropods and most of the filarial nematodes that cause disease in humans and dogs. After the introduction of *Wolbachia* into mosquito populations, infected individuals display reduced efficacy in the transmission of viral diseases. *Wolbachia* has been hypothesized to reduce the vectorial capability of host insect through the activation of its immune response. By focusing our studies on the *Wolbachia* surface protein (WSP) we reinforced this hypothesis and paved the way for the potential use of WSP in the control of vector-borne diseases, including malaria.

MATERIALS AND METHODS. We assayed the immune-stimulating properties of WSP in relation with three vector-borne diseases, following different approaches: i) using an engineered bacterium expressing WSP (*Asaia*-WSP), delivered to mosquitoes through the sugar meal; mosquitoes were then tested for their capability to transmit a filarial nematode; ii) the same bacterium *Asaia*-WSP was used to stimulate mouse macrophages, subsequently exposed to *Leishmania infantum*; iii) transgenic mosquitoes expressing WSP were generated, and were assayed for the their capability to transmit *Plasmodium berghei.*

RESULTS AND CONCLUSIONS. The results obtained on the three model-systems are coherent in indicating that *Wolbachia* possesses the capability to activate innate-immune responses, in both insects and mammals, with inhibitory or even killing effects on the tested parasites. On the one side, *Asaia*WSP proved to be as an easy-to-handle microorganism, suitable to be further developed as a tool for paratransgenesis-based control of vector-borne diseases. On the other side, transgenic mosquitoes expressing WSP strongly inhibited the transmission of *Plasmodium*, providing further evidence for the immune-stimulating properties of this molecule from *Wolbachia* and supporting the possibility of exploiting this bacterium as a tool for malaria control.

New insight into *Anopheles* swarming behavior through 3D tracking of mosquito trajectories: uncovering new avenues for vector control?

MA GUCCIARDINO[1], S MELILLO[2], G IACOMELLI[1], L PARISI[2], G MONACCHIA[1], R BESCHI[2], M LOMBARDI[1], S PAGNOTTA[1], M PEIRCE[1], I GIARDINA[3], A CAVAGNA[4], R SPACCAPELO[1]

[1]Dipartimento di Medicina e Chirurgia, University of Perugia, Perugia, Italy; [2]Istituto Sistemi Complessi, Consiglio Nazionale delle Ricerche, UOS Sapienza, Rome, Italy; [3]Dipartimento di Fisica, Università La Sapienza, Rome, Italy; [4]Istituto Sistemi Complessi, Consiglio Nazionale delle Ricerche, Rome, Italy.

Keywords: *Anopheles*, Swarming, Mating, 3D tracks

INTRODUCTION. The *Anopheles gambiae* (*An. gambiae*) complex includes the major African mosquito vectors of malaria, a disease which kills more than 400 000 people each year. Progress in reducing the human cost of malaria, driven by insecticide-based vector control measures, has been undermined by the rapid spread of insecticide resistance within the *gambiae* complex. This has spurred innovative new vector control strategies involving the release of male mosquitoes bearing genetic modifications. The successful spread of these traits among natural populations is entirely dependent on the mating fitness of the engineered mosquitoes. Mating in the An. *gambiae* takes place in crepuscular male swarms which females enter to mate. Here we describe the development of an approach to address several questions relating to swarming and mating in the *gambiae* complex.

MATERIALS AND METHODS. Three independent synchronized cameras were used to acquire high resolution video footage of lab-based swarms of the lab-adapted G3 *An. gambiae* strain in a single large cage (5.00 × 3.66 × 2.60 m) equipped with an artificial horizon and a ground swarming marker as described elsewhere (Facchinelli et al., 2015 Malar J. 14: 271) and illuminated with infra-red lighting. Using algorithms developed to study swarming in starlings (Attanasi et al., 2015 TPAMI. 37: 2451) we generated 3D tracks (detailing position and velocity) for each member of the swarm.

RESULTS AND CONCLUSIONS. Robust and repeatable swarming behaviour was observed despite using a lab-adapted strain. The number of participants varied up to a maximum of ~300 representing 15-20% of the released adults. Individual tracking enabled the identification of apparently successful and unsuccessful mating attempts when females were added to the cage. These findings represent a proof of principle that swarming behaviour can be usefully studied in a highly controlled, lab-based environment and offer the prospect of a detailed dissection of swarming and mating behaviour within and between members of the *gambiae* complex.

Immune factors in the salivary glands of the malaria mosquito *Anopheles coluzzii*

F LOMBARDO

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

Keywords: *Anopheles coluzzii*, Salivary glands, Antimicrobial peptides, Feeding behavior

INTRODUCTION. Mosquito salivary glands represent a key organ both in relation to physiological behaviors such as sugar and blood feeding and for the transmission of pathogens, as *Plasmodium* spp., to the human host. Indeed, mosquito females need a blood meal to attain nutrients for egg development, while both male and female mosquitoes rely on plant sugar to survive, to fly and to enhance reproduction. A successful blood meal depends mainly on the secretion of saliva once the mosquito successfully seeks a host and localizes the food source, landing on the human skin. Mosquitoes initially probe under the host skin and salivate, searching for a blood vessel to pierce and to start sucking the blood by exploiting anti-haemostatic activities in the saliva. This process exposes mosquito mouthparts to microbes on host skin surface that might contaminate the ingested blood meal. Moreover, when male and female mosquitoes feed on plants, they engorge nectar and honeydew that could be similarly contaminated by microbes. In the last decade, salivary gland transcriptomes of haematophagous arthropods proved the occurrence of salivary peptides with unknown function showing possible antimicrobial sequence features. Aim of this work is the identification of novel antimicrobial peptides (AMPs) in the salivary glands of the malaria vector *An. coluzzii*.

MATERIALS AND METHODS. From salivary transcriptome, we have initially selected 16 putative salivary AMPs, based on sequence properties and expression profiles (enrichment in female glands or female/male glands). A preliminary functional screening allowed focusing on 3 candidates from the initial list. Predicted precursors and mature peptides were obtained by chemical synthesis and employed in bacteria growth inhibition assays using Gram - (*Escherichia coli*, *Serratia marcescens*), Gram + (*Staphylococcus aureus*) bacteria and yeast (*Candida albicans*).

RESULTS AND CONCLUSIONS. Microbial *in vitro* growth inhibition assays performed employing different concentration (75 mM, 150 mM and 300 mM) of synthetic pro-peptides (Hyp6.2, Hyp13 and Hyp15) and mature peptides (Hyp6.2 and Hyp13) revealed activity for some of the peptides and relative MIC were calculated. In particular, the mature, short form of peptide Hyp6.2 impaired the growth of both Gram negative strains, thus suggesting the occurrence of specific antimicrobial activities in the salivary glands of *An. coluzzii* mosquitoes.

Entomological evidence of low community protection of LLINs in Burkina Faso

M POMBI

Sapienza Università di Roma, Dipartimento di Sanità Pubblica e Malattie Infettive, Roma, Italy.

Keywords: *Anopheles,* Vector control, Biting behavior, LLIN

INTRODUCTION. Long lasting insecticide treated bednets (LLINs) are considered by WHO as the main malaria vector control strategy, combining individual physical defense with the collective protection provided by the pyrethroids restrained in net fibers. Despite the success obtained in malaria prevention in the first 15 years from LLINs mass distribution (Bhatt et al., 2015 Nature. 526: 207-11), to date, a stalling of progress in the fight against malaria has been registered in 10 sub-Saharan countries (accounting for approximately 70% of the world malaria burden), where the malaria incidence remains still very high despite the large bednet coverage (World Malaria Report 2020). Among many causal factors affecting this scenario, mosquito physiological resistance to insecticide is crucial but a non-negligible role could be played by behavioral strategies adopted by vectors to elude the insecticidal effect of the net (Ransonet al., 2011 Trends Parasitol. 27: 2). Burkina Faso, where LLINs were introduced in 2010, is paradigmatic for the dramatic epidemiological situation and vector insecticide resistance status of West Africa. Here it is presented a longitudinal study conducted in a village of Burkina Faso, aimed to investigate the entomological factors contributing to explain the high malaria burden in a LLIN-covered area.

MATERIALS AND METHODS. Entomological collections have been carried out in Goden village in 2011, 2012, 2015 and 2019, i.e. at the turn of four bednet distribution campaigns. Resting and host seeking *Anopheles gambiae s.l.* were collected with different sampling methods, thus allowing to record several entomological parameters associated to malaria transmission: Sporozoite rate (SR); Entomological inoculation rate (EIR); Human Blood Index (HBI), rhythms of biting activity and allelic frequency of insecticide resistance (KDR allele).

RESULTS AND CONCLUSIONS. The consistently high SR of 5.5-7.1% detected in the local malaria vectors (*A. coluzzii* and *A. arabiensis*) through the years and the EIR of 10 infective bites/person/night assessed in 2015, confirm the high malaria transmission risk in the village, despite 10 years of net usage. Vectors are also characterized by: i) stable allelic KDR frequencies, ranging from 54 to 58%; ii) altered biting rhythms, without the typical peak during the middle of the night; iii) opportunistic host choice, as revealed by the HBI observed in 2011 and 2019 (15.8% and 33.8%). Overall, these data support the hypothesis that vector behavioural resistance to LLINs contributes in under mining bednet collective protection, with a considerable impact in malaria transmission of this hyperendemic savannah area.

Plasmodium vivax malaria resurgence risk in the European Region

C SEVERINI, M L'EPISCOPIA, E PERROTTI, D BOCCOLINI

Istituto Superiore di Sanità, Department of Infectious Diseases, Vector-borne Diseases Unit, Rome, Italy.

Keywords: Malaria, *Plasmodium vivax*, European region

Plasmodium vivax is the most widespread human *Plasmodium* species, thanks to its ability to complete the biological cycle even in areas characterized by temperate climate. So far, in the World Health Organization (WHO) European Region, most of the observed malaria endemicity was due to *P. vivax*. In 2015, this WHO Region achieved zero malaria reported cases and was declared malaria-free on April 25, 2016. Malaria elimination in the European Region has been achieved through to the implementation of integrated control strategies, such as accurate detection and surveillance of malaria cases, vector control programs, a tight collaboration with neighboring countries and a capillary activity of information among people living in at risk areas. However, this extremely positive result deserves steady attention. The continuous importation of malaria cases from endemic regions, the changes in both eco-epidemiological and climatic conditions and the presence throughout the territory of *Anopheles* mosquito species, potential vectors of malaria, could lead to a re-emergence of the disease. Other Regions in the world are prone to a possible re-emerging of malaria transmission, as the areas of China bordering North Korea, this latter the only temperate country where *P. vivax* is still endemic today. The massive movement of refugees entering in China and South Korea from North Korea and the impressive flight ability of the local *Anopheles* vector have been identified as major factors that can lead tothe reintroduction of *vivax* malaria in those areas (Cho et al., 2002 Korean J Parasitol. 40: 139-48). The risk of a possible re-emergence of malaria in a given area can be estimated by the evaluation of the malariogenic potential, which is determined by three factors, the receptivity, the infectivity and the vulnerability.The results of recent studies on malaria reintroduction evaluation risk carried out in the Mediterranean basin (Romi et al., 2012 Malar J. 11: 98) showed that although this risk appear to be low, outbreak events due to *P. vivax* cannot be excluded, as demonstrated by the *P. vivax* epidemic occurred in 2011 in Greece (Andriopoulos et al., 2013 Int J Infect Dis. 17: e125-e128). In Italy, between August and October 2017, seven suspected autochthonous cases of malaria were recorded, which raised some concern in public opinion. After the epidemiological investigations, two cases were identified as nosocomial while the other five were registered as cryptic, as it was not possible to establish a possible route of transmission (Boccolini et al., 2020 BMC Public Health. 20: 857). This unusual cluster of seven non-imported cases represented therefore a warning that should lead us to maintain a constant level of surveillance in a country where malaria was endemic until the early 1960s.

INVITED TALKS ESDA/SOIPA

DIROFILARIOSIS AND ANGIOSTRONGYLOSIS

SOIPA
Società Italiana di Parassitologia

ESDA
European Society of Dirofilariosis and Angiostrongylosis

Dirofilaria spp. and *Angiostrongylus vasorum*: current risk of spreading in Central and Northern Europe

HP FUEHRER

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

Keywords: *Dirofilaria immitis*, *Dirofilaria repens*, *Angiostrongylus vasorum*

INTRODUCTION. In the past decades the relevance of *Dirofilaria immitis* (causative agent of canine and feline cardiopulmonary dirofilariosis), *D. repens* (subcutaneous dirofilariosis), and *Angiostrongylus vasorum* (French heartworm causing canine angiostrongylosis) is steadily increasing in Central and Northern Europe (Capelli et al., 2018 Parasites Vectors. 11: 663; Alsarraf et al., 2021 Sci Rep. 11: 1068). On the one hand, these parasites are imported with dogs from endemic countries, on the other hand increasing temperatures benefit both vectors and parasites.

A summary of published articles dealing with these parasites in Central and Northern Europe is given.

It is obvious that the number of cases of *D. immitis*, *D. repens* and *Angiostrongylus vasorum* is increasing in Central and Northern Europe. Various studies report imported (Sonnberger et al., 2021 Pathogens. 10: 550), but also autochthonous cases (Alsarraf et al., 2021 Sci Rep. 11: 1068). Housing conditions of dogs, pet travel, climate change, and global change are important factors in the spread of these nematodes.

Exposure to *Angiostrongylus vasorum* in areas enzootic for VBDs

S MORELLI[1], **F GORI**[2], **M COLOMBO**[1], **A DI CESARE**[1], **G SIMONATO**[3], **F VERONESI**[4], **I RUSSI**[1], **M SCHNYDER**[2]

[1] Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy; [2]Institute of Parasitology, Vetsuisse-Faculty, University of Zurich, Zurich, Switzerland; [3]Department of Animal Medicine, Production and Health, University of Padua, Padua, Italy; [4]Department of Veterinary Medicine, University of Perugia, Perugia, Italy.

Keywords: *Angiostrongylus vasorum,* Antigen, Antibodies, Vector-borne diseases

INTRODUCTION. In the past years *Angiostrongylus vasorum* has been increasingly detected throughout Europe (Elsheikha et al., 2014 Vet. Res. 45:92). The same drivers involved in the spreading of canine angiostrongylosis, e.g. climatic changes and urbanization, may also spur the emergence of Vector-Borne Diseases (VBDs) (Beugnet et al., 2013 Comp. Immunol. Microbiol. Infect. Dis. 36:559-66). Thus, in many geographic areas dog populations may be at simultaneous risk of VBDs and angiostrongylosis. This study evaluated the exposure to *A. vasorum* in dogs tested for major canine VBDs in enzootic areas of Italy.

MATERIALS AND METHODS. Sera of 294 dogs previously tested for VBDs with i) SNAP® 4DX (IDEXX Laboratories Inc.) detecting *Dirofilaria immitis* antigens and *Borrrelia burgdorferi*, *Anaplasma* spp. and *Ehrlichia* spp. antibodies and ii) IFAT for *Leishmania infantum*, *Babesia canis* and *Rickettsia conorii*, were subjected to two ELISAs detecting *A. vasorum* circulating antigen (Ag) (Schnyder et al., 2011 Vet. Parasitol. 179:152-58) and antibodies (Ab) (Schucan et al., 2012 Vet. Parasitol. 185:216-24).

RESULTS AND CONCLUSIONS. Six (2%) and 22 (7.4%) dogs were seropositive for circulating *A. vasorum* Ag and Ab, respectively. Seventeen dogs (5.8%) were positive for *A. vasorum* Ab + at least one VBD, 3 (1%) were positive for *A. vasorum* Ag + at least one VBD, while 1 dog was positive for *A. vasorum* Ag + Ab + *B. canis* (Figure 1). Thus, dogs living in Italy are at risk of contemporaneous infection with both *A. vasorum* and VBDs. Knowledge on the simultaneous risk for VBDs and angiostrongylosis in dog populations of Europe is very poor, as similar data have been generated only in Italy (Sauda et al., 2018 Parasite 25:2) Portugal (Alho et al., 2016 Parasit. Vectors 9:225) and Bulgaria (Iliev et al., 2020 Helmintologia 57: 171-8). Further studies are thus warranted for a more comprehensive knowledge on this epidemiological risk in European territories and to understand if the routine use of broad-spectrum endo-ectoparasiticides should be re-considered.

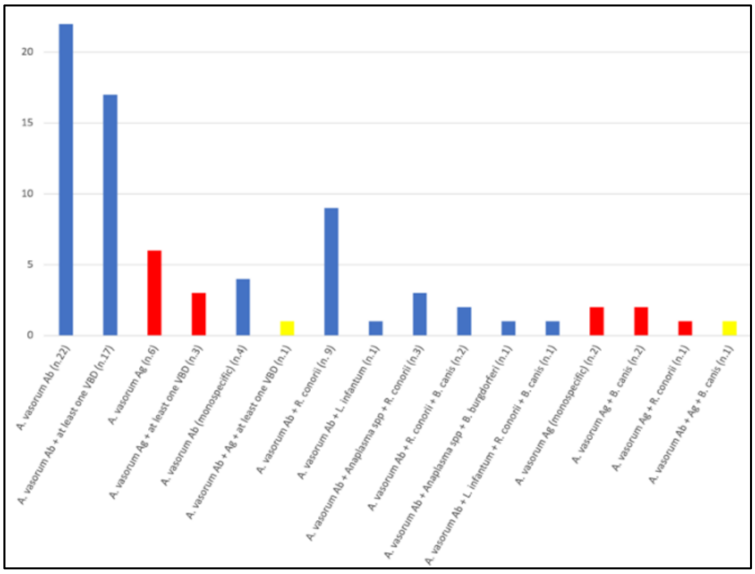

Figure 1. Number of dogs positive to *Angiostrongylus vasorum* antigen and/or antibodies and combinations and simultaneous co-exposure to vector-borne diseases.

INVITED TALKS SOIPA

Vectra 3D and canine vector-borne diseases: studies review

M VARLOUD

Ceva Santé Animale, Libourne, France.

Keywords: Ectoparasiticide, Repellency, Dog, Prevention

INTRODUCTION. Vector-borne diseases (VBDs) such as dirofilariosis, leishmaniosis, babesiosis and borreliosis are transmitted to dogs during arthropod blood-meals. Some are zoonotic. A repellent parasiticide (4.95 % w/w dinotefuran, 36.08 % w/w permethrin and 0.44 % w/w pyriproxyfen, Vectra®3D) reduces the risk of infection and spreading of VBDs.

MATERIALS AND METHODS. Four studies, of which 3 on *Phlebotomus perniciosus* and 1 on *Lutzomyia longipalpis*, involving 54 dogs evaluated its action against sandflies. Sedated dogs were exposed to unfed adult sandflies for 1 h starting from 1 day and at weekly intervals for 4 to 6 weeks after treatment. Two studies evaluated the transmission blocking effect of the product against dirofilariosis (McCall et al., 2017a Parasit Vectors. 511) and babesiosis (Varloud et al., 2018 IJID. 73: 390) while 3 evaluated the prevention of pathogen uptake from infected dogs by the vectors; of these latter, 2 involved dogs with leishmaniosis (Oliva et al., 2021 SOIPA) and dirofilariosis (McCall et al., 2017b Parasit Vectors. 511) respectively, and 1 was performed using an ex vivo model of borreliosis (Tahir et al., 2020 AAVP). A field investigation was performed in a highly endemic area for different VBDs (Laidoudi et al., 2020 IJID. 79).

RESULTS AND CONCLUSIONS. The immediate (1-2 days after treatment) anti-feeding efficacy of Vectra®3D was 88% against *L. longipalpis* (n=12 dogs) and 96.7% against *P. perniciosus* (n=42 dogs). It persisted for 4 weeks against both and for 5 weeks above 87% (n=16) against *P. perniciosus*. Studies involving pathogens, including in the field, confirmed a sustained repellent speed of action of Vectra®3D against vectors, whose disruption led to a transmission/uptake blocking effect against *Babesia canis*, *Borrelia burgdorferi*, *Dirofilaria immitis*, *Leishmania infantum*. Multimodal prevention strategies are often recommended. For instance, against heartworm disease, it is pivotal to combine macrocyclic lactones (killing larvae inside the dog) with mosquito repellents in both healthy (infectious bite prevention) and microfiariaemic dogs (uptake prevention). Repellency of vectors is the first line of defense in the control of VBDs. Vectra®3D, an advanced topical repellent, prevents the blood feeding of arthropod vectors on dogs with direct benefits in terms of prevention of the transmission or uptake of dangerous pathogens that the vectors may carry.

Vectra®3D against Leishmaniosis transmission

G BONGIORNO[1], A BOSCO[2], R BIANCHI[1], L RINALDI[2], V FOGLIA MANZILLO[2], M GIZZARELLI[2], MP MAURELLI[2], D GIAQUINTO[2], N EL HOUDA BEN FAYALA[2], M VARLOUD[3], A CRIPPA[3], G OLIVA[2], L GRADONI[1], G CRINGOLI[2]

[1]Reparto di Malattie trasmesse da vettori, Istituto superiore di Sanità, Roma, Italy; [2]Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli Federico II, Napoli, Italy; [3]Ceva Santé Animale, Libourne, France.

Keywords: Dinotefuran-pyriproxyfen-permethrin, Phlebotomine sand flies, Canine leishmaniosis, Xenodiagnosis

INTRODUCTION. Dogs are reservoir hosts of leishmaniasis caused by *Leishmania infantum* and transmitted by phlebotomine vectors. Dog protection from sand fly bites is universally considered to be the first-line approach to prevent CanL infections and transmission of zoonotic visceral leishmaniasis by infected dogs. Clinical trials of dinotefuran, pyriproxyfen and permethrin spot-on solution (Vectra®3D, Ceva Santé Animale) against *P. perniciosus*, have been found to confer elevated protection from sand-fly bites for one month in uninfected dogs under controlled conditions (Liénard et al., 2013 Parasit Res. 112: 3799-805; Varloud et al., 2015 FECAVA EuroCongress). The present study aimed to assess the effect of the spot-on formulation on the potential *L. infantum* transmissibility by infected dogs via the vector *P. perniciosus*.

MATERIALS AND METHODS. The study was part of a larger investigation on clinical characteristics of CanL associated with infectiousness to competent phlebotomine sand flies (Gizzarelli et al., 2021 Front Vet Sci. 8: 667290). Dogs affected by not severe clinical form of leishmaniasis (IRIS stage I, without or with mild proteinuria) were submitted to xenodiagnoses and 6 infecting >10% of insects were treated topically on Day 0. Antifeeding, insecticidal and anti-transmissibility effects were evaluated through xenodiagnoses performed on Days 1, 7 and 28, using individual pre-treatment parameters as control. Feeding and mortality rates were assessed at 24 h, whereas promastigote infection, maturation and burden were assessed up to 96 h post blood meal (potentially infectious rate).

RESULTS AND CONCLUSIONS. On Day 1 the anti-feeding efficacy was >95% in 4 dogs, insecticidal efficacy 100% in 4 dogs, and anti-transmissibility effect 100% in 6 dogs. Efficacy rates recorded on Day were very similar to Day 1. On Day 28, anti-feeding and insecticidal efficacy values were much broader, ranging 32.6-100% and 7.7-94.4%, respectively. Potentially infectious insects were recorded from 2 dogs, with sharp decrease in transmissibility rate as compared with pre-treatment condition. Altogether, Vectra®3D abrogated by >98% the potential *Leishmania* transmissibility by the examined pool of infected dogs over one month.

INVITED TALKS SOIPA

Boehringer
Ingelheim

The indoor cat: do the endoparasites knock at the door?

S MORELLI

Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy.

Keywords: Indoor, Cat, Endoparasites

Cats are extremely popular in Europe, with 106 million kept as pets in 2019 (https://www.statista.com/statistics/453880/pet-population-europe-by-animal/).

Although there is a growing trend towards a permanent indoor housing, European owners typically provide outdoor access to their cats. Data from a very recent survey indicate that the 59% of owned cats in Europe conduces an indoor-outdoor lifestyle (Foreman-Worsley et al., 2021 Animals 11:253). Lifestyle is crucial in putting cats at risk of infections and/or infestations with internal and external parasites. Outdoor access is associated with higher exposure to endoparasites such as *Toxocara cati*, Ancylostomatidae, *Dypilidium caninum*, *Aelurostrongylus abstrusus*, *Troglostrongylus brevior*, *Dirofilaria immitis* (Levy et al., 2017 J. Am. Vet. Med. Assoc. 250:873-880; Chalkowski et al., 2019 Biol. Lett. 15:20180840; Traversa et al., 2019 Acta. Trop. 193: 227-235; https://www.esccap.org/page/GL1+Worm+Control+in+Dogs+and+Cats/25/). However, also cats with no or limited outdoor access are at risk of infection with helminths or even ectoparasites (Coati et al., 2003 Par. Res. 3:146-7; Beugnet et al., 2014 Parasit. Vectors 7:291; Diakou et al., 2017 Par. Res. 116:3429-35; Traversa et al., 2019 Acta. Trop. 193: 227-235; Mirò et al. 2020 Parasit. Vectors 13:101), thus the misconception that indoor housing ensures a complete parasitological protection should be debunked. In fact, cats may become infected i) ingesting parasitic infective stages introduced mechanically into the domestic environment by the owners (e.g. dirty shoes), ii) preying intermediate or paratenic hosts (e.g. rodents, lizards, snails/slugs, earthworms) that find their way in the domestic environment (e.g. houses, terraces, gardens), iii) when fed with raw food by owners (e.g. BARF diet) (Papadopulos et al., 2020 Hell. J. Comp. Anim. 9:2), iv) when bitten by mosquitoes transmitting vector-borne helminths, e.g. *D. immitis* (Montoya-Alonso 2014 et al., Parasit. Vectors 7:506). Moreover, numerous owned cats are usually adopted from shelters or are found in the street and, if not properly parasitologically examined and dewormed before re-housing, they continue to harbor parasites, some of which have zoonotic potential. Finally, there is evidence that owners of indoor cats tend to a less accurate parasite management than owners of outdoor cats (Mirò et al. 2020 Parasit. Vectors 13:101). Therefore, adequate control strategies against feline endoparasitoses are of crucial relevance also for cats living indoor.

NexGard Combo: a new broad spectrum parasiticide for cats, supplied by Boehringer Ingelheim

A PEDE

Boehringer Ingelheim Animal Health.

Keywords: Cats, Nexgard Combo

Nexgard Combo features the first isoxazoline especially engineered for cats, esafoxolaner (active enantiomer of afoxolaner), combined with eprinomectin and praziquantel, in a topical product for cats suffering from, or at risk of, mixed parasitic infestations with ectoparasites (fleas/ticks/ear mites), gastrointestinal worms (nematodes and cestodes), cardiopulmonary worms, lungworms, and/or vesical worms.

Esafoxolaner binds GABA and glutamate receptors of chloride channels at synaptic level, blocking the entry of Cl- in nerve cells and resulting in uncontrolled activity of the central nervous system followed by death of insects and acarids. The high selectivity for arthropod receptors allow a high safety margin in cats.

Eprinomectin belongs to the macrocyclic lactones family and acts mainly on nematodes.

Praziquantel is a pyrazino- isoquinoline derivative anthelmintic targeting cestodes.

The product appears as a clear solution for topical use, supplied in a single-dose topical applicator, syringe-shaped. Multiparasitism is common in cats, co-infestation by fleas, nematodes and cestodes is a condition that occurs at a significant rate worldwide. Deworming of companion animals has become a routine recommendation of many veterinary practitioners, as well as continuous control of ectoparasites. Topical administration of such combination of actives is simple and may help to increase owner compliance.

INVITED TALKS SOIPA

Anthelmintic resistance in horses: Where is it a problem and what are the guidelines to prevent/postpone its development?

G VON SAMSON-HIMMELSTJERNA

Institute for Parasitology and Tropical Veterinary Medicine, Freie Universität Berlin, Berlin, Germany.

Keywords: Drug resistance, Cyathostomins, Tapeworms, Roundworms

INTRODUCTION. Gastro-intestinal parasites represent a serious health threat in horses and their effective control is a prerequisite for maintaining animal health in all equine settings. While in foals roundworms of the genus *Parascaris* are the most significant species, in yearlings and adults small strongyles (i.e. cyathostomins) have major importance, due to their ubiquitous prevalence and the potential of causing larval cyathostominosis. Also tapeworms such as *Anoplocephala perfoliata* occur frequently and can cause severe illness including colic. While being highly pathogenic large strongyles, namely *Strongylus vulgaris*, appear to show only very low prevalence, at least when tested coproscopically. Due to the event and increasing spread of anthelmintic resistance in cyathostomins and also *Parascaris* spp., current treatment recommendations aim at reducing treatment frequency and intensity to the least required while still preventing the development of parasite associated diseases.

MATERIALS AND METHODS. Recent comparative coproscopic as well as antibody testing (serum and saliva) findings from horse farms and clinic patients in Berlin/Brandenburg, Germany will be presented to highlight actual prevalences of major equine gastro-intestinal parasites and highlight the relevance of diagnostic test formats employed. An overview will be provided on selected studies providing insight on the occurrence of anthelmintic resistance in horses in different European countries. Current recommendations for sustainable parasite control in horses, specifically those from the European Scientific Counsel Companion Animal Parasites (ESCCAP), will be presented.

RESULTS AND CONCLUSIONS. The occurrence of key equine parasites such as *S. vulgaris* and *A. perfoliata* when diagnosed using antibody testing was found to be significantly higher as when tested coproscopically. This accounts for samples from German clinic patients as well as from equine settings. This has important implications concerning the design of parasite control regimes, particularly concerning the potential of re-emergence of large strongyle infections on farms employing selective treatment regimes. Novel and potential future opportunities for additional worm control components such as the use of nematophagous fungi, paraprobiotics or co-grazing strategies will be briefly discussed.

Parasitology & One Health – Perspectives from Africa and Beyond

V LORUSSO[1,2,3]

[1]Global Research & Intellectual Property Division, Vetoquinol, Paris, France; [2]University of Salford Tick Infections (USALTI)-Afrique, School of Science, Engineering & Environment, University of Salford, Greater Manchester, UK; [3]African Institute of One Health Research & Diagnostics (AIOHRD), Abuja, Nigeria.

Keywords: Africa, One health, Zoonoses, Parasitology, International cooperation

In a world dominated by the COVID-19 pandemic, the wording of “One Health” is now widely known and employed, beyond the mere scientific community. This locution is undoubtedly not novel to parasitologists, who, by vocation, study infections and their causative agents and hosts, at the interface with human and veterinary medicine. In Africa, although COVID-related cases and death rates have so far been lower than in other continents, the pandemic has still caused major disruptions, in the area of health and food security, that need to be addressed holistically, according to a One Health approach. Within this context, the present talk will provide an overview of issues pertaining to parasitic and vector-borne diseases, being either of animals or humans, or both, of topical relevance to the African continent as well as to neighboring and interconnected geographies. This analysis will be carried out against the background of the Sustainable Development Goals (SDGs), part of the UN’s 2030 Agenda, being mindful of the importance of tackling parasitic conditions for the attainment of SDGs such as, among others, #1 (‘no poverty’), #2 (‘zero hunger’) and #3 (‘good health and well-being’). In this view, the great significance of research and innovation as well as education in parasitology and entomology, will be highlighted, considering also their empowering role for youth and women. Finally, by taking the example of drugs like ivermectin, this presentation will underline the value of applying a One Health approach to drug discovery and development in the fight against neglected tropical diseases and zoonoses. All in all, this talk will uphold the adoption of a cross-sectoral and multi-disciplinary outlook, encompassing both life and social sciences, when dealing with parasitic conditions of humans and animals, in Africa and beyond, in COVID-times and further.

ORAL TALKS ESDA/SOIPA

DIROFILARIOSIS AND ANGIOSTRONGYLOSIS

Dirofilariosis: investigating the prevalence of the zoonotic parasitosis in dogs and humans from a hyper-enzootic area of Greece

D DIMZAS[1], S CHAINTOUTIS[1], A TAMVAKIS[2], S CHATZOUDI[3], A CHLICHLIA[4], M PANOPOULOU[5], A DIAKOU[1]

[1]School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece; [2]Department of Marine Sciences, University of the Aegean, Mytilene, Greece; [3]Private Veterinary Clinic, Xanthi, Greece; [4]School of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupolis, Greece; [5]School of Medicine, Democritus University of Thrace, Alexandroupolis, Greece.

Keywords: Dirofilariosis, Zoonosis, Prevalence, Greece

INTRODUCTION. Dirofilariosis is a mosquito-borne parasitic disease caused by nematodes of the genus *Dirofilaria*. Dogs get infected by the species *Dirofilaria immitis* and *Dirofilaria repens*, agents of heartworm disease and subcutaneous dirofilariosis, respectively. Both parasites, and particularly *D. repens*, have zoonotic implications, and humans living in enzootic areas are at increased risk of acquiring pulmonary and subcutaneous/ocular dirofilariosis (Simón et al., 2012 Clin Microbiol Rev. 25: 507-34). The aim of the present study was to assess the prevalence of *Dirofilaria* spp. infection in dogs living in Thrace, a known hyper-enzootic region of Northeastern Greece and to investigate the seroprevalence in humans in the same area at the same time-frame, in order to evaluate the risk of infection for humans in such epidemiological settings.

MATERIALS AND METHODS. Blood samples from 604 dogs were examined by the Knott's method for microfilariae identification and by serology (DiroCHEK®, Zoetis) for *D. immitis* antigen detection. Furthermore, 625 human serum samples were examined by an in-house ELISA for detection of anti-*Dirofilaria* IgG using a *D. immitis* crude antigen. Western blot analysis separately for *D. immitis* and *D. repens* was applied to confirm seropositive human samples (Santamaria et al., 1995 Clin Exp Dermatol. 20: 19-21). Statistical analysis was performed using the Chi-square test and calculating the Odds Ratio for several parameters, e.g. sex, age, living area and lifestyle (for dogs), and their association to infection.

RESULTS AND CONCLUSIONS. In total, 173 (28.6%) dogs tested positive for *D. immitis* by at least one examination method and *D. repens* microfilariae were found in 7 (1.2%) samples. IgG antibodies against *Dirofilaria* spp. were detected in 71 (11.36%) human samples. Western blot analysis results are pending. Sex, lifestyle and living area were associated with infection in dogs, while no associations were found for humans. This is the first seroprevalence study for dirofilariosis in humans in Greece. The findings of the present study suggest that humans living in hyper-enzootic areas are at risk of infection with *Dirofilaria* spp., emphasizing the need for preventive measures.

Atypical intraocular migration of *Angiostrongylus vasorum*

IE FILIPESCU[1], **S MANZOCCHI**[2], **M NERONI**[3], **S MORELLI**[4], **N PANTCHEV**[5], **M VERGARA**[1]

[1] Veterinary Clinic Cortonese, Perugia, Italy; [2] IDEXX Laboratories, Novara Day Lab, Granozzo con Monticello, Novara, Italy; [3]Veterinary Clinic Lungonera, Terni, Italy; [4]Faculty of Veterinary Medicine, Teramo, Italy; [5]IDEXX Laboratories, Kornwestheim, Germany.

Keywords: *Angiostrongylus vasorum*, Intraocular angiostrongylosis

INTRODUCTION. *Angiostrongylus vasorum* is a nematode which usually inhabitsthe pulmonary arteries and right ventricle of wild and domestic canids (Morgan et al., 2005 Trends Parasitol. 21: 49-51). Clinical manifestations vary from insidious and non-specific signs (mostly respiratory and neurological manifestations) to fatal cardiopulmonary insufficiency (Ferdushy et al., 2010 Parasitol Res. 107: 765-71). In extremely rare cases, larvae can migrate through the host's body and reach atypical tissues such as the anterior chamber of the eye, but the mechanism of migration is still unclear (Colella et al., 2016 Parasit Vectors. 9: 161). This report describes the case of two intraocular *A. vasorum* specimens in an 18-months old dog living in the countryside of Terni, Central Italy.

MATERIALS AND METHODS. The patient was referred for an ophthalmological examination due to the presence of two motile parasites in the anterior chamber of one eye. The medical historyshowed that hehad several episodes of recurrent cough in the past. Physical and imaging (thoracic X-ray and ultrasound) examinations were carried out, along with a complete hematobiochemical analysis and modified Knott test. The intraocular parasites were surgically removed and preserved in 70% ethanol for morphological and biomolecular identification. Serum and aqueous humor were tested forantigens of *Dirofilaria immitis* (SNAP 4DX Plus, IDEXX, Westbrook, US) and *A. vasorum* (AngioDetect, IDEXX, Westbrook, US). A stool sample has been examined using the Baermann's method.

RESULTS AND CONCLUSIONS. The intraocular parasites where morphologically consistent with metastrongylid and identified by PCR as *A. vasorum.* Both serum and aqueous humor yielded positive for AngioDetect test, and larvae of *A. vasorum* were retrieved at the Baermann'stest. All tests for *D. immitis* were negative. Thoracic X-ray and ultrasound showed findings compatible with angiostrongylosis, i.e. areas of subpleural lung consolidation. This clinical case demonstrates the importance of including *A. vasorum* in the differential diagnosis of canine ocular diseases. Furthermore, an update of the current epidemiological distribution of *A. vasorum* in Italy would be of great help for local vets towards appropriate diagnostic, prevention, and treatment approaches for angiostrongylosis.

Highly variable clinical pictures in dogs naturally infected with *Angiostrongylus vasorum*

S MORELLI[1], M COLOMBO[1] E GRILLOTTI[1,2], C PEZZUTO[3], C DE TOMMASO[4], F PAMPURINI[5], R SCHAPER[5], J DRAKE[5], PE CRISI[1], A DI CESARE[1]

[1]Faculty of Veterinary Medicine of Teramo, Italy; [2]Ambulatorio Veterinario Reate, Rieti, Italy; [3]Ambulatorio Veterinario Pezzuto Carlo/Piano Noemi, Campobasso, Italy; [4]Labforvet, Caserta, Italy; [5]Elanco Animal Health.

Keywords: *Angiostrongylus vasorum*, Clinical signs, Dog, Italy, Lungworm

INTRODUCTION. In the past few years, canine angiostrongylosis by *Angiostrongylus vasorum* has spread in many Countries of Europe. Though awareness of angiostrongylosis is constantly increasing, veterinarians are often faced with the challenging variability of its clinical course (Schnyder et al., 2017 Parasitol. Res. 116: 31-40). In fact, adult parasites, larvae and eggs elicit complex pathogenetic mechanisms which cause unpredictable clinical signs. This case series presents a high variability of clinical pictures showed by naturally infected dogs living in Italy.

MATERIALS AND METHODS. Clinical data of 36 dogs with a copromicroscopic diagnosis of angiostrongylosis and referred as individual clinical cases from 4 regions of Central and Southern Italy are here reported.

RESULTS AND CONCLUSIONS. Twenty-three (63.9%) dogs showed variable clinical pictures, while 13 (36.1%) were subclinically infected (Figure 1). Cardio-respiratory signs were present in 19 dogs (52.8%) while 14 (38.9%) had non-specific signs (e.g. diarrhea, weight loss, lethargy). Two dogs (5.6%) showed blood disorders (e.g. hematochezia, hemorrhages) and 1 (2.8%) neurological signs (e.g. shivering, hypersalivation). Cough was the predominant sign, showed by 11 dogs (30.5%), followed by dyspnea (8/22.2%), weight loss (6/16.7%), diarrhea (4/11.1%), anorexia (4/11.1%), syncope (3/8.3%) and lethargy (3/8.3%). Other signs were less frequent (Figure 2). There were 17 (47.2%) dogs shedding L1 while exhibiting no cardio-respiratory signs. While 13 (36.1%) had no clinical signs of infection, 4/19 dogs presenting with clinical signs had neither cough nor dyspnea. These data show the extreme unpredictability of angiostrongylosis, which must be included in the differential even when dogs living in endemic areas have non-specific clinical signs. The frequency of subclinical infections renders routine parasitological analysis mandatory for a timely diagnosis and a prompt treatment with efficacious formulations, to minimize the risk of a sudden onset of a potentially fatal disease.

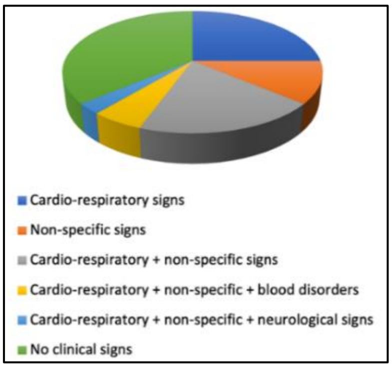

Figure 1. Clinical pictures of 36 dogs with angiostrongylosis

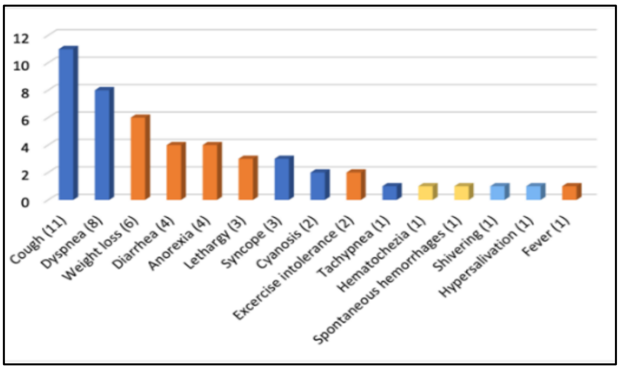

Figure 2. Specific clinical signs displayed by 36 dogs with angiostrongylosis (numbers in brackets indicate the number of dogs displaying the relative clinical sign)

Cardio-pulmonary nematodes of the red fox in Sardinia island, Italy

F NONNIS[1], C TAMPONI[1], A VARCASIA[1], L MELONI[1], S KNOLL[1], L CAVALLO[1], G DESSÌ[1], F PORCU[1], S CARTA[1], C PASINI[1], MF SINI[1], S CAFIERO[1], PA CABRAS[2], P FOIS[3], A VIGLIETTI[3], A SCALA[1]

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Italy; [2]Istituto Zooprofilattico Sperimentale della Sardegna, Tortolì; [3]Libero Professionista.

Keywords: Red fox, Bronchopulmonary nematodes, Sardinia, Wildlife

INTRODUCTION. Cardiopulmonary parasites *Angiostrongylus vasorum*, *Crenosoma vulpis* and *Eucoleus aerophilus* are a source of increasing concern among the veterinary community due to the clinical manifestations they may cause in dogs, from mild respiratory signs tomore severe ones (Conboy, 2009 Vet Clin North Am Small Anim Pract. 39: 1109-26; Traversa et al., 2010 Parasit Vectors. 3: 62). Even though it is already known that the red fox is an important reservoir for *A. vasorum* and might play a key role also in the transmission of *C. vulpis* and *E. aerophilus* to dogs, there are no recent studies involving the red fox population of Sardinia, and indeed, the last study dates back to 1986 (Leoni et al., 1986 Parassitologia. 28: 268-69). Thus, with this study we aimed to determinethe role that these wild canids might play in the transmission of these parasites to dogs in Sardinian territory.

MATERIALS AND METHODS. Between 2015 and 2021 fifty foxes, found dead in road accidents, were collected. Hearts and lungs were dissected in order to extract adult worms, which were identified using morphometrical features and PCR amplification. Faecal samples of each animal were collected and processed by Baermann technique and the identification of L1s was based on morphological keys (McGarry et al., 2009 Vet Rec. 165: 258-61).

RESULTS AND CONCLUSIONS. An overall prevalence of 54% was detected at the dissection of lungs and heart: 46% of the foxes scored positive for *E. aerophilus*, 16% for *C. vulpis* and 12% for *A. vasorum*. In the 14% of animals a concurrent infestation of different species was found (in all cases involving *E. aerophilus*). Baermann exam revealed an overall prevalence of 8% (2% *A. vasorum,* 2% *C. vulpis,* 4% both species). PCR amplification confirmed the morphological identification of the parasites. The prevalence of *C. vulpis* and *E. aerophilus* were found to be higher compared to the previous study (Leoni et al., 1986 Parassitologia. 28: 268-69), while *A. vasorum* prevalence seemed slightly decreased. Our study revealed that the red fox represents a reservoir for *A. vasorum*, *C. vulpis* and *E. aerophilus* also in Sardinia, and this should be considered by veterinary clinicians in the differential diagnosis of respiratory distress syndrome in dogs, especially when domestic and wild canids share the same territory and the risks of transmission are higher.

Selection of new diagnostic markers for *Dirofilaria repens* infections with the use of phage display technology

M PĘKACZ[1,2], K BASAŁAJ[1], P BĄSKA[3], M KLOCKIEWICZ[2], D STOPKA[4], M WIŚNIEWSKI[2], A ZAWISTOWSKA-DENIZIAK[1]

[1]Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Twarda 51/55, 00-818 Warsaw, Poland; [2]Division of Parasitology, Department of Preclinical Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland; [3]Division of Pharmacology and Toxicology, Department of Preclinical Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland; [4]Department of Pathology and Veterinary Diagnostics, Division of Pathology, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland.

Keywords: Dirofilariosis, Phage display technology, Diagnostic

INTRODUCTION. *Dirofilaria repens* is a parasitic nematode causing vector-borne zoonotic infection (dirofilariosis), considered an emerging problem in human and veterinary medicine. Currently, diagnosis is based on the detection of the adult parasite and microfilariae in the host tissues. However, the efficacy of test relying on microfilariae detection is limited by microfilariae periodic occurrence. Therefore, a new reliable diagnostic method is needed.

MATERIALS AND METHODS. With the use of Ph.D.-12^{TM} Phage Display Peptide Library (NEB), we selected highly immunogenic 12-mer peptides reacting with IgG and IgM antibodies from dogs infected with *Dirofilaria repens*. After prescreening steps, we used antibodies pooled from 5 dogs infected with *Dirofilaria repens*. Selected peptides were further tested with positive and negative sera. Collected blood samples were classified as positive or negative based on detection of microfilariae in the bloodstream (Knott's method/PCR) or by adult *Dirofilaria repens* somatic antigen (*Dr*SA) ELISA test**.**

RESULTS AND CONCLUSIONS. We selected several IgG and IgM specific peptides with diagnostic potential in ELISA test. Interestingly, our approach enables us to detect infections in dogs with no clear microfilariae presence in the bloodstream or showing a weak signal in the *Dr*SA ELISA test, which might indicate occult/prepatent infection. Our method could be considered as a new specific diagnostic tool for subcutaneous dirofilariosis.

Financial support for this study was provided by The National Centre for Research and development, Poland (grant LIDER IX 0106/L-9/2017).

First autochthonous infection of a cat with *Dirofilaria immitis* in Austria

<u>MS UNTERKÖFLER</u>[1], LM KULMER[2], HP FUEHRER[1], M LESCHNIK[2]

[1]Institute of Parasitology, Department of Pathobiology, University of Veterinary Medicine Vienna, Vienna, Austria; [2]University Hospital for Small Animals, Department for Companion Animals and Horses, University of Veterinary Medicine Vienna, Vienna, Austria.

Keywords: heartworm, *Dirofilaria immitis,* Cardiopulmonary dirofilariosis

INTRODUCTION. In Austria increasing numbers of *Dirofilaria* cases in humans (Riebenbauer et al., 2021 Parasites Vectors. 14: 227) and dogs (Sonnberger et al., 2020 Parasitol Res. 119: 1001-09) are reported. Most cases are imported from endemic regions, but autochthonous *Dirofilaria repens* has been found in humans, dogs and mosquitoes in Austria. No autochthonous case of *Dirofilaria immitis* has been reported so far (Fuehrer et al., 2016 PLoS Negl Trop Dis. 10: e0004547).

MATERIALS AND METHODS. This is the first report of an autochthonous infection of a cat with *Dirofilaria immitis* in Austria.

RESULTS AND CONCLUSIONS. The case of a 7-year-old male neutered European Shorthair cat is presented. Originating from Burgenland the cat had unrestricted outdoor access and was presented with severe cardiopulmonary symptoms. Subsequent echocardiography lead to the tentative diagnosis of dirofilariosis. Surgical removal of the heartworms was performed following medical treatment with pimobendan (0.25 mg/kg BW, BID, p.o.) and milbemycin oxim (4 mg/kg BW, once per month, p.o.), which was maintained as lifelong therapy. The cat fully recovered and showed no symptoms during the follow up period of one year.

SESSIONE

PARASSITOLOGIA VETERINARIA

In vitro evaluation of acute toxicity of five essential oils towards the parasitic mite *Varroa destructor*

R BAVA[1], F CASTAGNA[1], C PIRAS[1], E PALMA[1,2], G CRINGOLI[3], V MUSOLINO[1], D BRITTI[1], EV MUSELLA[1]

[1]Department of Health Sciences-University of Catanzaro Magna Græcia, CISVetSUA, Catanzaro, Italy; [2]Nutramed S.c.a.r.l. Complesso Ninì Barbieri, Roccelletta di Borgia, Catanzaro, Italy; [3]Department of VeterinaryMedicine and Animal Production, University of Naples Federico II, CREMOPAR, Regione Campania, Naples, Italy.

Keywords: *Apis mellifera, Varroa destructor*, Calabria region, Essential oils

INTRODUCTION. *Varroa destructor* now represents the greatest parasite threat to honeybee health. Its presence within farms led to a decreased profitability, to an increased incidence of pathogens (bacterial and viral) and to a decreasing number of non-reared hives (Le Conte et al., 2010 Apidologie. 41: 353-63). Chemicals that are widely used to control this parasitosis have pushed towards the spread of drug resistant mites (Panini et al., 2019 Bull Insectology 72: 227-32). The research of alternative products for *Varroa* control is now mandatory. Calabria is a southern Italy region with a peculiar agricultural vocation, where *Citrus* species grow both spontaneously and cultured for commercial purposes. The aim of this study is to test whether Calabrian *Citrus* spp. essential oils could inhibit the survival of *V. destructor* mite.

MATERIALS AND METHODS. *Citrus* spp. used in this study were *Citrus paradise* (grapefruit), *Citrus reticulate* (tangerine), *Citrus sinensis* (orange), *Citrus bergamia* (bergamot), *Citrus limon* (lemon). For each experiment, 5 viable adult female *Varroa*, collected the same day from an infested apiary, were used and for each essential oil tested were transferred in tubes and stored at 35 °C. Each experiment included a negative (acetone only) and a positive (Amitraz diluted in acetone) control was performed in 6 technical replicates and performed in different days. *Citrus* essential oils and Amitraz were diluted in HPLC grade acetone (2 mg/ml) to obtain solution to be tested (50 µl/tube). Mortality was manually assessed after 1 hour exposure by pocking. In the absence of any movement, the mite was recorded as dead. If the mite was not able to walk, but still able to move at least one leg, it was recorded as inactivated.

RESULTS AND CONCLUSIONS. Results showed 100% viability of the parasites in every technical replicate when *Varroa* was exposed to only acetone and a 100% mortality when Amitraz was used. Calabrian orange (*C. sinensis*) essential oil killed 76.6% ($p \leq 0.001$) of the parasites and neutralized (dead + inactivated) 100%. Bergamot (*C. bergamia*) oil killed 70% ($p=0.001$) and neutralized 76.6% ($p \leq 0.001$). Interestingly, lemon oil killed 30% ($p=0.06$) of the parasites and neutralized 100%. The results demonstrate that Calabrian orange, bergamot and lemon extracts consistently reduced *V. destructor* viability and open the possibility to use them to control this parasitosis in bees farming.

Immunological response of songbirds to tick infestation: tolerance or resistance?

B BISAGLIA[1,2], E MATTHYSEN[1], E PRINSEN[3], D HEYLEN[1]

[1]University of Antwerp, Department of Biology, Evolutionary Ecology Research Group (EVECO), Belgium; [2]University of Milan, Department of Biosciences, University of Milan, Italy; [3]University of Antwerp, Department of Biology, Integrated Molecular Plant Physiology Research (IMPRES), Belgium.

Keywords: *Ixodes ricinus*, Birds, Immune system, ELISA

INTRODUCTION. Many wild bird species act as a reservoir for tick-transmitted pathogens. To gain knowledge into the epidemiology of tick-transmitted diseases in the wild, it is important to investigate the host immune response to the tick species to which they are naturally exposed. The processes that occur at the tick-host interface may indeed influence the biology of ticks and the pathogen transmission, as well as the health of the host.

MATERIALS AND METHODS. To investigate the birds' immune response to ticks' infestations, we performed several ELISA tests using sera from birds (*Cyanistes caeruleus* and *Parus major*), previously parasitized with *Ixodes ricinus*, and salivary glands extract (SGE) from ticks. In addition, we performed statistical analysis to compare the ELISA results with some selected birds' health parameters.

RESULTS AND CONCLUSIONS. Firstly, we turned out that the recognition between the secondary (rabbit anti-chicken AP serum) and the primary (birds' sera) antibodies occurred. Then, we provided serological evidence that it is possible to distinguish previously parasitized from not parasitized birds. Furthermore, we observed an increase of the birds' IgY level after subsequent infestations. Regarding the correlations between the ELISA results and the birds' health parameters, we observed that birds' body condition and haematocrit level increased through subsequent infestations too. Our results suggest that *C. caeruleus* and *P. major* developed tolerance toward tick's infestations rather than resistance since they cannot reduce or prevent tick harm. These findings are important since birds, as others wild animals, represent an important reservoir for ticks and for the pathogens they harbour.

Efficacy of condensed tannins against gastrointestinal nematodes of goats: *in vitro* evaluation of bioactive fodder and products

AL GAZZONIS[1], S PANSERI[2], SA ZANZANI[1], L VILLA[1], L RAPETTI[3], L CHIESA[2], MT MANFREDI[1]

[1]Department of Veterinary Medicine, Università degli Studi di Milano, Milan, Italy; [2]Department of Health, Animal Science and Food Safety "Carlo Cantoni", Università degli Studi di Milano, Milan, Italy; [3]Department of Agricultural and Environmental Sciences – Production, Landscape, Agroenergy, Università degli Studi di Milano, Milan, Italy.

Keywords: Condensed tannins, Anthelmintic activity, Eggs hatch essay, Larval migration inhibition test

INTRODUCTION. In dairy goats breeding, the use of alternative methods, e.g. supplementing the diet with bioactive fodder containing condensed tannins (CT), to anthelmintic drugs for the control of gastrointestinal nematodes (GIN) is becoming increasingly important. The present study aimed to evaluate the in vitro anthelmintic efficacy against GIN' eggs and third stage larvae (L3) of CT contained in commercial products (Silvafeed®ByPro, SBP and Silvafeed®Quebracho, SQ) and in sainfoin hay (SH).

MATERIALS AND METHODS. CT were extracted from each tested sample by using two methods for the extraction of the solely CT (extraction in acetone/water) and of both CT and other compounds (e.g., flavonoids, extraction in ethanol). CT concentration values was determined by the acetone-HCl-butanol method (Grabber et al., 2013 J Agric and Food Chem. 61: 2669-78). The Eggs Hatch Essay (EHA) and the Larval Migration Inhibition Test (LMIT) were conducted, adding the CT inhibitor polyvinylpolypyrrolidone (PVPP) as internal control. The inhibition of hatching percentage (%EHI) and the inhibition of larval migration percentage (%LMI) were calculated (Demeler et al., 2012 Parasitol Int. 61: 614-18). Data were analysed by one-way analysis of variance, setting the level of significance at 0.05.

RESULTS AND CONCLUSIONS. Concerning the water extracts, SBP was effective against the eggs hatching at higher concentration (1200µg/ml, %EHI:54.07%) than SQ and SH (SQ:300µg/ml, %EHI:31.75%, SH:150µg/ml, %EHI:40.87%). Concerning LMIT, SBP and SQ water extracts showed effect at lower concentration (600µg/ml, %LMI:69.67% and 88%, respectively) than SH (1200µg/ml, %LMI:70.42%). For both tests, the ethanol extracts were effective against GIN eggs and L3 starting from the first dilution tested. Data on PVPP indicated that the inhibiting effect on the migration of the L3 was entirely attributable to CT only for SQ water extract. Data obtained suggest that the integration of CT-rich fodder into the diet may be considered and implemented for the control of gastrointestinal nematode infections in the goats.

Cytauxzoon sp. and *Hepatozoon* spp. in cats in North-eastern Italy: Preliminary results

M GRILLINI, A FRANGIPANE DI REGALBONO, G SIMONATO, C TESSARIN, G DOTTO

Department of Animal Medicine, Production and Health, University of Padua, Vialedell'Università 16, Legnaro (Padua).

Keywords: *Cytauxzoon*, *Hepatozoon*, Cat, North-eastern Italy

INTRODUCTION. In Italy, data on presence and distribution of tick-borne protozoa, such as *Cytauxzoon* sp. and *Hepatozoon* spp., are scarce and limited to single areas, e.g. in Trieste province in North-eastern (Carli et al., 2012 Vet Parasitol. 183: 343-52) and Southern regions (Giannelli et al., 2017 Ticks Tick Borne Dis. 8: 721-24), respectively. In the present study, we investigated the occurrence these protozoa in domestic cats from North-Eastern (NE) Italy.

MATERIALS AND METHODS. This study was carried out in Veneto (V), Friuli-Venezia Giulia (FVG) and Trentino Alto-Adige (TAA) regions. K_3EDTA blood samples were collected from cats of all age-classes, exposed to at least one season at risk for VBDs, without clinical signs and any treatment against ectoparasites. Blood smears were observed for hemoparasites according to existing key (Hodžić et al., 2017 Parasitology 144: 650-61; Baneth et al., 2013 Parasit Vectors. 6: 102). A conventional PCR was performed to detect *Hepatozoon* and *Cytauxzoon* DNA targeting 18S-rRNA gene (Tabar et al., 2008 Vet Parasitol. 151: 338-36). Nucleotide sequences were compared in GenBank® dataset. A preliminary evaluation of potential risk factors associated with hemoprotozoan infection in relation to epidemiological data (provenance, owned/free-ranging cats, in/outdoor lifestyle) was done by Chi-square test (SPSS for Windows, version 27.0).

RESULTS AND CONCLUSIONS. A total of 158 cats (103 owned, 55 free-ranging) was recruited. *Cytauxzoon* and *Hepatozoon* DNA was detected in 6 (3.8%) and 26 (16.5%) cats, respectively. No *Hepatozoon* gamonts were detected in blood smears, while all *Cytauxzoon* PCR-positive samples evidenced parasitaemia. No co-infections were detected. Two species of *Hepatozoon* were found: *Hepatozoon felis* (n=10) and *Hepatozoon silvestris* (n=16). No significant differences were showed between *H. felis* prevalence and epidemiological data, whereas *H. silvestris* prevalence was significantly ($p<0.05$) higher in FVG and in free-ranging cats. *Cytauxzoon*sp. was detected only in free-ranging cats from FVG, with prevalence value (6/39, 15.4%) close to that previously reported in the same area (Carli et al., 2012 Vet Parasitol. 183: 343-52). In conclusion, this study indicates that two species of *Hepatozoon* (i.e. *H. felis*, *H. silvestris*) can infect domestic cats in NE Italy and *Cytauxzoon* sp. is still present in FVG region.

Eucoleus garfiai in wild boar (*Sus scrofa*) from Italy

L PACIFICO[1], MF SGADARI[1], N D'ALESSIO[2], F BUONO[1], E CASTALDO[1], B RESTUCCI[1], M OTTAVIANO[1], M ANTONICIELLO[1], G GAGLIO[3], A VARCASIA[4], V VENEZIANO[1]

[1]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II; [2]Istituto Zooprofilattico Sperimentale del Mezzogiorno; [3]Department of Veterinary Sciences, University of Messina, [4]Department of Veterinary Medicine, University of Sassari.

Keywords: *Eucoleus* spp., Wildboar, Italy, *Capillaria garfiai*

INTRODUCTION. *Eucoleus garfiai* (Syn. *Capillaria garfiai*) (Gallego and Mas-Coma, 1975 Vie MilieuXXV 237-48) is a nematode infecting lingual tissue of domestic and wild swine. Data on the prevalence are scant and often the report of *E. garfiai* is related to an accidental finding, moreover the presence of this nematodeis reported only in Europe and Japan (Masuda et al., 2019 Parasitol Int. 73: 101972). In this study an epidemiological survey was performed in order to identify *E. garfiai* in wild boar from Italy.

MATERIALS AND METHODS. During two hunting seasons (2019 – 2020) a total of 153 wild boars were inspected in four different provinces of Campania region. Histological examinations were performed on tongue samples fixed and stained with haematoxylin and eosin. From positive samples a scraping of dorsal tongue tissue was carried out to collect adult worms for parasitological examinations.

RESULTS AND CONCLUSIONS. Forty out of 153 wild boar examined revealed the presence of helminths and/or eggs in tongue tissues, for an overall prevalence of 26.14%. Nematodes were observed in the basal layer of the lingual epithelium while eggs were found both in the corneal layer and in prickle cell layers in proximity of adult worms. Nematodes presented long esophagus with presence of stichosome. Males were 11 mm in length and 0.08 mm in width; the caudal end had two lateral lobes, connected by a membrane. Female were 15.4 mm in length and 0.10 mm in width, presenting short distance between esophagus and vulva and rounded caudal end with sub-terminal anus. Eggs were barrel shaped, with two polar end measuring 61.04 x 27.22. Molecular analyses of 18S rRNA gene shown a homology of 99% with *E. garfiai* sequences deposited in GenBank (accession number MW947272). No significant differences were found at statistical analysis for age, sex and hunting provinces. Our findings agree with histopathological features described in previous studies and seems to confirm the low pathogenic impact of this nematode (Ferrer and Castellà, 1996 Res Rev Parasitol 56: 65-67). The present study represents the first report of *E. garfiai* in wild boar from Italy.

Summer sores secondary to a hoof crack in an Andalusian stallion

A PALOZZO, D TRAVERSA, G MARRUCHELLA, G CELANI, S MORELLI, L PETRIZZI

Faculty of Veterinary Medicine, University of Teramo, Italy.

Keywords: Horse, Habronemosis, Hoof crack

INTRODUCTION. Horse cutaneous habronemosis caused by larvae of the spirurid nematodes *Habronema microstoma* and *Habronema muscae* is also known as “summer sores’’. These lesions are often severe and disfiguring (Scott and Miller, 2003 Equine Dermatology, pp. 357-360). Although *Habronema*-caused lesions at the distal hind coronary grooves have been described (Schuster et al., 2010 Vet Parasitol. 174:170-174), cases of hoof cracks with secondary summer sores have never been reported. The present report describes clinic-pathological and surgical features of a quarter crack case complicated by cutaneous habronemosis at the dermal layers.

MATERIALS AND METHODS. A 15-years-old, Andalusian stallion was referred to the Veterinary Teaching Hospital (VTH) of the University of Teramo for a neoformation of the hoof and severe lameness. The clinical examination revealed a total thickness vertical quarter crack and a protruding and exuberant granulomatous mass. The mass was surgically removed and subjected to histopathological, microbiological, and parasitological examinations. In particular, a conventional copromicroscopical analysis was performed, and faecal and skin samples were subjected to a duplex semi-nested PCR able to differentiate *H. muscae* and *H. microstoma*.

RESULTS AND CONCLUSION. The molecular positivity of faecal (*H. muscae*) and skin (*H. muscae* and *H. microstoma*) samples indicates the primary role of *Habronema* in causing the hoof granulomatous mass and confirms previous data which showed the correlation between animals with summer sores and the concomitant occurrence of gastric infections (Vasey, 1981 Compend Cont Educ Pract Vet. 3:290-298). A septic pododermatitis with granulation tissue reaction was confirmed by histology. The secondary intention healing, the persistent bacterial infection and the parasitic infection have fostered the exuberant granulation tissue reaction, which occurs very rarely in the healing of foot wounds (Parks, 1997 Equine Vet Educ. 9:317-327). This is the first description of a hoof wall crack complicated by summer sores in the simultaneous presence of gastric habronemosis. A prompt diagnosis during fly activity especially in horses with a history of "summer sores" and / or which live in endemic areas, is imperative for timely treatment and efficacious prevention of disfiguring cutaneous habronemosis.

Molecular detection of *Acarapis woodi* in honey bee colonies of Italy, Latium region

S TOFANI[1], M PIETROPAOLI[1], C RUBINO[1], C DI RUGGIERO[1], M MILITO[1], A CERSINI[1], C MEROLA[2], M AMORENA[2], G FORMATO[1]

[1]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Rome, Italy; [2]Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Teramo, Italy.

Keywords: Tracheal mite, *Apis mellifera*, Diagnostic methods

INTRODUCTION. *Acarapis woodi* is the etiological agent of acarapisosis, a parasitic disease of the adult honey bee *Apis mellifera* L. and other Apis species. This Tarsonemid mite, also known as "tracheal mite", inhabits the respiratory system of honey bees and, in particular, the large prothoracic trachea of the worker honey bees (OIE, 2008). *Acarapis woodi* has been reported worldwide, and it is recognized as a cause of significant economic losses in the beekeeping sector (Garrido-Bailón et al., 2012 Exp Parasitol. 132: 530-36). However, little is known regarding its real prevalence in honey bee colonies in Mediterranean countries, including Italy. To fill this knowledge gap, the present study aimed to determine the occurrence of *A. woodi* in honey bee colonies of central Italy, using molecular diagnostic techniques.

MATERIAL AND METHODS. The study was carried out in Latiumregion (Central Italy) from May to June, for two consecutive years, i.e.2018, 2019. Overall, 44 adult honey bees and 111 hive debris were collected from 17 apiaries by the veterinary services in the context of a bee health monitoring study. Two specific molecular techniques for the identification of *A. woodi* were executed. The first protocol of PCR amplifies a region of 118 bp in the subunit I of cytochrome oxidase (COI) of honeybee samples. In addition, the presence of *A. woodi* from honey bees and debris matrices was further investigated through Real time PCR, amplifying a sequence of 113bp in the subunit I of COI. The amplicons of positive samples were sequenced using Sanger sequencing analyses.

RESULTS AND CONCLUSIONS. Overall, 9.1% of adult honey bee samples (all belonging to one apiary) and 6.3% of hive debris samples (belonging to 6 apiaries) were positive for molecular detection. All adult honey bee samples had a sequence identity of 95% and a query cover of 100% with the accession number HF945444.1. One debris sample had a sequence identity of 100% with accession number KX790788.1. The results of the present study confirm the presence of *A. woodi* in honey bee colonies of Italy and points out the underestimation of its prevalence inItalian apiaries. In fact, their last report dates back to 20 years ago. This evidence could be related to the time-consuming traditional methods used for its diagnosis (e.g. microscopy), while molecular tools have higher sensitivity and specificity in the diagnosis of acarapisosis. Further studies are needed to clarify the actual prevalence of *A. woodi* in Europe and the potential relationship between its presence and colony loss.

Updates on the distribution of *Eustrongylides excisus* Jägerskiöld, 1909 (Nematoda: Dioctophymatoidea) in lake fish from northern Italy

P TEDESCO[1], V MENCONI[2], M PREARO[2], M TOMASONI[2], A GUSTINELLI[1], ML FIORAVANTI[1]

[1]Dipartimento di Scienze Mediche Veterinarie, Alma Mater Studiorum – Università di Bologna; [2]Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d’Aosta.

Keywords: *Eustrongylides*, Fish, Fish-borne parasitic zoonosis, Italian lakes

INTRODUCTION. *Eustrongylides* spp. are parasitic nematodes of piscivorous birds and freshwater fish, which act as their definitive and intermediate/paratenic hosts respectively, and are as well recognized as potentially zoonotic parasites. To date, *Eustrongylides* has been reported from fish and birds from different areas of central Italy (Dezfuli et al., 2015Parasites Vectors. 8: 227; Mazzone et al., 2019 J Parasitol. 105: 882-89; Guardone et al., 2021 Food Control. 120: 107517); therefore, the purpose of this study was to investigate the presence of the parasite in fish populations from areas not yet studied in northern Italy.

MATERIALS AND METHODS. A total of 2130 specimens belonging to a wide range of fish species (*Perca fluviatilis*, *Lepomis gibbosus*, *Micropterus salmoides, Squalius cephalus, Scardinius erythrophthalmus, Carassius carassius, Rutilus rutilus, Alburnus arborella, Ameiurus melas, Silurus glanis*) were collected with the help of local fishermen from six lakes in northern Italy (Garda, Nero, Alice, Pistono, Sirio, Candia) and subjected to a full parasitological examination. Collected nematodes were fixed in 70% ethanol and identified morphologically through light and scanning electron microscopy; a subset of larvae was analyzed with molecular methods (amplification and sequencing of ITS rDNA) for species confirmation.

RESULTS AND CONCLUSIONS. Overall, *Eustrongylides* larvae were recovered in 4.88% of the examined fish and were exclusively found in the species *P. fluviatilis, L. gibbosus* and *M. salmoides*, with particularly high prevalence in *L. gibbosus* (as high as 95.45% in Candia lake). The parasite occurrence was confirmed in all six surveyed lakes with prevalence values higher in Candia lake (65.15%) and lower in Sirio lake (1.42%). Morphological and molecular analyses allowed to confirm that all larvae belonged to the species *Eustrongylides excisus*.

The distribution of *E. excisus* in all the lakes under study and the high prevalence values reported in some fish species throughout the present investigation highlight a possible emergence of this parasitic infection, which could represent an important health issue for piscivorous birds, being particularly pathogenic for nestlings, as well as a potential zoonotic risk.

SESSIONE

PARASSITOLOGIA UMANA

Inflammatory mechanisms in Caco-2 cells stimulated with *Anisakis*-derived messengers of pathogenicity

I BELLINI, D SCRIBANO, M SARSHAR, C AMBROSI, A PIZZARELLI, AT PALAMARA, S D'AMELIO, S CAVALLERO

Department of Public Health and Infectious Diseases, Sapienza University of Rome.

Keywords: *Anisakis*, Caco-2, Crude extract, Exosomes

INTRODUCTION. Anisakiasis is a zoonotic disease caused by consumption of raw fish parasitized with *Anisakis* spp. third stage larvae (L3). Larval migration in the gastrointestinal tract, the excreted/secreted products, and extracellular vesicles (EVs) can progressively determine allergic symptoms, erosive ulcerous lesions and granulomas (Audicana et al., 2008 Clin Microbiol Rev. 21: 360–379). Reports of tumors co-occurrence with *Anisakis* L3 are increasing (Sonoda et al., 2015 Surg Today. 45: 1321-1325; Murata et al., 2018 BMC Infect Dis. 2018 4;18: 619) but pathogenic mechanisms, role of host's immune response and potential implications are still unknown. The aim of this study was to investigate the inflammatory pathway in *in-vitro* human epithelial colorectal adenocarcinoma cells (Caco-2) exposed to the live L3, the crude extract (CE) and the exosomes enriched fraction, as representative of mechanical action of larval motility, whole body of senescent larvae and inflammation silencing, respectively. In particular, the activation of inflammatory response key molecules (ERK1/2, NF-kB), and the amounts of the pro-inflammatory cytokines (IL-6, IL-8) were analyzed.

MATERIAL AND METHODS. L3 collected were used for incubation as live L3 (38), for CE preparation (36) and for EVs isolation (136). Samples were incubated with Caco-2 cells (6h/24h) at 37°C in the presence of 5%CO_2. Cell lysates were used for Western Blot (WB) and Real-Time PCR analyses and supernatants were used for ELISA tests.

RESULTS AND CONCLUSION. ELISA tests carried out on Caco-2+L3 showed a progressive decrease of IL-6 (P<0.01) and IL-8 if compared to non-treated cells. Differently, no signals were obtained for IL-6 in Caco-2+EVs (P<0.01) and IL-8 was strongly downregulated (P<0.01). On the contrary, CE induced a strongly increased secretion of IL-6 (P<0.01) and a decreasing trend in IL-8. Accordingly, WB analyses showed an increased phosphorylation level of ERK1/2 in Caco-2+CE (P<0.05) and in Caco-2+L3, with a slight increased level of activated NF-kB. No differences were observed in the phosphorylation levels of ERK1/2 in cells incubated with EVs respect to the controls. Furthermore, real-time PCR preliminary results on Caco-2+L3 suggested an early effect on cytokines expression. The results obtained showed an intricate host-parasite interplay, characterized by an early phase where active L3 and its released EVs modulate the immune response to find a long-lasting niche, and a second phase where L3 senescence may lead to the activation of host immune response leading to the formation of granuloma.

Small-RNA analysis from third-stage larvae and exosomes provides the first miRNAs catalogue from anisakid nematodes

S CAVALLERO, A PIZZARELLI, I BELLINI, S D'AMELIO, B ARCA'

Department of Public Health and Infectious Diseases, Sapienza University of Rome.

Keywords: *Anisakis pegreffii*, miRNAs, Extracellular vesicles, Exosomes

INTRODUCTION. Nematodes of the genus *Anisakis* are the causative agents of the emerging fish-borne zoonosis known as anisakiasis. Despite the increasing public health awareness, most of the mechanisms of infection and clinical outcomes in humans are still obscure. Establishment of successful long-term infections by pathogens as nematodes usually involves manipulation of host immune response. Extracellular vesicles (EV) carrying proteins, DNA and non-coding RNAs recently emerged as relevant players in intercellular signaling and parasite-host interactions (Hansen et al., 2019, J Extracell Vesicles. 8: 1578116). Among ncRNAs, miRNAs play relevant roles in post-transcriptional gene regulation and are the most extensively studied among EV cargo categories (Lefebvre and Lécuyer 2017, Front Microbiol. 8: 377). To obtain a deeper understanding of strategies involved in host manipulation, we characterized miRNAs from *Anisakis pegreffii* infective third stage larvae (L3) and the released exosomes (EX).

MATERIAL AND METHODS. Small-RNA fractions were isolated from three biological samples of L3 and EX of *A. pegreffii* from *Merluccius merluccius* (FAO37) and sequenced on an Illumina platform (single-end, 50bp). After trimming and filtering, raw reads were mapped to the *Anisakis simplex* genome (As14, Wormbase) and then to a collection of hairpins and mature *A. pegreffii* miRNAs predicted by a double approach: i) in-silico, using hairpins and mature miRNAs from *Ascaris suum* and ii) miRDeep* software. Mapping reads were used for assembling a catalogue of *A. pegreffii* miRNAs and for the differential expression analysis by EdgeR. The expression profile of selected miRNAs was validated by Stem&Loop PCR.

RESULTS AND CONCLUSIONS. A mean of 33 million and 24 million reads were obtained for *A. pegreffii* L3 and EX, respectively. After merging miRDeep* analysis and in-silico prediction results, we obtained a preliminary list of 206 predicted miRNAs, 156 of which showed a match in the samples (76%). So far, most of Stem&Loop RT-PCR confirmed results from sequencing. Forty miRNAs (25.6%) were found significantly differentially expressed (FC>2.0, FDR<0.05) in the pairwise comparison between L3 and EX. The most abundant miRNAs in the L3 sample showed identical seed regions with miRNAs described in other parasitic helminths suggesting a possible conserved function across evolutionary distant taxa. Among the 13 miRNAs found enriched in exosomes, some showed potential targets related to host immune system and inflammation.

Leishmania promastigotes and endothelial cells: production of CXCL8 and neutrophils recruitment

S D'ALESSANDRO[1,2], S PARAPINI[3,2], Y CORBETT[4,2], S DELBUE[1], A MODENESE[5], M GRAMICCIA[6], D ILBOUDO[7], P FERRANTE[1], D TARAMELLI[4,2], N BASILICO[1,2]

[1]Università degli Studi di Milano, Dipartimento di Scienze Biomediche, Chirurgiche e Odontoiatriche, Via Pascal, 36, 20133, Milano, Italy; [2]Centro Interuniversitario di Ricerca sulla Malaria-Italian Malaria Network (CIRM-IMN), Milano, Italy; [3]Università degli Studi di Milano, Dipartimento di Scienze Biomediche per la Salute, Via Pascal, 36, 20133, Milano, Italy; [4]Università degli Studi di Milano, Dipartimento di Scienze Farmacologiche e Biomolecolari, Via Pascal, 36, 20133, Milano, Italy; [5]Laboratorio Analisi, Istituto Clinico Città Studi, Milano, Italy; [6]Department of Infectious Diseases, Unit of Vector-borne Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy; [7]Université de Fada N'gourma, Institut Supérieur du Développement Durable, BP 54 Fada N'gourma, Burkina Faso.

Keywords: *Leishmania* promastigotes, Endothelial cells, CXCL8, Neutrophils

INTRODUCTION. *Leishmania* promastigotes are inoculated into the skin of the human hosts by the bite of phlebotomine sandflies and internalized by phagocytic cells, first neutrophils, then macrophages, where they develop into amastigotes and multiply. Endothelial cells are likely to represent an additional cell type encountered by *Leishmania* promastigotes. However, little is known on their role in the establishment of the infection. The aim of the study was to investigate the in vitro interaction between human endothelial cells and *Leishmania* promastigotes.

MATERIALS AND METHODS. Co-cultures of human microvascular endothelial cells (HMEC-1) with promastigotes were established, and morphological changes and phagocytosis determined by microscopy after Giemsa staining. The production of CXCL8 by HMEC-1 was evaluated by ELISA. Migration of leukocytes isolated from fresh peripheral blood human lymphocytes (PBL) was evaluated using the transwell migration assay.

RESULTS AND CONCLUSIONS. Promastigote of different *Leishmania* species showed morphological changes and loss of infectivity by co-incubation with HMEC-1 cells, and a dose-dependent ability to stimulate the production of CXCL8 by HMEC-1. PBL migration was evaluated using conditioned media from unstimulated or *Leishmania*-stimulated HMEC-1, as chemo-attractants. Significant increase in cells migrating toward conditioned medium from *Leishmania*-stimulated cells was observed. After 2 hours of chemotaxis, the migrated cells were mostly neutrophils in all groups, while after 24 hours, a higher percentage of monocytes was detected in conditioned media from unstimulated cells, whereas neutrophils still predominated in conditioned medium from *Leishmania*-stimulated cells. These data indicate that CXCL8 produced by endothelial cells may contribute to the establishment of *Leishmania* infection, through neutrophils recruitment to the site where promastigotes are deposited by the sandflies.

Prevalence and genotyping of *Pneumocystis jirovecii* pneumonia in patients with previously untreated acute myeloid leukemia and during induction therapy

D DI CAVE[2], V DEL PRETE[2,3], F BERRILLI[2], G PATERNO[1], L GUARNERA[1], R PALMIERI[1], F BONANNI[1], E BUZZATTI[1], F MORETTI[1], L MAURILLO[1], F BUCCISANO[1], A VENDITTI[1], MI DEL PRINCIPE[1]

[1]Department of Biomedicine and Prevention, University of Rome Tor Vergata, Rome, Italy; [2]Department of Clinical Sciences and Translational Medicine, University of Rome Tor Vergata, Rome, Italy; [3]PhD Program in Microbiology, Immunology, Infectious Diseases, and Transplants (MIMIT), University of RomeTor Vergata, Rome, Italy.

Keywords: *Pneumocystis jirovecii*, Acute myeloid leukemia, Bronchoalveolar lavage, *Pneumocystis jirovecii* pneumonia

INTRODUCTION. Pneumocystis Pneumonia (PJP) is an opportunistic fungal infection commonly found in lymphoproliferative disorders. The association between PJP and acute myeloid leukemia (AML) is not clearly defined. In our study, we performed a molecular diagnosis and genotyping of *Pneumocystis jirovecii* in bronchoalveolar lavage (BAL) samples obtained from patients with AML.

MATERIALS AND METHODS. This study included the analysis of 67 BAL samples received at the parasitology laboratory of the Policlinico Tor Vergata- Rome, between 2010 to 2020. The large subunit mitochondrial ribosomal RNA (*mtLSU*-rRNA) gene was used as a diagnostic marker for the detection of *P. jirovecii* and for the determination of the specific genotype of the species. Molecular analysis is carried out by PCR amplification and subsequent sequencing to highlight single nucleotide polymorphisms (SNP) in the sequence. Amplified DNA was sequenced by Bio-Fab research and multiple alignments were performed using Clustal W2 and MEGA7 software to find a correlation between the genotype and the patient's characteristics.

RESULTS AND CONCLUSIONS. Among 251 consecutive patients with non M3-AML, 67 BAL was performed. PJ presence was demonstrated in 11/67 (16,7%) of BAL, with a prevalence of 4,3% among our series of patients. As concerns the molecular analysis, in the *mtLSU*-rRNA locus 4 genotypes were distinguished on the basis of polymorphisms in positions 85 and 248: genotype 1 (85:C/248:T), genotype 2 (85:A/248:C), genotype 3 (85:T/248:C) and genotype 4 (85:C/248:T). In our patients, 70% presented the genotype 1 and 30% the genotype 3. No statistically significant correlations have yet been found between these patients. In our experience, PJP is not uncommon among patients with AML. In clinical care of AML, awareness of PJP should be heightened and prophylaxis should be considered, particularly in older patients. From a molecular point of view, our study confirms that the most widespread genotype in Europe is genotype 1.

Genetic variability of human *Giardia duodenalis* in Italy: what's new from a neglected parasite?

D DI CAVE[1], I GUADANO PROCESI[1,2], F BERRILLI[1]

[1]Department of Clinical Sciences and Translational Medicine, Parasitology, Faculty of Medicine, University of Rome "Tor Vergata", Via Montpellier 1, 00133 Rome, Italy; [2]PhD Program in Evolutionary Biology and Ecology, Department of Biology, University of "Tor Vergata", Rome, Italy.

Keywords: *Giardia duodenalis*, Human, MLST, Assemblages

INTRODUCTION. Although *Giardia duodenalis* is recognized as one of the most common intestinal parasites in Italy and many data are available from animals, genetic diversity and transmission dynamics in human are unknown. The aim of this study is to make available data on genetic variability of *G. duodenalis* from patients in our country, thereby starting to fill data gaps within the European scenario, and to provide food for thought regarding its transmission routes.

MATERIALS AND METHODS. Faecal samples were collected from symptomatic patients at the Laboratory of Parasitology of the Polyclinic Tor Vergata of Rome, between 2001 and 2019. Faeces resulted positive to *Giardia* cysts by microscopic investigation and/or by immunofluorescence were subjected to PCR by a MLST strategy and sequenced. Phenetic analysis was performed by comparison of the obtained sequences with those retrieved from NCBI GenBank by MEGAX. Due to the allelic sequence heterogeneity, analysis of Assemblage A and B were performed independently.

RESULTS AND CONCLUSIONS. The study involved 56 human *Giardia* specimens, grouped in two-time frames: 44 samples (frame 1: years 2001-2008) and 12 samples (frame 2: years 2018-2019). In frame 1, 26 samples were typed as Assemblage A (0.6), 16 as Assemblage B (0.34) and two isolates showed mixed Assemblages A+B (0.04). In frame 2, 8 samples were typed as Assemblage A (0.6), 3 as Assemblage B (0.25) and one isolate showed mixed Assemblages A+B (0.08). Sub-assemblage AII and, at lower frequency AIII, were identified in both time frames. As expected, within Assemblage B a larger sequences variability at sub-assemblage level was detected, making complex a proper isolates allocation.

Data obtained from our long-term analysis showed the same A and B assemblage frequencies in both time frames and 100% identity with human and animal *Giardia* isolates from different geographical areas. These results evidenced: i) no temporal and spatial segregations of genetic variants identified in Italy, widely distributed worldwide; ii) the necessity of increasing *G. duodenalis* sequences availability from Italy to understand the transmission pathways in our country at different epidemiological scales.

Interactions between *Acanthamoeba* and Shiga toxin-producing *Escherichia coli* (STEC): a possible relevant aspect of public health importance?

M MONTALBANO DI FILIPPO[1], A BONI [1], P CHIANI[1], F MINELLI[1], F BERRILLI[2], D DI CAVE[2], S MORABITO[1]

[1]Istituto Superiore di Sanità, Dipartimento di Sicurezza Alimentare, Nutrizione e Sanità Pubblica Veterinaria, Laboratorio di Riferimento Europeo per *Escherichia coli*, Roma, Italia; [2]Dipartimento di Scienze Cliniche e Medicina Traslazionale, Università di Roma Tor Vergata, Roma, Italia.

Keywords: Zoonosis, FLA, STEC, Public health

INTRODUCTION. *Acanthamoeba* is a widely distributed free-living amoeba (FLA), known to potentially cause an infection of the cornea, central nervous system, and skin. This opportunistic pathogen, feeds different microorganisms, including potentially pathogenic prokaryotes, and can carry them intracellularly as “Trojan Horse”. Shiga toxin-producing *Escherichia coli* (STEC) are foodborne pathogens that are the major causative agents of hemorrhagic colitis (HC) and post-diarrheal hemolytic uremic syndrome (HUS) leading to severe kidney disease and even death. STEC infections are zoonosis with ruminants being their natural reservoir, and transmission to humans occurs as a result of the ingestion of contaminated food, person-to-person and direct contact with infected animals or contaminated environment (especially water). We investigated the interaction of *Acanthamoeba* with STEC by co-culturing the two organisms and report on the survival rates of STEC and non-pathogenic *E. coli* upon predation by the protozoa.

MATERIALS AND METHODS. Here, we used a wild strain of *Acanthamoeba* sp. (isolated from thermal water - belonging to the T4 genotype) and studied its interactions with different strains of STEC (O157, O121, O26) and with a K-12 laboratory strain – BL21. Assays were performed at different tome points, to determine bacterial association with and invasion of *Acanthamoeba*. Additionally, intracellular survival of bacteria within *Acanthamoeba* trophozoites was determined.

RESULTS AND CONCLUSIONS. All bacterial isolates tested, associated with *Acanthamoeba* trophozoites. After 24 h (invasion assays), we observed only K-12 laboratory strain – BL21growth. After 48 hours (bacterial survival intracellular assays), two STEC strains (O157 and O121), as well as the non-pathogenic *E. coli* (BL21), survived inside *Acanthamoeba.*

Even if these results are preliminary, these findings suggest that STEC can survive within *Acanthamoeba,* at least for 48 hours. Thus, this ubiquitous amoeba could represent an environmental niche for STEC survival and transmission.

Development of a sensitive molecular assay for *Plasmodium* spp. for low parasitaemic samples

MG PRATO, M DEIANA, F PERANDIN, M DEGANI, G LA MARCA, B PAJOLA, M LEONARDI, C PIUBELLI

Department of Infectious, Tropical Diseases and Microbiology, IRCCS Sacro Cuore Don Calabria Hospital, Negrara di Valpolicella, 37024 Verona, Italy.

Keywords: Plasmodium, rt-PCR, Droplet digital PCR, Microscopy, DNA

INTRODUCTION. Malaria is a mosquito-borne disease caused by mainly five species of *Plasmodium* parasites. Accurate diagnosis plays an essential role in malaria control. Microscopy of blood smears is the gold standard for diagnosis. Malaria diagnosis is currently carried out clinical criteria associated to microscopy and rapid diagnostic test (RDT), though the performance of these methods are strongly related to parasitaemia and to the ability of the operator. Thus, low parasite density could lead to misdiagnosis. Molecular biology techniques such as real time-PCR (rt-PCR) and loop-mediated isothermal amplification (LAMP), represent the alternative strategies for malaria diagnosis. Therefore, employment of molecular assays could allow to improve sensitivity and specificity for malaria diagnosis. Recently, droplet digital PCR (ddPCR) has been reported as promising quantitative and sensitive technique for the molecular quantitation of *Plasmodium*. The purpose of our study is to implement a ddPCR method able to detect the DNA of *Plasmodium* species in samples with low parasitaemia, in order to increase the sensitivity and allow a correct diagnosis mostly for critical cases.

MATERIALS AND METHODS. We designed a “Pan-plasmodium” set of primer and probe on a 18S rRNA gene region common to all the 5 *Plasmodium* species (*P. falciparum, P. ovale, P. malariae, P. vivax*, *P. knowlesi*). We set up and evaluated both an rt-PCR and a ddPCR assays using four clinical samples of human blood archived in our “Tropica Biobank”, classified as positive for *P. falciparum, P. ovale, P. malariae, P. vivax*. We determined the limit of detection (LOD) and the limit of quantification (LOQ) of both rtPCR and ddPCR assays using 10-fold serial dilutions of each sample. The same assays were then evaluated on a set of 10 low-parasitaemia samples, which showed discordant results at microscopy and QBC tests.

RESULTS AND CONCLUSIONS. Both rt-PCR and ddPCR assays for *Plasmodium* species showed good reproducibility and a linearity without non-specific signals until 0.1 trophozoites/µl. The assays were able to detect the *Plasmodium* signal in the low parasitaemia samples, and copy number was determined by ddPCR. The results indicate that this rt-PCR or ddPCR pan-plasmodium test can be used as reliable assays when sensitivity is fundamental, as for example in case of evaluation of transfusional samples.

The response of *Anisakis simplex* s.s. to the anthelmintic drugs - specific changes in the metabolism of invasive larvae

R STRYIŃSKI[1], I POLAK[1], A GAWRYLUK, P ROSA[2], E ŁOPIEŃSKA-BIERNAT[1]

[1]Department of Biochemistry, Faculty of Biology and Biotechnology, University of Warmia and Mazury inOlsztyn, Poland; [2]National Marine Fisheries Research Institute, Research Station in Świnoujście, Poland.

Keywords: Parasitic nematode, Ivermectin, Oxidative stress, ABC transporters

INTRODUCTION. Anisakiasis is a fish-borne parasitic disease caused by the consumption of raw or undercooked fish, as well as cephalopods, contaminated by invasive larvae (L3) of species belonging to the genus *Anisakis* (Anisakidae). The main objective of this study was to investigate the metabolic status of *A. simplex* s.s. under the influence of anthelminthic drugs, ivermectin (IVM) and pyrantel (PYR). The mRNA expression of ABC transporters, antioxidative enzymes, GABA and acetylcholine receptors, as well oxidative status was determined.

MATERIALS AND METHODS. The L3 larvae of *A. simplex* s.s from Baltic herring (*Clupea harengusmembras*) were cultured *in vitro* with IVM or PYR at different concentrations: 1.56, 3.125 and 6.25 µg/mL of culture medium for 3, 6, 9, and 12 hrs under anaerobic conditions (37 °C, 5% CO_2). The *in vitro* culture was carried out as described by Iglesias et al., (2001 Parasitology 123: 285-91). The real-time PCR reaction was performed with the use of Sensitive RT HS-PCR Mix SYBR Kit (A & A Biotechnology, Poland) on a QuantStudio 3 (Applied Biosystems, USA). The relative expression of selected genes, presented as the fold change relative to the untreated control, as well as normalized to an endogenous reference gene, elongation factor 1-alpha (relative quantification RQ = 1), was calculated using the comparative Pfaffl method (Pfaffl, 2001 Nucleic Acids Res. 29: e45). The biochemical analyses: peroxiredoxins (PRDXs) activity, total antioxidant capacity (TAC), and glutathione S-transferase (GST) activity were also performed. Statistical analysis was done using analysis of variance in Prism 8 software (GraphPad Software Inc., USA).

RESULTS AND CONCLUSIONS. The invasive larvae of *A. simplex* s.s. showed lower mobility in the culture with PYR. The Real-time PCR analysis showed significant differences in mRNA expression of the selected ABC transporters after IVM and PYR treatment, while a similar picture of the antioxidant metabolism in response to both drugs. These results indicate a relationship between the expression of the studied genes, including oxidative metabolism genes, and the effectiveness of the tested drugs. Therefore, further research into parasitic nematodes' gene expression, including gene silencing, seems necessary to find new methods to prevent parasites' invasive larvae survival in the host organism.

Funding: This work was funded by the "Development Program of the University of Warmia and Mazury in Olsztyn" (POWR.03.05.00-00-Z310/17).

SESSIONE

ZOONOSI PARASSITARIE

Detection of *Toxocara* larvae in ostrich meat intended for “carpaccio” preparation

V CAGNIN[1], S PASQUALOTTO[1], S SGUBIN[1], E PORCELLATO[1], A ZOROASTER[1], C FALCARO[1], P DANESI[1], A MICHELUTTI[1]

[1]Istituto Zooprofilattico Sperimentale delle Venezie, Laboratory of Parasitology, Mycology and Medical Entomology, Legnaro (Padua), Italy.

Keywords: *Toxocara*, Ostrich, Muscle digestion

INTRODUCTION. The routine diagnostic activity of the IZSVe Parasitology Laboratory includes, among others, the enzymatic digestion of striated muscle samples from susceptible species, collected at the slaughterhouse, for the prevention of human trichinellosis (EC Regulation 1375/15, ISO 18743:2015). In 2019, more than 24,000 muscle samples were analyzed, from pigs (n=15,804), horses and wild boars (n=8,291) and ostriches (n=3). The aim of this study is the evaluation of the transmission risk of *Toxocara* spp. larvae detected by enzymatic digestion from ostrich meat.

MATERIALS AND METHODS. Three ostrich pectoral muscle samples (10 g each) were examined for the presence of *Trichinella* larvae by magnetic stirrer digestion. The method consists in the enzymatic digestion of meat pools, followed by filtration, sedimentation and observation of the digested material under a stereomicroscope for nematode larvae detection (Mayer-Scholl et al., 2017. J Vis Exp. 3: 55354). Nematode larvae were molecularly identified by PCR and amplicon sequencing of the 18S rRNA gene.

RESULTS AND CONCLUSIONS. Eight larvae of *Toxocara* spp. were isolated from two of the three ostrich samples. All larvae were alive and mobile. The 18S rRNA sequences identified *Toxocara cati* (99.87% similarity) and *T. canis* (99.75% similarity), when blasted in Genbank database. *Toxocara* nematodes are zoonotic agents. Infection in humans may occur asymptomatically or it may cause visceral or ocular migrating larva syndromes, or neurotoxocariasis syndromes. People can become infected by accidentally ingesting eggs from the environment (soil, water, or raw vegetables) or third-stage larvae, from raw or undercooked meat or organs of paratenic hosts. The ostriches of this study were farmed outdoors and may have become infected by ingesting eggs from the environment. This result is relevant for public health, since ostrich meat should have been utilized for raw preparation ("carpaccio"). The storage at minus 20 °C was observed to be an effective preventive measure for inactivating *Toxocara* larvae, as confirmed by the absence of larvae in samples re-tested after 2 months by enzymatic digestion.

This work was funded by Italian Ministry of Health (RC IZSVE 18/16).

Characterization of the bacterial flora in Swedish ticks using 16S amplicon sequencing

G GRANDI[1,2], G CHIAPPA[1,2,3], A OMAZIC[1], K ULLMAN[1], PE LINDGREN[4,5], A ALBIHN[1,2], P CHOKLIKITUMNUEY[2], E ÖSTLUND[1], R SÖDERLUND[1]

[1]National Veterinary Institute (SVA), Uppsala, Sweden; [2]Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden; [3]University of Milan, Lodi, Italy; [4]Linköping University, Linköping, Sweden; [5]Department of Clinical Microbiology, Region Jönköping County, Jönköping, Sweden.

Keywords: Ticks, Microbiome, Endosymbionts, 16S

INTRODUCTION. Studying tick microbiome offers the possibility to better understand tick parasitism, as well as to identify potential tools for an innovative approach to tick control. The present study was aimed at characterizing the bacterial microbiome in ticks from northern Sweden.

MATERIALS AND METHODS. Ticks collected from their hosts (mainly domestic animals or humans) in north of river Dalälvenin 2018 were separately washed and morphological identification of tick species was performed before homogenisation and extraction of total nucleic acids. A microfluidic real-time PCR was used for screening of individual samples for tick-borne pathogens. Subsequently, 16S ribosomal RNA community profiling was performed according to the Illumina standard protocol on a MiSeq instrument generating >125000 2x300 bp read pairs from each of 63 samples.

RESULTS AND DISCUSSION. A total of 727 operational taxonomic units (OTUs) occurred and principal coordinate plots revealed that for the most part microbiomes clustered by tick species, but there was no consistent difference between *Ixodes ricinus* ticks from cats and dogs. Likely endosymbiont 16S sequences were present in all samples, with *Midichloria* especially prevalent in *I. ricinus* samples and *Lariskella* in most *Ixodes persulcatus*. Potential endosymbionts from the *Rickettsiella* genus were detected in two *I. ricinus* samples. Compared to real-time PCR detection data for the same pathogens in the analysed samples there was good agreement but as expected 16S sequencing was less sensitive. *Pseudomonas* were relatively prevalent in all tick species, with *Pseudomonas fragi*, *Pseudomonas putida* and *Pseudomonas fluorescens* the most common species. Other recurring findings were *Enterobacteriaceae* including *Serratia* and *Enterobacter*, *Staphylococcus, Sphingobacterium* and *Herbaspirillum*, with the latter two more common in *I. persulcatus* or ticks positive for both *I. ricinus* and *I. persulcatus* markers by PCR. The presented data confirms previous results obtained from the same tick species in other geographical areas and adds new data to a growing knowledge about the association between the ticks' microbiome, pathogens and hosts.

Source of funding: Swedish Research Council, TICKBIOCON, grant nr. 2018-03830

Modified BG sentinel trap as an alternative to CDC for Flavivirus surveillance in Italy

S MANZI[1], L NELLI[2], A MICHELUTTI[3], M BERTOLA[3], F TONIOLO[3], F GRADONI[3], F LISTA[5], M PAZIENZA[6], F SEVERINI[4], C FORTUNA[4], F MONTARSI[3], M POMBI[1]

[1]Sapienza Università di Roma, Dipartimento di Sanità Pubblica e Malattie Infettive, Roma, Italy; [2]University of Glasgow, Institute of Biodiversity, Animal Health and Comparative Medicine, Glasgow, UK; [3]Istituto Zooprofilattico Sperimentale delle Venezie, Laboratorio di Parassitologia, Legnaro, Italy; [4]Istituto Superiore di Sanità, Dipartimento di Malattie Infettive, Rome, Italy; [5]Policlinico Militare di Roma, Dipartimento Scientifico, Roma, Italy; [6]Stato Maggiore Difesa, Distaccamento Ponte Galeria, Roma, Italy.

Keywords: Arbovirus, Sampling, Epidemiology, Vector-borne disease

INTRODUCTION. Early detection of pathogens in arthropod vectors is highly important in prevention of vector-borne diseases and has the potential of providing the timely indicator of pathogens circulation before reaching humans. Here we propose a BG Sentinel trapping method based on the maintenance of mosquitoes alive through a sugar delivery system that avoids the need of cool chain for their preservation. This approach might be particularly useful in arbovirus surveillance and can be a possible alternative to CDC traps used for WNV surveillance in Italy.

MATERIALS AND METHODS. BG-sentinel trap baited with BG-lure and CO_2, modified to collect mosquitoes in a chamber containing a honey-based solution as a feeding source (Timmins et al., 2018 J Med Entomol. 55: 1638-41), was compared to CDC-CO_2 trap in 10 sites of Veneto region characterized by both high vector densities and viral circulation of WNV and USUV. Overall, 4 captures per site have been carried out on alternate weeks from July to August 2019. BG traps worked for two consecutive days while CDC were active for one day. Mosquitoes were identified and RNA was extracted from pools and screened for the presence of Flavivirus using a one-step SYBR Green-Based rRT-PCR and sequencing (Scaramozzino et al., 2001 J Clin Microb. 39: 1922–27). Data were statistically analysed through GLM models.

RESULTS AND CONCLUSIONS. In total, 39,313 mosquitoes were collected. The BG caught higher species diversity (Shannon H, BG:0.98 CDC:0.40) with better identification rates (BG:0.02%, CDC:3.2%) but with lower 1-day median trapped mosquitoes (BG:169, CDC:267), comparable only at low density. Median abundances of *Culex pipiens* were higher for CDC (BG:96, CDC:189) but no differences were observed at 2-days sampling. Equal or greater abundance of secondary vectors was observed in BGat1-day sampling (*Ochlerotatus caspius* BG:8.5, CDC:10.5; *Aedes albopictus* BG:7.5, CDC:1). Flavivirus prevalences were equivalent at 2-days sampling (WNV, BG: 0.027% CDC: 0.03%; USUV, BG: 0.054%, CDC: 0.06%). In conclusion, this modified BG shows useful performance in arbovirus surveillance: i) working for consecutive days without the need of cool chain; ii) collecting a higher number of species -and potentially viruses- than CDC ensuring an increase in species identification.

The zoonotic nematode *Anisakis pegreffii* releases extracellular vesicles: microscopic and proteomic characterization

M PALOMBA[1,2], A RUGHETTI[3], ME SCHININA[4], H RAHIMI[3], L MASUELLI[3], G MIGNOGNA[4], A GIORGI[4], T CASTRIGNANO[5], M SANTORO[2], S GABRIELLI[1], S MATTIUCCI[1]

[1]Dpt. Public Health and Infectious Diseases, Sapienza-University of Rome [2]Dpt. of Integrative Marine Ecology, Anton Dohrn; [3]Dpt. Experimental Medicine, Sapienza-University ofRome; [4]Dpt. of Biochemical Sciences, Sapienza-University of Rome; [5]Dpt of Ecological and Biological Sciences, Tuscia University.

Keywords: *Anisakis pegreffii*, Extracellular vesicles, TEM, Proteomics

INTRODUCTION. Nematodes of genus *Anisakis* have developed strategies to adapt to their hosts, survive, and counteract the host-immune response (Mattiucci et al., 2018 Adv Parasitol 99:93-226). The biological mechanisms and molecular signals involved in the differential hosts' response to the zoonotic species *A. pegreffii* in both natural and accidental (human) hosts, still remain largely unknown. Extracellular vesicles (EVs) play an important role as conveyors of molecular and biological signals to host cell and tissues. Aim of the study was to characterize morphologically and the protein content of EVs released by third stage larvae of *A. pegreffii*.

MATERIALS AND METHODS. LiveL3 of *A. pegreffii*, removed from fish, were cultured for 24h in PBS (37°C, 5% CO_2). EVs were isolated by serial centrifugation and ultracentrifugation of culture media. *Anisakis*EVs were characterized for size and morphology by Transmission Electron Microscopy (TEM) and Nanoparticle Tracking Analysis (NTA). Protein content was determined on isolated EVs by shot-gun proteomics (3 technical replicates), with protein identification searched against a robust de-novo transcriptome assembly of *A. pegreffii*. Functional annotation was achieved by using the function blastx and blastp of Diamond algorithm (Buchfink et al., Nat Meth12:59–60) against three databases (Nr, SwissProt and Trembl) for homology annotation and by performing a domain search with the Interproscan38 tool (Mulder & Apweiler, 2007 Met Mol Biol 396:59-70) to detect conserved domain signatures.

RESULTS AND CONCLUSIONS. A predominance of EVs of 80-140 nm in diameter and a concentration of 1.50-3.60 particles/ml, was found. A high percentage of proteins with catalytic and/or binding activity and involved in metabolic and cellular processes was found in the EVs proteome. Among the others, *Anis 13* and *Anis 1*, target antigens of the human IgE response (Mattiucci et al., 2017 Parasit Immunol *39*: e12440), were identified. Heat shock proteins (HSPs) and C-type lectins as galectin, that are involved in host immune activation and pathogen recognition, were also detected. Results suggest that the released EVs may deliver antigenic and immunomodulatory cargo to the host tissue microenvironment.

Research granted by: Ist. Pasteur Italy - Fond.Cenci-Bolognetti (2018) and Ministry of Public Health RF 2018.

First evidence of pyrethroid resistance in West Nile Virus vector, *Culex pipiens*, across Italy

V PICHLER[1], C GIAMMARIOLI[1], R BELLINI[2], R VERONESI[2], D ARNOLDI[3], A RIZZOLI[3], RP LIA[4], D OTRANTO[4], M BALLARDINI[5], P COBRE[1], P SERINI[1], A DELLA TORRE[1], B CAPUTO[1]

[1]Dipartimento di Sanità Pubblica e Malattie Infettive, Università Sapienza, Rome, Italy; [2]Centro Agricoltura Ambiente "G. Nicoli", Crevalcore, Italy; [3]Department of Biodiversity and Molecular Ecology, Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Trento, Italy; [4]Dipartimento di Medicina Veterinaria, Università di Bari, Valenzano, Italia; [5]Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy.

Keywords: Insecticide-resistance, *Culex pipiens*, Mosquitoes

INTRODUCTION. *Culex pipiens* is the major native species in urban areas in Italy and the vector responsible of transmission of several cases of West Nile virus to human each year. Insecticide ground spraying is the only tool for interrupting virus transmission, with pyrethroids being the only compounds authorized for adult mosquito control in Europe. Evidence of phenotypic resistance to pyrethroids was anyway reported in *Cx pipiens* populations from Greece and Spain, possibly limiting the effectiveness of these control tools.

MATERIALS AND METHODS. We carried out WHO-bioassays to assess the phenotypic susceptibility to permethrin (0.75%) in adult specimens from 10 *Cx pipiens* populations collected in 6 Italian regions (Trentino, Piemonte, Liguria, Emilia Romagna, Lazio e Puglia). In addition we investigated the possible mechanism underlying the resistance phenotype by genotyping a subset of the tested specimens for the presence of mutation L1014F in the voltage-sensitive sodium channel gene, strongly associated with pyrethroid resistance.

RESULTS AND CONCLUSIONS. Results provide first evidence of widespread resistance to permethrin in *Cx pipiens* populations across Italy with lowest mortality observed for 2 populations from Ferrara province (Emilia Romagna; mortality: 16.2% and 14%, respectively), Bari (Puglia; 27.1%) and Anzio (Lazio, 53.9%). For all other populations mortality ranged between 80 and 98%, indicating possible resistance, with exception of the population from Frosinone province (Lazio), resulting completely susceptible (mortality >98%). Genotyping of 121 specimens exposed to permethrin in the biossays allowed to detect the presence of the mutated allele 1014F in all regions and suggested a clear association with the permethrin resistance phenotype (chi-square:32.77; df: 1; p-value <0.0001). Overall the results obtained represent an alarm on the status of pyrethroid resistance of Italian *Cx pipiens* populations and urge the implementation of insecticide resistance management practices in order to limit, or possibly revert, this phenomenon which risks to strongly affect the possibility to intervene in case of West Nile virus transmission.

The drug's efficacy on zoonotic nematodes of Family Anisakidae - new data about metabolic status

I POLAK[1], R STRYIŃSKI[1], M PODOLSKA[2], J PAWLAK[2], MW BITTNER[1], T BORKOWSKI[1], E ŁOPIEŃSKA-BIERNAT[1]

[1]Department of Biochemistry, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland; [2]National Marine Fisheries Research Institute, Gdynia, Poland.

Keywords: Parasitic nematodes, Pyrantel, Oxidative stress, Drug's receptors

INTRODUCTION. In the family Anisakidae there are marine nematodes, most of which are paratenic hosts for important commercial fish species. Both, public health risks and socio-economic problems are attributed to these parasites. Despite these concerns, the knowledge of their metabolism remains unknown. Therefore, the main objective of this study was to investigate the metabolic status of two Anisakidae species, *Pseudoterranova decipiens* and *Contracaecum osculatum*, under the influence of anthelminthic drugs, ivermectin (IVM) and pyrantel (PYR). The mRNA expression of GABA and acetylcholine receptors, ABC transporters, antioxidative enzymes, as well oxidative status were determined. To our knowledge, this is the first time that the IVM and PYR have been tested against these parasitic nematodes.

MATERIALS AND METHODS. The L3 larvae of *P. decipiens* and *C. osculatum* from Atlantic cod (*Gadus morhua*) and Baltic cod (*Gadus morhuacallarias*), respectively, were cultured *in vitro* with IVM or PYR at different concentrations: 1.56, 3.125 and 6.25 µg per mL of culture medium for 3, 6, 9, 12 and 72 hrs. The relative expression of selected genes, presented as the fold change relative to the untreated control, as well as normalized to an endogenous reference gene, elongation factor 1-alpha (relative quantification RQ = 1), was calculated using the comparative Pfaffl method (Pfaffl, 2001 Nucleic Acids Res. 29: e45). The biochemical analyses: peroxiredoxins (PRDXs) activity, total antioxidant capacity (TAC), and glutathione S-transferase (GST) activity were also performed. The data were expressed as means ± standard deviations. Statistical analysis was performed using ANOVA in Prism 8 software (GraphPad Software Inc., USA).

RESULTS AND CONCLUSIONS. There were significant differences in metabolism status in response to drugs between those species. The *P. decipiens* has a longer lifespan than *C. osculatum* after drug treatment, 72 hours, and 12 hours, respectively. The IVM caused a stronger oxidative response in *C. osculatum* than in *P. decipens*. The mRNA expression of selected ABC transporters was higher in *P. decipens* than *C. osculatum* in response to tested drugs. With environmental and geographic variation, the response of the metabolism of both nematode species to anthelminthic drugs is different and important for further targeted research.

SESSIONE

PARASSITOLOGIA VETERINARIA E UMANA

Fresh produce contaminated by pathogenic parasites: what is new in Italy?

A BARLAAM[1,2], **TT TEMESGEN**[3], **KR TYSNES**[3], **L RINALDI**[4], **N FERRARI**[5], **AR SANNELLA**[6], **G NORMANNO**[1], **SM CACCIÒ**[6], **LJ ROBERTSON**[3], **A GIANGASPERO**[1]

[1]Department of Agriculture, Food, Natural Resources and Engineering, University of Foggia, Foggia, Italy; [2]Faculty of Veterinary Medicine, University of Teramo, Italy; [3]Department of Paraclinical Sciences, Norwegian University of Life Sciences, Oslo, Norway; [4]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy; [5]Department of Veterinary Medicine, University of Milan, Milan, Italy; [6]Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy.

Keywords: Prevalence, Pathogenic parasites, Fresh produce, Italy

INTRODUCTION. The consumption of ready-to-eat (RTE) salads and berries has significantly increased in industrialized countries; these products can be contaminated by pathogenic parasites that have been responsible for foodborne outbreaks worldwide (Trevisan et al., 2019 Trends Parasitol. 35: 695-703). In Italy, while very few data are available on RTE salads, there are no data on berries.

MATERIALS AND METHODS. Throughout 2019, 648 packages of berries (blueberries from Peru, blackberries from Mexico, raspberries from Italy) and three brands of local RTE mixed salads were bought from supermarkets. To estimate the prevalence (desired precision of 1.15% and interval of confidence of 95%) for each fresh produce, a pool size of nine packages each month and 72 pools per one year were processed. After washing (Murphyet al., 2017BAM Chapter 19b U.S. FDA), the pellets were examined by microscopy (FLOTAC) and tested using conventional PCR (Ryan et al., 2003 J Parasitol. 89: 809-13; Lalle et al., 2005 J Parasitol. 91: 203-205; Santos et al., 2010 Parasitol Res. 106: 883-88), sequencing and simplex or multiplex *q*PCR (Temesgen et al., 2019 Front Microbiol. 10: 1939; Temesgen et al., 2019 Food Res Int. 125: 108636).

RESULTS AND CONCLUSIONS. By microscopy, a *Cyclospora*-like oocyst and an *Entamoeba*-like cyst in imported blueberries were detected, *Giardia*-like cysts in local raspberries, and a taeniid egg in a RTE salad sample. *Cyclosporacayetanensis*, *Entamoeba histolytica*and the taeniid (*Echinococcus multilocularis*)were molecularly confirmed with prevalence of 0.15% (95% C.I. 0.003-0.862), 0.96% (95% C.I. 0.351-2.083) and 0.15% (95% C.I. 0.003-0.862), respectively.*Giardia duodenalis* (Assemblages A, B and E) and *Cryptosporidium*(*Cryptosporidium ryanae*, *Cryptosporidium bovis*, *Cryptosporidium xiaoi*, *Cryptosporidium ubiquitum*) were molecularly identified in both matrices with prevalences of 4.6% (95% C.I. 2.986-6.784) and 5.81% (95% C.I. 3.909-8.242), respectively. *Cryptosporidium* and *Giardia* showed seasonal differences, with higher values in winter and spring ($p<0.044$ and $p<0.012$), respectively. These results indicate a new epidemiological scenario and highlight that inadequate management of fresh produce, both locally produced and imported, along the food chain may have potential consequences on human health.

Feeding behaviour of *Anopheles messeae/daciae* and *Anopheles maculipennis* s.s. in North-eastern Italy

M BERTOLA[1], F GRADONI[1], A MICHELUTTI[1], S SGUBIN[1], S MANZI[2], M POMBI[2], F MONTARSI[1,2]

[1]Laboratory of Parasitology, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy; [2]Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy.

Keywords: Maculipennis complex, Bloodmeal, Host preference, Malaria vector

INTRODUCTION. Since the malaria eradication, the species composition of the *Anopheles maculipennis* complex in North-eastern Italy has changed considerably and, to date, the two main species are represented by *Anopheles messeae/daciae* and *Anopheles maculipennis s.s.* (Calzolari et al., 2021 Sci Rep. 11: 6421). The aim of this study was to investigate the feeding choices of these two species.

MATERIALS AND METHODS. Mosquitoes belonging to Maculipennis complex were collected in 19 sites in Veneto region (2018-2020) by hand aspiration and trapping. All specimens were morphologically identified and a sub-sample of engorged *An. maculipennis s.l.* females was identified at species level by molecular analysis. Blood meal origin was identified by PCR and *cox1* gene sequencing. Feeding choice of *An. messeae/daciae* was investigated in a site rich in animal diversity by calculating the forage ratio (FR) and feeding index (FI).

RESULTS AND CONCLUSION. A total of 1,094 engorged *An. maculipennis s.l.* were collected, 267 of which were identified at species level (242 *An. messeae/daciae*, 25 *An. maculipennis s.s.*). For 127 specimens (115 *An. messeae/daciae* and 12 *An. maculipennis s.s.*) it was possible to identify the origin of the blood meal, from 13 and 3 different host species respectively. Both *Anopheles* species fed mainly on horse (45.2% *An. messeae/daciae* and 33.3% *An. maculipennis s.s.*) and cow (30.4% *An. messeae/daciae* and 58.3% *An. maculipennis s.s*). Despite these proportions, *An. messeae/daciae* FR indicates host preference for buffalo (21.2), horse (8.4), man and pig (5.3); the pairwise-comparison of human FI with each host reveals its preference for humans compared to chicken (79.5), rabbit (7.3) and dog (8.0). Our results show that, due to its opportunistic feeding behavior, *An. messeae/daciae* shows its potential role as a vector of human and zoonotic diseases in Italy.

Genome investigations on neglected representatives provide novel perspectives on *Rickettsiales* evolution

M CASTELLI[1], T NARDI[1], G PETRONI[2], D SASSERA[1]

[1]Department of Biology and Biotechnology, University of Pavia, Italy; [2]Department of Biology, University of Pisa, Italy.

Keywords: *Rickettsiales*, Evolution, Vector-borne bacteria, Genomics

INTRODUCTION. *Rickettsiales* are a lineage of intracellular bacteria, best known for encompassing vector-borne human and animal pathogens, such as *Rickettsia*, *Anaplasma*, and *Ehrlichia*, as well as *Wolbachia*, a symbiont showing complex interactions with arthropods and filarial nematodes. It has recently become evident that these members share highly interwoven evolutionary relationships with a much wider number of bacteria hosted by a variety of unicellular or multicellular eukaryotes from aquatic environments. In-depth investigations on the features and role in the host biology of such "aquatic" bacteria are important for a better understanding of the evolution of the whole *Rickettsiales* lineage, including the mechanisms at the basis of the recurrent emergence of pathogens.

MATERIALS AND METHODS. In this study, multiple samples of free-living aquatic protozoa hosting *Rickettsiales* bacteria were shotgun sequenced, and dedicated bioinformatic procedures were applied to selectively assemble the *Rickettsiales* genomes. Subsequently, in-depth phylogenetic and comparative genomic analyses were applied, in order to elucidate the evolutionary relationships within *Rickettsiales*, as well as aiming to reconstruct their ancestral functional and metabolic repertoire and the variations in different sub-lineages.

RESULTS AND CONCLUSIONS. The complete genome sequences of12 novel *Rickettsiales* species were obtained. A much wider ancestral repertoire and flexibility than previously recognised could be inferred, both concerning metabolism and host-interaction mechanisms. In particular, a bacterium living in a stable extracellular relationship with its host and showing distinctive metabolic features was discovered, questioning strict intracellular replication in ancestral *Rickettsiales*, intriguingly hinting for possible multiple independent adaptations of sub-lineages including pathogenic representatives. Moreover, the dataset allows deep investigations on horizontal gene transfer events, for which preliminary analyses indicate a fundamental role in the acquisition of key novel functions.

Aspergillus fumigatus pneumonia and oxalate nephropathy in a foal

J HATTAB[1], A VULCANO[2], S D'AREZZO[2], F VERNI[3], PG TISCAR[1], G LANTERI[4], E GJURCEVIC[5], U TOSI[1], G MARRUCHELLA[1]

[1]Faculty of Veterinary Medicine, University of Teramo, Italy; [2]Istituto Nazionale Malattie Infettive "Lazzaro Spallanzani", Laboratorio di Microbiologia Banca Biologica e Cell Factory, Rome, Italy; [3]Veterinary Practitioner, Mosciano Sant'Angelo, Italy; [4]Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche e Ambientali, University of Messina, Italy; [5]Faculty of Veterinary Medicine, University of Zagreb, Croatia.

Keywords: Horse, *Aspergillus fumigatus*, Pneumonia, Oxalate nephropathy

INTRODUCTION. *Aspergillus* sp. are filamentous fungi, which thrive in soil as saprophytes and occasionally act as opportunistic pathogens. Equine aspergillosis is an uncommon, often severe respiratory disease, which can affect the sinonasal cavities, the guttural pouches or the lung parenchyma (Cafarchia et al., 2013 Vet Microbiol. 167: 215-34). We describe herein the peculiar pathological features observed in a horse affected by pulmonary aspergillosis.

MATERIALS AND METHODS. A 10-month-old Quarter Horse foal suddenly got prostrate showing fever, dyspnea, tachycardia, and profuse diarrhea. Despite antimicrobial and fluid therapy, the clinical picture further worsened, the animal spontaneously died and was then necropsied under field condition. Tissue samples were routinely processed for histopathological investigation.

RESULTS AND CONCLUSIONS. At necropsy, disseminated nodules (2-5 mm in size) were detected throughout the lung parenchyma. Microscopically, such nodules appeared as necrotic-hemorrhagic lesion, densely infiltrated with fungal hyphae. Biomolecular investigations carried out on paraffin-embedded tissue yielded the identification of *Aspergillus fumigatus*. The lumen of the large intestine was filled with abundant watery content, while the mucosa appeared necrotic. Remarkably, a severe and diffuse nephropathy was observed, large amount of oxalate crystals filling the renal tubules, with no evidence of nephritis and/or renal fibrosis. Under polarized light, such crystal showed their typical birefringence. According to literature, this case of pulmonary aspergillosis likely occurred as a sequela of acute enteritis, after the hematogenous spreading of fungi (Seyedmousavi et al., 2015 Med Mycol. 53: 765-97). Interestingly, oxalate crystals have been reported in sites of *Aspergillus* infection both in humans and animals (Payne et al., 2017 J Vet Diagn Invest. 29: 51-8), but never in "ectopic" sites and in such a large amount. In our opinion, the timing of lesions argues in favor of a direct link between mycotic pneumonia and oxalate nephritis. The absence of any disease condition in horses living in the same stable makes unlikely alternative etiological hypothesis.

Comparative genomics and phylogeny of *Coxiella* symbionts of ticks, and their relation to *Coxiella burnetii*: acquired or ancestral pathogenicity?

T NARDI[1], E OLIVIERI[1], E KARIUKI[2], D SASSERA[1], M CASTELLI[1]

[1]Department of Biology and Biotechnology, University of Pavia, Italy; [2]Veterinary and Capture Service Department, Kenya Wildlife Service, Kenya.

Keywords: Ticks, Vector-borne pathogens, Pathogen evolution, Symbiosis

INTRODUCTION. Ticks require bacterial symbionts for the provision of compounds absent in their hematophagous diet. Such symbionts are frequently vertically transmitted and often are strongly related to pathogens, for example *Francisella tularensis* or *Rickettsia* spp.. The most common symbionts belong to the *Coxiella* genus, which also includes the human pathogen *Coxiella burnetii.*

The current knowledge on the evolution of *Coxiella* is hampered by the limited genomic resources available, leaving open fundamental questions such as: was the ancestor of *Coxiella* a pathogen or a symbiont? How did this genus evolve in terms of interaction with ticks and mammalian hosts?

In order to try to shed light on this topic, we sequenced a *Coxiella symbiont* (CE) from a previously unstudied clade and used it for comparative genomics and phylogenetics.

MATERIALS AND METHODS. We used Illumina technology to sequence DNA extract from a whole *Amblyomma nuttalli* tick from Kenya. We applied bioinformatic analyses to extract and assemble the genome of the *Coxiella* endosymbiont from the tick sequences. We used the genome for phylogenetics and genomics analysis comparing it to the available genomes of *Coxiella* endosymbionts and *C. burnetii.*

RESULTS AND CONCLUSIONS. Our data allow to infer an evolutionary scenario of parallel genome reduction among *Coxiella* endosymbionts, with similar constraints, leading to selective retention of biosynthetic pathways beneficial for the host. The combined predicted functional capabilities of the symbionts appear to be a subset of those of *C. burnetii*. Accordingly, this pathogen could be closer to the ancestral state of the genus, rather than being derived from an endosymbiotic ancestor, as previously hypothesized.

Entomological surveillance in former malaria-endemic areas of Southern Italy

I VASCO[1], F SEVERINI[2], D BOCCOLINI[2], M MENEGON[2], L TOMA[2], DA RAELE[1], P MICCOLIS[3], E FRANCO[3], F DESIANTE[3], V NOLA[4], P SALERNO[4], MA CAFIERO[1], M DI LUCA[2]

[1]Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata; [2]Istituto Superiore di Sanità, Dipartimento Malattie Infettive, Reparto Malattie Trasmesse da Vettori, Roma; [3]Dipartimento di Prevenzione, Azienda Sanitaria Locale Taranto; [4]Dipartimento di Prevenzione, Sanità e Benessere Animale, Azienda Sanitaria Locale Matera.

Keywords: Malaria, *Anopheles*, Puglia, Basilicata

INTRODUCTION. Malaria still represents a potential public health issue in Italy since the presence of former *Anopheles* vectors and the increasing number of yearly imported human cases. In areas no longer endemic, the concurrent presence of gametocyte carriers and competent vectors makes reemergence of local transmission possible, as recently reported in Greece. Due to the occurrence of 4 cases of suspected indigenous malaria in October 2017 in Taranto province (Apulia region), entomological investigations were performed by ISS, IZSPB and ASL of Taranto, to verify the possible involvement of local mosquitoes. Afterwards, in 2019-2020 entomological surveillance was implemented, funded by the IZSPB 1/18RC Project.

MATERIALS AND METHODS. Entomological surveys were carried out in 2017 and 2019-2020 in the areas where the four cases occurred and in other nearby sites, between the provinces of Matera and Taranto. Furthermore, in 2020 the areas around the Lesina and Salso Lakes (Foggia province) were also investigated. Resting mosquito females were collected in animal shelters and human dwellings by using battery powered aspirators and CDC light-traps and BG-sentinel traps. Larvae were sampled using a standard 500mL enamel dipper in natural or artificial breeding sites. Mosquitoes were both morphologically and molecularly identified, when needed.

RESULTS AND CONCLUSIONS. A total of 2,227 mosquitoes of 19 species belonging to 7 genera were collected; 1,208 (54.2%) of these were anophelines, identified as *Anopheles labranchiae*, *Anopheles algeriensis*, *Anopheles superpictus* and *Anopheles plumbeus*. In all investigated areas, *An. labranchiae* was the most abundant species, *An. algeriensis* was predominant in the Gargano sites, *An. superpictus* was recorded only in 2017 in Matera province, while *An. plumbeus* was found in a wooded area of Salandra locality (Matera province). Our findings show the high receptivity of the investigated areas, where the former malaria vectors, *An. labranchiae* and *An. superpictus* occur at different densities related to the kind of environment, climatic parameters, and anthropic activities.

POSTER

POSTER ESDA

DIROFILARIOSIS AND ANGIOSTRONGYLOSIS

Cardiopulmonary evaluations in *Dirofilaria immitis* naturally infected cats

B ALBERIGI[1], **A BENDAS**[1], **D SOARES**[2], **N LABARTHE**[3]

[1]Instituto de Veterinária, Departamento de Medicina e Cirurgia Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, Brazil; [2]Instituto de Especialidades em Medicina Veterinária, Diagnostic imaging service, Rio de Janeiro, Brazil; [3]Programa de Pós-Graduação Bioética, Ética e Saúde Coletiva, Escola Nacional de Saúde Pública, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil.

Keywords: Heartworm, ProBNP, Echodopplercardiogram, Radiography

INTRODUCTION. Cats are less susceptible to *Dirofilaria immitis* infections than dogs, and mostly infected cats are asymptomatic or present non-specific signs (Litster et al., 2008 J Feline Med Surg. 10: 137-44). The aim of this work was to describe thoracic radiographs, ecodopplercardiogram (ECO) and the proBNP level of *D. immitis* antigen seropositive cats.

MATERIALS AND METHODS. Seven *D. immitis* antigen positive cats (Feline Triple®, IDEXX) presenting no clinical signs of infection were submitted to thoracic radiographs, ECO and proBNP testing (SNAP Feline proBNP®, IDEXX) in blood samples.

RESULTS AND CONCLUSIONS. The included cats age ranged from 2-12 years old (x=5), including three males one intact and five females one intact. X-rays showed that all cats presented bronchial and interstitial pattern simultaneously. Two of them presented alveolar pattern and one of these two also presented micronodular pattern. Another cat presented hyperinflated lungs. Main pulmonary artery (MPA) enlargement was noticed in two cats and one also presented caudal pulmonary artery (CPA) enlargement. Another cat presented CPA enlargement without abnormalities on MPA area. Evaluation of CPA was impaired for one cat because alveolar pattern overlapped its topography. Heart x-rays revealed right atrioventricular enlargement in five cats with no left side enlargement. Only five cats were examined with ECO. None of them presented signs of cardiomyopathy (CM). Worms could be visualized in one cat only, which was also the only one presenting pulmonary and tricuspid insufficiency. All cats presented asymmetric pulmonic flow. The mean results of other right ventricle parameter analyzed are presented as following: right pulmonary artery distensibility index: $\bar{x}$=37,2%±4,7; pulmonary artery and aorta ratio: $\bar{x}$= 0.69±0.38; and tricuspid annular plane systolic excursion (TAPSE) $\bar{x}$= 7,4 ±1.8. TAPSE results are similar to those of subclinical CM cats reported before (Spalla et al., 2018 J Vet Cardiol. 20: 154-64) suggesting that these cats could present reduced TAPSE due to HWD. Levels of proBNPwere within the normal range for six cats and elevated in one, suggesting that although lung disease was evident, heart lesions were incipient. The results suggest that feline *D. immitis* infection can cause lesions that are only detected by specific exams and that ECO of HTW infected cats needs to be further studied.

Canine heartworm in a cardio-respiratory unit routine

B ALBERIGI[1], **N LEMOS**[2], **M ALBUQUERQUE**[1], **N LABARTHE**[3]

[1]Instituto de Veterinária, Departamento de Medicina e Cirurgia Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, Brazil; [2]Programa de Pós-graduação em Medicina Veterinária, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, Brazil; [3]Programa de Pós-Graduação Bioética, Ética e Saúde Coletiva, Escola Nacional de Saúde Pública, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil.

Keywords: Heartworm, Ecodopplercardiogram, Clinical sign

INTRODUCTION. Canine infected with *Dirofilaria immitis* (HTW) are frequently presented with coughing, dyspnea, abnormal lung and heart sounds and, exercise intolerance (ESDA Canine Heartworm Guideline, 2017). Since those clinical signs are unspecific, the aim of this work was to compare the severity of the clinical signs among HTW infected and HTW free dogs presented at the cardio-respiratory unit of the Veterinary Hospital of the Universidade Federal Rural do Rio de Janeiro, Brazil.

MATERIALS AND METHODS. After the owner's formal consent, by convenience sampling, dogs were submitted to clinical examination, HTW antigen (Snap 4DX plus, Idexx®) and microfilariae (Newton et al., 1956 J Parasitol. 42: 246-58) testing, ecodopplercardiogram (ECO), and thoracic radiograph.

RESULTS AND CONCLUSIONS. A total of 26 dogs was included. Most of them presented cough (65.4%) and abnormal lung sounds (81%). Eleven of the dogs were HTW infected (42.3 %). Murmur at the tricuspid area was present in seven dogs (26.9%; 7/26), 57.1% infected (4/7). The ECO showed tricuspid insufficiency in 30.8% of the dogs (8/26), 37.5% being infected (3/8); and pulmonary insufficiency in 46.1% (12/26), (50% infected (6/12)). Worms were detected by ECO in 45.5% of the infected dogs. The x-rays showed that 88.5% of the dogs presented altered pulmonary pattern (23/26). The bronchial pattern was similar in both sets of dogs (45.5% of the infected and 46.7% of the free of infection). The interstitial pattern was present in 18.2% of the infected (2/11) and in 6.7% (1/15) of the uninfected. High pulmonary pressure was indicated by scarce parameters although pulmonary hypertension could not be confirmed. These results suggest that HTW infection could not be either included or excluded from the differential diagnosis by the clinical presentation, radiographic exam or ECO unless the worms could be identified. Therefore parasitological and serological testing cannot be discarded.

Efficacy of Simparica Trio™ against L5 stages of *Angiostrongylus vasorum* in experimentally infected dogs

C BECSKEI[1], **P DOHERTY**[2], **SP MAHABIR**[3]

[1]Veterinary Medicine Research and Development, Zoetis, Mercuriusstraat 20, Zaventem, 1930, Belgium; [2]Pre-Clinical Services, Charles River Laboratories, Glenamoy Co. Mayo, Ireland; [3]Veterinary Medicine Research and Development, Zoetis, 333 Portage Street, Kalamazoo, MI 49007, USA.

Keywords: Moxidectin, Oral chewable, French heartworm, Prophylaxis

INTRODUCTION. Dogs living in endemic regions require regular treatment against the larval stages of *A. vasorum* to prevent the development of angiostrongylosis.

MATERIALS AND METHODS. Three placebo-controlled, randomized, masked studies were conducted in experimentally infected dogs to demonstrate the efficacy of Simparica Trio™ (containing moxidectin, sarolaner and pyrantel) against the L5 stages of *A. vasorum*. In the first study three different dose levels of moxidectin (3, 12 and 24 µg/kg) were evaluated in the combination. Two follow-up studies were conducted to confirm the efficacy of the selected dose of 24 µg/kg moxidectin in Simparica Trio™. In all studies, dogs (n=8/group) were infected orally with approx. 200 third stage larvae of *A. vasorum* and were treated 28 days later with the combination of moxidectin, sarolaner and pyrantel or placebo. Animals were humanely euthanised once patency was reached in the placebo group i.e. L1 was detected in the faeces. At necropsy the extent and severity of the lung damage were scored, and worms were recovered by reverse perfusion of the lungs and heart. Efficacy of the combination was calculated as the percent reduction in adult worm counts vs. placebo.

RESULTS AND CONCLUSIONS. A moxidectin dose of 24 µg/kg provided >90% efficacy against the L5 stages of *A. vasorum* following a single treatment administration, while efficacy was 7.2% and 54.5% when 3 and 12 µg/kg moxidectin was included in the combination product, respectively. In the two dose confirmation studies, Simparica Trio™ reduced adult worm counts by ≥92.9% vs. placebo (P=0.0002) and reduced L1 excretion by ≥98.7%. In the placebo-treated groups ≥45.3% of the total area of the lung lobes showed *A. vasorum*-induced macroscopical lesions, while in the Simparica Trio™-treated groups this was reduced to ≤3.1%. A single administration of Simparica Trio™ was highly efficacious against the L5 stages of *A. vasorum* and should thus provide effective prophylaxis of canine angiostrongylosis.

Efficacy of orally administered combination of moxidectin, sarolaner and pyrantel (Simparica Trio™) for the prevention of *Angiostrongylus vasorum* infection in dogs

C BECSKEI[1], JL WILLESEN[2], M SCHNYDER[3], N MIROSHNIKOVA[3], SP MAHABIR[4]

[1]Veterinary Medicine Research and Development, Zoetis, Mercuriusstraat 20, Zaventem, 1930, Belgium; [2]Department of Veterinary Clinical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, 16 Dyrlægevej, 1870 Frederiksberg C, Denmark; [3]Institute of Parasitology, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 266A, 8057 Zürich, Switzerland; [4]Veterinary Medicine Research and Development, Zoetis, 333 Portage Street, Kalamazoo, MI 49007, USA.

Keywords: Canine angiostrongylosis, French heartworm, Lungworm, Prophylaxis

INTRODUCTION. The efficacy of Simparica Trio™ (containing moxidectin, sarolaner and pyrantel) for the prevention of canine angiostrongylosis was investigated under natural infection pressure.

MATERIALS AND METHODS. A double-masked, placebo-controlled study was conducted in regional hot-spots of *A. vasorum* in Italy and Denmark where a concurrent survey study of 1628 dogs found 12.2% infection rate. Client-owned dogs at risk of *A. vasorum* infection received ten monthly oral treatments with Simparica Trio™ at the approved dose to deliver 24 to 48 µg/kg moxidectin, 1.2 to 2.4 mg/kg sarolaner and 5 to 10 mg/kg pyrantel (n=315) or placebo tablets (n=307). Monthly blood samples were tested for *A. vasorum* in the clinics using an antigen quick test and in a central laboratory using ELISA for antigen and specific antibody detection. If any one of these tests was positive, then three daily faecal samples were collected from the dog and were tested for first-stage larvae (L1) using the modified Baermann method. To verify a diagnosis of canine angiostrongylosis, the suspected dog had to be positive on the antibody test and also in at least one of the two antigen tests. Dogs with L1 in the faeces were also considered positive, independent of the serology results.

RESULTS AND CONCLUSIONS. Two of the 307 placebo-treated client-owned dogs developed adult *A. vasorum* infections during the ten-month study period, while none of the 315 Simparica Trio™-treated dogs became infected with *A. vasorum*. Oral administration of Simparica Trio™ provided 100% efficacy in the prevention of natural infection with *A. vasorum* in dogs in endemic areas.

Seroprevalence of *Angiostrongylus vasorum* in dogs in Serbia

LJ CURCIN[1], N MILOJKOVIĆ[2], M SCHNYDER[3], R SCHAPER[4], LJ SPASOJEVIC KOSIC[5], M ZEKIC STOSIC[6], S SAVIC[6]

[1]Veterinary Clinic "Intervet", Belgrade, Serbia; [2]Veterinary Clinic "Vet Centar", Belgrade Serbia; [3]Institute of Parasitology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland; [4]Elanco Animal Health, Germany; [5]Department for Veterinary Medicine, Faculty of Agriculture, University of Novi Sad, Novi Sad, Serbia; [6]Scientific Veterinary Institute "Novi Sad", Novi Sad, Serbia.

Keywords: *Angiostrongylus vasorum*, Seroprevalence, Dogs, ELISAs

INTRODUCTION. Angiostrongylosis in dogs is caused by the nematode *Angiostrongylus vasorum*. It was initially found in France and is often referred to as "the French Heartworm". The parasite occurs in most of the European countries. In Serbia, little is known about the presence in dogs, although there were single case reports (Simin et al., 2014 Parasit Vectors. 27: 396) and detection in wild canids (Gavrilović et al., 2017). The aim of this study was to investigate the seroprevalence of *A. vasorum* in dogs in Serbia.

MATERIALS AND METHODS. Between 2017 and 2021, 1095 dog blood samples were collected from patients presented in different veterinary clinics in Serbia and analyzed at the Institute for Parasitology in Zurich for the presence of circulating *A. vasorum* antigens and specific antibodies against the parasite, using monoclonal and polyclonal antibodies.

RESULTS AND CONCLUSIONS. Overall, 25 samples (2,3%) were positive for *A. vasorum* antigen and 41 (3,7%) were positive for *A. vasorum* antibodies. Of these tested samples, two dogs (0,2%) were positive in both tests (antigen and antibodies).

These preliminary results confirm that *A. vasorum* is present in Serbian dogs and therefore support earlier studies. Veterinarians and pet owners should be made aware of these findings and alerted to look for the symptoms indicating canine angiostrongylosis in dogs.

Serum acute phase proteins in dogs with heartworm disease (*Dirofilaria immitis*) before and after adulticide treatment

Y FALCÓN[1], JA MONTOYA-ALONSO[1], N COSTA[1], S MARTÍNEZ[2], L FRANCO[2], A CARO[3], SN GARCÍA RODRÍGUEZ[1], JI MATOS-RIVERO [1], E CARRETÓN[1]

[1]Internal Medicine, Faculty of Veterinary Medicine, Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain; [2]Interdisciplinary Laboratory of Clinical Analysis, Interlab-UMU, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, Espinardo, Murcia, Spain; [3]Department of Animal Medicine and Surgery, Veterinary Faculty, Complutense University of Madrid, Madrid, Spain.

Keywords: Pulmonary hypertension, Endarteritis, Biomarkers

INTRODUCTION. Pulmonary hypertension (PH) is one of the most serious complications in dogs with heartworm (HW). An acute phase response and the utility of acute phase proteins (APP) as early biomarkers of PH have been demonstrated in infected dogs (Venco et al., 2014 Vet Parasitol. 206: 48-54; Carretón et al., 2017 Parasit Vectors. 10: 477). Since PH seems to persist in dogs up to 10 months after the end of adulticide treatment (Falcón-Cordón et al., 2019 Vet Parasitol. 273: 1-4), the aim was to evaluate serum concentrations of positive APP [C-reactive protein (CRP), haptoglobin and ferritin] and negative APP [albumin and paraoxonase-1 (PON-1)] to assess their utility as diagnostic biomarkers of PH and its progression throughout adulticide treatment.

MATERIALS AND METHODS. 25 HW-infected dogs were subjected to adulticide treatment (Carretón et al., 2019 Vet Parasitol. 271: 54-56). On days 0, 90 (end of treatment) and 270 (6 months after completion), the presence/absence of PH was echocardiographically determined using the Right Pulmonary Artery Distensibility Index (RPAD Index), and serum concentrations of APP were determined.

RESULTS AND CONCLUSIONS. PH was present in 44%, 44% and 48% of the dogs on days 0, 90 and 270 respectively. On days 0 and 90 all APP but haptoglobin (day 0) and albumin (day 90) differed significantly from the reference values, while the alterations in Ferritin and PON-1 persisted on day 270. Only CRP showed significant different concentrations on day 0, 90 and 270 between dogs with/without PH, although on day 270 concentrations were within the reference values. There may still be a persistent damage at the vascular level 6 months after the patient is discharged, regardless of the presence or absence of PH, while the presence of PH could be a consequence in those dogs with more severe endarteritis. The results confirm that HW disease has a strong inflammatory component that seems to persist for at least 6 months after discharge, whether or not there is presence of PH.

Canine *Dirofilaria* infection – from species to disease in the last decade

S GIUBEGA[1], M IMRE[1], MS ILIE[1], K IMRE[2], I LUCA[1], T FLOREA[1], GH DARABUS[1], S MORARIU[1]

[1]Department of Parasitology and Dermatology, Banat`s University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, no. 119, Calea Aradului, 300645, Timisoara, Romania; [2]Department of Animal Production and Veterinary Public Health, Banat`s University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, no. 119, Calea Aradului, 300645, Timisoara, Romania.

Keywords: *Dirofilaria*, *Acanthocheilonema*, Dogs, Dirofilariosis

INTRODUCTION. *Dirofilaria* infections in dogs are recognized as widespread mosquito-borne diseases caused by the filarioid nematodes (*Onchocercidae*) species *Dirofilaria immitis* and *Dirofilaria repens*, with zoonotic potential. The encountered clinical manifestations can largely vary, from asymptomatic to severe, depending by the involved microfilariae species. The study was undertaken to provide valuable data on the occurrence of *Dirofilaria* infections in dogs during a long-term survey achieved in Western and South-Western Romania.

MATERIALS AND METHODS. Blood samples (~3 ml) of 1088 dogs, originating from 73 localities of 11 Western and South-Western counties of Romania, were collected in sterile vacutainer tubes containing EDTA, and assayed by the modified Knott test for the presence and differentiation of *Dirofilaria* spp. based on their morphological features. Subsequently, all of the microscopically positive samples were subjected to molecular analysis for confirmation.

RESULTS AND CONCLUSIONS. Altogether, the obtained data showed a prevalence value of 21.42% (233/1088) of canine dirofilariosis in the investigated region. Thus, the identified species in case of monoinfections were *D. immitis*, *D. repens* and *Acanthocheilonema reconditum* in 106 (9.74%), 102 (9.38%) and 1 (0.09%) sample, respectively. Twenty-four (2.21%) samples exhibited simultaneous presence of *D. immitis* and *D. repens*. No correlation was found ($p>0.05$) between the infection status and the breed of the dogs, but animals older than one-year, male gender, rural habitat and shelter dogs were positively associated ($p<0.05$) with the pathogen prevalence, and can be considered risk factors in acquiring infection. The results of the present investigation suggest the possible zoonotic risks for humans in the screened area, and consequently, it becomes imperative to increase the awareness of veterinarians, but also of physicians regarding the continuous spread of these zoonotic filariae.

Echocardiographic evaluation of pulmonary hypertension in dogs with heartworm disease using the pulmonary vein to pulmonary artery ratio

A MELIÁN HENRÍQUEZ, JI MATOS-RIVERO, JA MONTOYA-ALONSO, SN GARCÍA RODRÍGUEZ, N COSTA-RODRÍGUEZ, E CARRETÓN

Internal Veterinary Medicine, Faculty of Veterinary Medicine, Research Institute of Biomedical and Health Sciences, University of Las Palmas de Gran Canaria.

Keywords: *Dirofilaria immitis*, Pulmonary hypertension, VP/AP ratio

INTRODUCTION. *Dirofilaria immitis* is a nematode that produces proliferative pulmonary endarteritis in dogs, due to direct contact of the adult parasites with the intima layer of the pulmonary arteries. The vascular lesions lead to an irreversible structural damage and a sustained pulmonary hypertension (PH) which can produce dyspnea, exercise intolerance, cough, or even right heart failure (Falcón-Cordón *et al*., 2019 J Vet parasitol. 273: 1-4). Therefore, the purpose of this study was to assess the diagnostic value of the pulmonary vein to pulmonary artery ratio (PV/PA ratio) measure (Birettoni *et al.,* 2016 J Vet Cardiol. 18: 326-335) obtained by echocardiography to determine moderate or severe PH (>55mmhg) in dogs with heartworm disease.

MATERIALS AND METHODS. 29 heartworm-infected dogs (group A) and 11 healthy dogs (group B) were included in the study. Based on the Right Pulmonary Artery Distensibility index (RPAD index), PH was present in 58.6% of dogs infected by *D. immitis* (group A). The PV/PA ratio was measured by M mode and 2D mode, and compared with the RPAD index (Gold Standard) and with other alternative measures for estimation of PH (Main pulmonary Artery to Aorta ratio, worm load score and the PV/PA ratio via x-ray) to value their sensitivity and specificity in the detection of pulmonary hypertension.

RESULTS AND CONCLUSIONS. The statistical study of the results showed a high positive correlation between RPAD index and AP/VP ratio, with a sensitivity and specificity of 1.00. Therefore, the PV/PA ratio may be useful as a complementary diagnostic method for the determination of moderate or severe PH in dogs with *D. immitis,* when the measure is ≤ 0.615 measured as M mode and ≤0.675 measured as 2D Mode (Similar results of Roel *et al*., 2019 J Vet Cardiol. 24: 85-94). However, further research with a greater number of dogs must be carried out to confirm these results.

Usage of rigid alligator forceps in surgical treatment of caval syndrome due to heartworm disease

N MILOJKOVIĆ[1], D MILOSAVLJEVIĆ[1]

[1]Veterinary clinic Vet Centar, Belgrade, Serbia.

Keywords: Caval syndrome, Rigid alligator forceps

INTRODUCTION. Caval Syndrome is well described in some dogs with cardiopulmonary dirofillariosis as a serious condition with poor prognosis. The main goal for practitioners who are dealing with such patients is the urgent removal of the worms from the right atrium and the right heart chamber. Sometimes it is important to remove the worms in general practice without sophisticated equipment (fluoroscopic guidance and “Ishihara forceps” or similar device) because in suburban and rural hyper endemic areas (especially in low-income countries) many such owners can not afford to treat their pets in a referral clinic.

MATERIALS AND METHODS. We want to present our experience with dogs which underwent surgical removal of heartworms from right heart chambers by using rigid alligator forceps without fluoroscopic guidance. During the last three years, 14 dogs with caval syndrome were presented in our clinic. Besides clinical signs and positive HW testing, presence of caval syndrome was confirmed by echocardiography. In one dog, after stabilization (fliuds and prednisolon) and sildenafil administration, the worms returned to the pulmonary arteries. Owners of 10 dogs accepted the surgery. We performed jugular phlebotomy on the left side and carefully inserted the rigid alligator forceps to the point when ictus cordis was palpable on the forceps. Then we proceeded by pushing the forceps a little bit ventrally and gently grasped the worms and pull them out. After several unsuccessful attempts to catch the worms, we halted with our further attempts and checked for the worm presence in the right atrium and right heart chamber by ultrasonography. We concluded our intervention if no more worms are visible.

RESULTS AND CONCLUSIONS. Two dogs didn’t survive the procedure – one because of iatrogenic tearing of the right atrium and cardiac tamponade, the second one died due to cardiac arrest during surgery. Five dogs, after removal of the worms received three step adulticide treatment and were on careful follow up and are in good condition at the moment. One dog died due to acute kidney failure (probably induced by severe pigmenturia) five days after the procedure. Two dogs survived the surgery but died within six months due to unknown causes, because of the low owner compliance there were no regular check-ups and adequate treatments.

In our opinion, removing worms with rigid alligator forceps is an acceptable procedure for any general practitioner with basic echocardiography and surgical skills who works in hyper-endemic areas with high incidence of dogs with caval syndrome.

Dirofilaria immitis in stray dogs in the municipality of Puebla, Mexico

R MORCHÓN[1], JA MONTOYA-ALONSO[2], E CARRETÓN[3], J VICENTE-BENGOCHEA[1], I RODRÍGUEZ-ESCOLAR[1], X MURCIA-MARTÍNEZ[1], P PÉREZ[1], JL ZUMAQUERO[3]

[1]Animal and human dirofilariosis group, University of Salamanca, Spain; [2]Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas de Gran Canaria, Spain; [3]Faculty of Biology. Benemerita Autonoma University of Puebla, Mexico.

Keywords: *Dirofilaria immitis*, Puebla, Mexico, Dogs

INTRODUCTION. *Dirofilaria immitis* is the causative agent of cardiopulmonary dirofilariasis in dogs and cats. Several species of culicid mosquitoes act as vectors of this parasitosis. The existence of canine dirofilariosis implies a risk for human populations living in endemic areas in which, the parasite can cause pulmonary dirofilariosis. In Puebla (Mexico), there is evidence of dirofilariasis in domestic dogs and seroprevalent cases in humans. The aim of this study is to study the presence of *D. immitis* in stray dogs and to investigate the zoonotic risk posed by the uncontrolled presence of the parasite in these animals.

MATERIALS AND METHODS. Blood samples were collected from 453 dog samples were collected. Canine samples were tested for the detection of *D. immitis* antigen with a immunochromatographic test kit (UranotestDirofilaria®, Urano Vet SL, Barcelona, Spain) and Knott test. A map of the sampling area was constructed using ArcMapv.10.8(ESRI, 2020 Redlands, California, USA), including the following layers of relevant environmental information that have been considered to be relevant for the dynamics of the analyzed organisms and their transmission vectors: climate, potential vegetation, and surface waters and surface and edaphic humidity (rivers, lakes, lagoons, irrigated croplands and parks).

RESULTS AND CONCLUSIONS. The prevalence of heartworm in the canine population was 9.4% and, of theses, 52.5% were microfilaremic. Positivity was most frequently found in colonies with drainage features, stagnant water, rivers, ponds and near markets, etc. Given the zoonotic potential of this disease, as their presence in humans in Mexico has been described as well as their link to canine infections, a close relationship between veterinarians, physicians and public health administrations under the concept of One Health is needed. Prophylactic measures among dogs and cats must be taken to prevent the animal infection in the state of Puebla and avoid zoonotic infections.

Epidemiological study of distribution of *Dirofilaria immitis* in dogs from Castilla y León (Spain)

R MORCHÓN[1], **JA MONTOYA-ALONSO**[2], **P PÉREZ-PÉREZ**[1], **JA SÁNCHEZ AGUDO**[3], **I ESCOLAR-RODRÍGUEZ**[1], **X MURCIA-MARTÍNEZ**[1], **M RUIZ-SOMACARRERA**[1], **E CARRETÓN**[2]

[1]Animal and human dirofilariosis group, University of Salamanca, Spain; [2]Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas de Gran Canaria, Spain; [3]Research Group on Biodiversity, Human Diversity and Conservation Biology, University of Salamanca, Spain.

Keywords: *Dirofilaria immitis*, Prevalence, Epidemiology, GIS

INTRODUCTION. *Dirofilaria immitis* is the causative agent of canine cardiopulmonary dirofilariosis, a chronic disease which can lead to congestive heart failure and death of the animal. The most recent mean prevalence reported in Spain in dogs is 6.25% but data providing the influence of environmental factors (i.e. orography, vegetation, climate) of each of the areas studied have not been published. For this reason, the aim of this study wasto analyze the prevalence and distribution of *D. immitis* in Castilla y León (Spain), the largest region of the Iberian Peninsula, providing a geospatial approach based on a geographical information systems (GIS) analysis.

MATERIALS AND METHODS. A total of 1475 canine blood samples were collected between September 2019 and December 2020 from the 9 provinces of Castilla y León. All samples were tested for the detection of *D. immitis* antigens (Uranovet, Barcelona Spain).

RESULTS AND CONCLUSIONS. The overall prevalence was7.19%, showing an increase from previous results and demonstrating the expansion of canine dirofilariosis in Spain, especially towards northern areas of the Iberian Peninsula (such as Castilla y León) which were previously considered free of the disease. The highest prevalences were located in the center and west of Castilla y León, where climates with mild summers and winters >10ºCare present, while no infected dogs were found in the east. The prevalence in outdoor dogs (8.51%) or indoor/outdoor dogs (9.54%) was significantly higher than the prevalence of indoor dogs (4.40%). Most of the infected animals were located in areas with high edaphic availability of water, either in the form of stagnant water, irrigation systems or riverbanks, always in close proximity with forest and groves vegetation, which contribute to the development of mosquito vectors. Given the zoonotic potential of *D. immitis*, a close relationship between veterinarians, physicians and public health administrations under the concept of One Health is needed. This would allow effective control measures to be carried out on infected animals and vectors, mainly focused on prophylactic measures to be applied routinely on dogs.

Presence of *Angiostrongylus vasorum* in dogs in one of the largest regions of the Iberian Peninsula and Europe

R MORCHÓN[1], JA MONTOYA-ALONSO[2], JA SÁNCHEZ AGUDO[3], J VICENTE-BENGOCHEA[1], I ESCOLAR-RODRÍGUEZ[1], X MURCIA-MARTÍNEZ[1], E CARRETÓN[2]

[1]Animal and human dirofilariosis group, University of Salamanca, Spain; [2]Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas de Gran Canaria, Spain; [3]Research Group on Biodiversity, Human Diversity and Conservation Biology, University of Salamanca, Spain.

Keywords: *Angiostrongylus vasorum*, Spain, Europe, Epidemiology

INTRODUCTION. *Angiostrongylus vasorum* is the causative agent of canine angiostrongylosis, a disease affecting domestic and wild canids. In Europe it is an emerging disease, mainly reported in red foxes. In Spain there are few studies that address the prevalence and pathology of this disease. Castilla y León is the largest region of the Iberian Peninsula. Its great extension surpasses the surface of several European countries, and it present a vast climatic and orographic diversity; however, it lacks epidemiological studies. The aim of this study was focused on the analysis of the prevalence and distribution of *A. vasorum* in dogs living in this territory.

MATERIALS AND METHODS. From September 2019 to December 2020, a total of 1475 blood samples from domestic dogs from all the9 provinces of Castilla y León were collected. All samples were tested for the presence of circulating antigens of *A. vasorum* using Angio Detect^{TM} (IDEXX Laboratories Inc.; Westbrook, Maine, USA).

RESULTS AND CONCLUSIONS. The overall prevalence of canine angiostrongylosis in the studied areas of Castilla y León was 0.75%. Of the provinces analyzed, the highest prevalences were shown in Palencia (1.79%), followed by León (1.80%), Burgos (1.06%), Zamora (0.92%) and Salamanca (0.73%). No infected animals were found in the other provinces. The location of infected dogs and the highest prevalences corresponded mostly with sub-climatescharacterized by mild summers and cold winters but above 10ºC, that in the bioclimatic context show a trend towards a less seasonal character with an oceanic influence, moderate temperatures and greater precipitation. All infected dogs were located in areas with high presence of water or soil humidity, such as stagnant water, irrigated lands or riverbanks which would favor the presence of the intermediate host. Furthermore, the prevalence was higher in outdoors dogs, being mainly guard and hunting breed dogs. The results obtained show the scarce control that is carried out of the *A. vasorum* parasite in Castilla y León. As angiostrongylosis is an emerging disease in Europe and, in particular, in Spain, prevention and control measures are important.

Dirofilaria immitis infection in the Pelagie archipelago: the southernmost hyperendemic focus in Europe

E NAPOLI[1], R PANARESE[2], G DE BENEDETTO[1], G GAGLIO[1], MA BEZERRA-SANTOS[2], JA MENDOZA-ROLDAN[2], D OTRANTO[2], E BRIANTI[1]

[1]Department of Veterinary Sciences, University of Messina, Messina Italy; [2]Department of Veterinary Medicine, University of Bari, Bari, Italy.

Keywords: *Dirofilaria immitis*, Canine heartworm disease, *Dirofilaria repens,* Dirofilariosis

INTRODUCTION. *Dirofilaria immitis* and *Dirofilaria repens* are mosquito-borne filarioids of zoonotic concern, that cause canine heartworm disease (HWD) and subcutaneous dirofilariosis, respectively. The geographical distribution of these filaroids is constantly changing, and although HWD has been considered endemic in northern Italy, an increasing prevalence of the disease has been recorded in the last decades in central and southern regions. Here we report the southernmost hyperendemic European focus HW infection in the Pelagie archipelago.

MATERIALS AND METHODS. From June to November 2020, an epidemiological survey on dirofilariosis in dogs and cats was conducted in two islands of the Pelagie archipelago. Fifty-six dogs and 17 cats (*i.e.,* 94.9% and 7.1% of the whole dog and cat populations, respectively) in Linosa, and 101 dogs and 29 cats in Lampedusa were enrolled in the survey. All the enrolled animals were kept on the islands since the year before due to the COVID-19 pandemic restrictions, and no history of travel was reported by the owners. Blood samples were collected from animals and for the detection of *D. immitis* and *D. repens* microfilariae by the modified Knott's test, rapid antigen (SNAP® 4Dx® Plus Test) and duplex real-time PCR (qPCR).

RESULTS AND CONCLUSIONS. Of the 56 dogs from Linosa, 33 (*i.e.,* 58.9%, 95% CI: 0.46–0.72) tested positive for *D. immitis* at least by one diagnostic method, whereas 8 (*i.e.,* 7.9%, 95% CI: 0.04–0.15) from Lampedusa were Knott positive for *D. repens*. All dogs positive for *D. immitis* by Knott's test were also positive to the HW antigen test, except for one. The qPCR confirmed the morphological identification and allowed to the detection 3 cats positive for *D. immitis* (*i.e.,* 17.6%) in Linosa. With a *D. immitis* prevalence in dogs of almost 59%, Linosa represents the southernmost hyperendemic focus for HWD in Europe. This study further support the spreading of *D. immitis* to previously non-endemic territories in southern Europe and highlight the need of embracing efficacious control measures towards the protection of animal and human health.

Insecticidal efficacy of afoxolaner (NexGard®) in the prevention of *Leishmania infantum* and *Dirofilaria immitis* transmission to sheltered dogs in a high endemic area

R PANARESE[1], R IATTA[1], JA MENDOZA-ROLDAN[1], A ZATELLI[1], F BEUGNET[2], D OTRANTO[1]

[1]Dipartimento di Medicina Veterinaria, Università Degli Studi di Bari Aldo Moro, Valenzano, Italia; [2]Boehringer Ingelheim Animal Health, Lyon, France.

Keywords: Dirofilariosis, Leishmaniosis, Afoxolaner, NexGard®

INTRODUCTION. The presence of *Leishmania infantum* and *Dirofilaria immitis* in endemic areas represents a real threat for animal and human health. Chemoprophylaxis measures based on the use of systemic ectoparasiticides, such as afoxolaner (NexGard®), may impact on the transmission of those infections. Thus, this study aimed to assess, for the first time, the insecticidal efficacy of afoxolaner (NexGard®) in decreasing the incidence of *D. immitis* and *L. infantum* infection to sheltered dogs living in a hyperendemic area, compared to the year before treatment (i.e., 63.9% and 10%, respectively), as well as its effect on the abundance of mosquito and sand fly populations.

MATERIALS AND METHODS. Based on their infection status, 179 dogs enrolled in the study were included in two groups: no-infected (i.e., Group 1, G1) and infected (i.e., Group 2, G2) by *D. immitis* and/or *L. infantum*. After the enrolment in March 2020 (T0), all animals included in G1 were sampled in June (T1, +90) and in October (T2, +210), to exclude those infected before T0, whereas clinical evaluations were conducted for both groups at each time point. From March to September 2020, all animals (G1 and G2) were weighted and monthly treated with NexGard®. The post-treatment incidence was assessed for dogs in G1 on March 2021 (T3, +330) to evaluate the prevention efficacy of afoxolaner. From May to November 2020, the study was corroborated by an entomological survey.

RESULTS AND CONCLUSIONS. The post-treatment incidence of *D. immitis* was 3.7% (i.e., 1/27; 95% CI: 0.2-18.1), and of *L. infantum* was 3.6% (i.e., 3/83; 95% CI: 1.0-10.1). The protective efficacy against the *D. immitis* and *L. infantum* infections was 94.2% and 64%, respectively. Afoxolaner is efficacious in decreasing the seasonal incidence of both *D. immitis* and *L. infantum* infections, as well as the occurrence of infection in the mosquito population, though comparing to the pre- and post-treatment period, there is a significant difference only in the transmission rate of the heartworm infection.

Autochthonous *Angiostrongylus cantonensis*, *Angiostrongylus vasorum* and *Aelurostrongylus abstrusus* infections in terrestrial gastropods from the Macaronesian Archipelago, Spain

L SEGERITZ[1], A CARDONA[2], A TAUBERT[1], C HERMOSILLA[1], A RUIZ[2]

[1]Institute of Parasitology, Faculty of Veterinary Medicine, Justus Liebig University Giessen; Giessen, Germany; [2]Parasitology Laboratory Unit, Faculty of Veterinary Medicine, University of Las Palmas de Gran Canaria, Las Palmas, Spain.

Keywords: Gastropod-borne disease, Lungworm infections, Metastrongyloid nematodes

INTRODUCTION. The presence of zoonotic relevant *Angiostrongylus cantonensis* infections has recently been reported in rat final hosts and gastropod intermediate hosts in Tenerife, Spain. However, data on *A. cantonensis*-, *Angiostrongylus vasorum*- and *Aelurostrongylus abstrusus* prevalences in endemic gastropods for other islands of the Macaronesian Archipelago are still missing. In order to fill this gap, we conducted an epidemiological study with native terrestrial gastropods.

MATERIALS AND METHODS. Slug (*Plutonia lamarckii*) and snail (*Cornu aspersum*, *Theba pisana*, *Rumina decollata*) species were collected in 27 selected locations of Tenerife, Gran Canaria, El Hierro, Lanzarote, La Palma and Fuerteventura. Overall, 131 terrestrial gastropods were obtained in winter/spring season 2018/2019 and examined for the presence of metastrongyloid lungworm larvae via artificial digestion.

RESULTS AND CONCLUSIONS. The current data revealed a total prevalence of 4.6 % for *A. vasorum*, 3.8 % for *A. abstrusus*, 0.8% for *A. cantonensis*. In Tenerife, three lungworm species were detected, thereby re-confirming *A. cantonensis* endemicity for this island. The prevalence of snails (*C. aspersum*) originating from El Hierro was 5% for *A. abstrusus* and 15% for *A. vasorum*, respectively, with larval burdens up to 290 larvae per specimen. This epidemiological study indicates the presence of human, canine and feline lungworm species in Macaronesia, Spain. The current data - particularly those on anthropozoonotic *A. cantonensis* - call for a regular large-scale monitoring on intermediate hosts, paratenic hosts and definitive hosts to prevent further spread of lungworm-related diseases in man and animal.

Wolbachia detection in dogs naturally infected with *Dirofilaria* spp. and treated for the heartworm disease with an alternative therapy

LJ SPASOJEVIĆ KOSIĆ[1], **V LALOŠEVIĆ**[1], **V VRAČAR**[1], **G KOZODEROVIĆ**[2], **A POTKONJAK**[1], **S SIMIN**[1]

[1]University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, Novi Sad, Serbia; [2]University of Novi Sad, Faculty of Education in Sombor, Sombor, Serbia.

Keywords: *Dirofilaria* spp., *Wolbachia*, Alternative therapy

INTRODUCTION. *Wolbachia* was first reported in Serbia in dogs naturally infected with *Dirofilaria* spp. using a *Wsp* gene PCR (Kosić et al., 2018 Parasit. Vectors 11(Suppl 1):623). This study investigates if the usage of PCR for two genes (*Wsp*, 16S rRNA) improves *Wolbachia* detection in dogs, especially in those treated with antibiotics for heartworm disease (HWD).

MATERIALS AND METHODS. Retrospective analyses of blood samples of dogs infected (N1=26) and non-infected (N2=10) with *Dirofilaria* spp. were performed for the presence of *Wolbachia* by PCR deterction of *Wsp* and 16S rRNA genes (Simsek and Turcan, 2016 J Arthropod Borne Dis. 10: 445-53; Foster et al., 2008 Parasit Vectors. 1: 31). Clinical and parasitological examinations (modified Knott test for circulating microfilariae and SNAP Test IDEXX for circulating *Dirofilaria immitis* antigen) had been used for diagnosing and differentiating *Dirofilaria* spp.. All dogs had been older than 7 months, exposed to at least one mosquito season and never treated with macrocyclic lactones. Nine dogs with HWD, which had been treated with an alternative therapy (oral doxycycline 10 mg/kg b.w., SID for 6 weeks, then alternately 4 weeks without and 2 weeks with the medication, and oral ivermectin 6-14 µg/kg b.w., every 2 weeks) until antigenic negativization (Bazzocchi et al., 2008 Intern J Parasitol. 38: 1401-10), were analyzed again for *Wolbachia* at the end of therapy.

RESULTS AND CONCLUSIONS. *Wolbachia* was detected only in *Dirofilaria* spp. infected dogs, with the prevalence of 53.85% (14/26) either with both (n.7) or one gene (1 dog was *Wsp* positive whilst 6 were 16SrRNA positive). Of the 9 dogs treated with the alternative therapy for HWD, 6 were D+W+ and 3 were D+W-. After the therapy, 6 dogs were D-W-, 2 were D-W+ and one was D+W-. The effectiveness of the alternative therapy for HWD and *Wolbachia* infection was 88.89% (n.8/9) and 83.33% (n.5/6), respectively. A higher number of dogs infected with *Wolbachia* were detected when PCR for two genes were used. The effectiveness of the alternative therapy for HWD and for *Wolbachia* infection was similar.

Dirofilaria repens from the subcutaneous tissue of a dog native to the Abruzzo region, Italy

<u>EE TIERI</u>[1], G GIOVANNINI[2], D SANTOLERI[2], M PRIMAVERA[2], GC TELERA[1], D MALATESTA[1], S DEFOURNY[1], M DE ASCENTIS[1], S D'ALESSIO[1], A COCCO[1], P BADAGLIACCA[1], MS LATROFA[3], D OTRANTO[3], A PETRINI[1]

[1]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy; [2]Clinica Veterinaria Guardiese, Guardiagrele (CH), Italy; [3]Department of Veterinary Medicine, University of Bari "Aldo Moro", Valenzano (BA), Italy.

Keywords: *Dirofilaria repens*, Dog, Microfilariae, Abruzzo region

INTRODUCTION. *Dirofilaria repens* is endemic throughout European countries. The infection is frequently asymptomatic in dogs, and only a few reports of clinically manifest disease have been reported. Currently, *D. repens* is showing a faster and more intense spreading through Europe. Since 1965 this parasite has been found in the native dogs of the Abruzzo region (Mantovani et al., 1965 Parassitologia, 7:117-21), however scant recent information is available. The aim of this study is to fill this gap of information and to assess the pathogenicity caused by microfilariae (mfs).

MATERIALS AND METHODS. In August 2020, in a veterinary clinic in the province of Chieti, the presence of an adult *D. repens* in the subcutaneous tissue of an adult male Briquet Griffon Vendèen dog has been observed and filmed, during the suturing operation of a chest wound caused by a wild boar. The parasite and the mfs present in the dog's blood have been identified morphologically and molecularly by conventional PCR (Latrofa et al., 2012 Vet. Parasitol., 185:181-5). The animal died after a few days and has been subjected to necropsy. Other 3 adult filariae from the subcutis of the thoracic region were collected and identified and fragments of tissues were subjected to histological examination. Throughout 2020, mosquito collections have been performed in the same territory within the Abruzzo entomological surveillance for arboviroses in four collection sites.

RESULTS AND CONCLUSIONS. Adult parasites and mfs have been identified as *D. repens*. During entomological surveillance, *Aedes albopictus*, *Ae. vexans*, *Anopheles maculipennis* s.l., *Culex pipiens* s.l., *Culiseta annulata* have been found. Histological examination revealed panniculitis, hepatitis and interstitial nephritis associated to the presence of vascular mfs in the liver and in the kidney. The findings suggest the implication of *D. repens* mfs in the aetiology of histopathological lesions.

POSTER SOIPA

PARASSITOLOGIA VETERINARIA

Characterisation of equine pastern dermatitis (greasy heel, mud fever) in Nordic breeds Døla and North Swedish coldblooded horse

K BERGVALL[1], M ISAKSSON[2], I SKAAR[3], G GRÖNDAHL[1], E OSTERMAN-LIND[2], S BORNSTEIN[2], G GRANDI[1,2]

[1]Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden; [2]National Veterinary Institute (SVA), Uppsala, Sweden; [3]Norwegian Veterinary Institute, Oslo, Norway.

Keywords: Pastern dermatitis, *Chorioptes*, Chorioptic mange, Horse

INTRODUCTION. The painful, crusty and sore-causing inflammation of the skin associated to pastern dermatitis and the powerful and insufferable itch associated to leg mange caused by *Chorioptes bovis* are undesirable and often chronic animal welfare problems in horses. The overall aim for this study was to gain deeper knowledge about the occurrence and etiology of pastern dermatitis and associated conditions in horses.

MATERIALS AND METHODS. A field sampling and clinical study in 103 horses (58% belonging to North Swedish Draught and Dølahorses) in Sweden and Norway. The distal leg cutaneous health was clinically examined in each horse. An Equine Leg Dermatitis Score protocol was developed for nine different types of lesions, the degree of pain was noted, and the owner indicated the degree of itch on a visual analog scale. *Chorioptes* mites were detected from skin scraping with both a PCR method and microscopically. A total of 53 samples from 43 horses with pastern dermatitis were examined for bacteria and fungi.

RESULTS AND CONCLUSIONS. The highest score for skin lesions was reached on the palmar aspect of the cannon bones, followed by the palmar aspect of the pastern region and the flex region of the carpus and hock. Pruritus (itching) was reported in 46% and pain in 28% of all the horses. In 41/106 horses (38.7%) chorioptic mange was diagnosed by at least one test method. *Staphylococcus aureus* and *Streptococcus dysgalactia equisimilis* were detected in 51% and 23% of the examined horses respectively; yeasts and moulds were detected in 48% and 69% of horses respectively, while no dermatophytes were detected.65% of horses were affected by pastern dermatitis. Chorioptic mange was part of the problem in many horses, often characterized by itching behavior but in 19.5% and 9.7% of *Chorioptes*-positive horses, respectively, neither pruritus nor skin lesions were noticed. This makes necessary to have in place a correct diagnostic protocol and an effective and safe treatment protocol. Bacterial infections might develop in pastern dermatitis areas, while mycological infections seem to be a minor problem.

Source of funding: Swedish-Norwegian Foundation for Equine Research

Flea mite phoresy and high prevalence of *Rickettsia asembonensis* in *Archaeopsylla erinacei* fleas from European hedgehogs

MA BEZERRA-SANTOS, JA MENDOZA-ROLDAN, G SGROI, A CAMARDA, R IATTA, D OTRANTO

[1] Dipartimento di Medicina Veterinaria, Università Degli Studi di Bari Aldo Moro, Valenzano, Italia.

Keywords: Mites, Fleas, Hedgehogs, Phoresy

INTRODUCTION. The European hedgehog (*Erinaceus europaeus*) is a synanthropic species in urban, rural and wild environments. This mammal harbors diverse ectoparasites (e.g., ticks, fleas, mites) and it is suggested to be reservoir of diverse vector-borne pathogens (e.g., *Rickettsia* spp., *Bartonella* spp.). The phoretic association of *Caparinia tripilis* mites and *Archaeopsylla erinacei* fleas has been herein described on *E. europaeus* hedgehogs. In addition, arthropod-borne pathogens have also been investigated in ticks, fleas and mites collected on this host species.

MATERIALS AND METHODS. Animals (*n* = 47) were captured in two regions of South Italy (i.e., Basilicata and Apulia region) and individually inspected for the presence of ectoparasites, which were morphologically identified at genus and species levels. Vector-borne pathogens (*Rickettsia* spp*., Borrelia* spp. and Anaplasmataceae) have been molecularly screened.

RESULTS AND CONCLUSIONS. Of 47 hedgehogs examined, 21 (44.68%) were positive for ectoparasite infestation, with 18 (38.30%) presenting infestation by fleas, 7 (14.89%) by ticks and 6 (12.77%) by mites. Phoretic behavior of *C. tripilis* mites on *A. erinacei* was detected in two female flea specimens, with mites observed on their legs and head. *Rickettsia* spp. was detected in 93.33% (42/45) of the fleas evaluated. Ticks and mites scored all negative for the pathogens tested. According to BLAST analysis, all sequences of *Rickettsia* spp. presented 100% nucleotide identity with *Rickettsia asembonensis* sequences available at the GenBank database. Data suggest the occurrence of a new phoretic association between *C. tripilis* mites and *A. erinacei* fleas, which could be an important route for the spreading of this mite species through hedgehog populations. Additionally, we report a high prevalence of *R. asembonensis* on the same flea species, suggesting that *E. europaeus* hedgehogs may play a role as a reservoir host for this bacterium.

Development of a Reverse Line Blot for simultaneous detection of Tick-Borne Pathogens in equines

V BLANDA, A CARUANA, S BONACCORSO, F LA RUSSA, R D'AGOSTINO, S VILLARI, G CAMMILLERI, A TORINA, V FERRANTELLI

Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy.

Keywords: Reverse line blot, Equines, Tick borne pathogens

INTRODUCTION. *Babesia caballi*, *Theileria equi*, *Anaplasma phagocytophilum* and *Rickettsia* spp. are the main etiologic agents of Tick Borne Diseases (TBDs) in equines. Due to the nonspecific symptoms of TBDs, several molecular assays, each specific for a different Tick Borne Pathogen (TBP), are required for the diagnosis. This work aimed at developing a single Reverse Line Blot (RLB) for simultaneous detection and identification of equine TBPs.

MATERIALS AND METHODS. Overall, 148 DNA samples, from equine blood samples and ticks positive for TBPs were used to set up the method. PCRs with biotinylated primers amplifying DNA from *Anaplasma* spp. (Schouls et al., 1999 J Clin Microbiol. 37: 2215-22), *Rickettsia spp.* (Jado et al., 2006 J Clin Microbiol. 44: 4572-76), *Babesia* spp. and *Theileria* spp. (Nagore et al., 2004 Vet Parasitol. 123: 41-54) were carried out. *Anaplasma* spp. and *Rickettsia* spp. DNA amplification was also performed with a new optimized multiplex PCR. Obtained amplicons were hybridized with 5 genus-specific probes (*Babesia/Theileria*, *Anaplasma*, *Rickettsia* species, Spotted Fever Group *Rickettsia*) and 10 species-specific probes (*T. equi, T. equi like, B. caballi, B. caballi like, A. phagocytophilum, Ehrlichia deer, R. slovaca, R. conorii, R. aeschlimannii, R. helvetica*), previously immobilized in a nitrocellulose membrane. Different probe concentrations and incubation times were tested. Amplicons for different TBPs from the same sample were differently combined and tested in a single membrane channel for simultaneous detection of TBPs. Sensitivity was determined by 10-fold serial dilutions of positive samples. Specificity was tested with samples positive for other relatedpathogens.

RESULTS AND CONCLUSIONS. The new RLB was optimized with 15 different probes. Two optimal concentrations for each probe (1200 and 2400 pmol/150 µl for *Babesia*, *Theileria* and *Anaplasma* and 400 and 1200 pmol/150 µl for *Rickettsia* probes) were defined and the assayresulted sensitive and specific. The new multiplex PCR for *Anaplasma* spp. and *Rickettsia*spp. Made the assay faster, maintaining the sensitivity and specificity of the single PCRs. The combination in a single tube of the amplicons obtained by the PCRs for different TBPs allowed the hybridization and the detection of different TBPs in a unique membrane channel. Financed by the Italian Ministry of Health (RC IZS SI 07/18).

A national survey on equine intestinal strongyle infections in Italy and Parasitological Assistance Program in Equids (PAPE)

F BUONO[1], L PACIFICO[1], C RONCORONI[2], D PIANTEDOSI[1], E CASTALDO[1], G CRINGOLI[1], V VENEZIANO[1]

[1]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Naples, Italy; [2]Istituto Zooprofilattico Sperimentale Lazio e Toscana, Roma, Italy.

Keywords: Parasite control, Horse, Selective treatment, Anthelmintic resistance

INTRODUCTION. Horses are naturally parasitized by several genus/group of helminths and intestinal strongyles are the most common. Presently three anthelmintic classes are registered in horses: tetrahydropyrimidines, benzimidazoles and macrocyclic lactones (Gokbulut and McKellar, 2018 Vet Parasitol. 261: 27-52). The traditional approach to helminths control is based on interval dose program without a diagnosis, furthermore the frequent and reason less drug use has led to development of anthelmintic resistance (Leathwick et al., 2019 Vet Parasitol. 209: 210-20). Equine deworming scheme for adult horses is based on strongyle Fecal Egg Count (FEC) and on treatment of horses with FEC>200 egg per gram of feces (EPG) (Selective Treatment) (AAEP Internal Parasite Control Guidelines, 2019). FEC is also crucial to evaluate the treatment efficacy. The aims of this work were to carry out a survey on intestinal strongyles in horses in Italy and to propose a veterinary assistance considering a Parasitological Assistance Program in Equids (PAPE) based on coprological diagnosis (FEC).

MATERIALS AND METHODS. The study was performed on 7,796 horses bred in 542 Italian farms. Individual FECs were performed using Mini-FLOTAC technique and a Sheather's sugar solution with a specific gravity of 1.250. Individual coprocultures were set up for each horse with FEC>200EPG.

RESULTS AND CONCLUSIONS. Intestinal strongyle eggs were found in 420 (77.5%) tested farms with a prevalence at animal level of 57.6% (4,492/7,796). The average EPG was 441.2±622.0 (min 5 – max 7,100). Regarding the egg shedding level, 71.4% of horses had a FEC <200 EPG (low contaminators), 11.3% between 200-500 EPG (moderate contaminators) and 17.3% >500 EPG (high contaminators). Coprocultures revealed L3 of Cyathostominae (100%), *Poteriostomum* (3.2%), *Gyalocephalus* (2.1%), *Strongylus vulgaris* (1.9%), *Strongylus edentates* (1.1%), *Triodontophorus* (0.7%) and *Oesophagodontus* (0.2%). Considering that FEC was <200 EPG in 71.4% (5,566) of horses, only 28.6% should be treated. Veterinarians must play an active role considering a Parasitological Program in Equids (PAPE) based on parasitological monitoring and Selective Treatment suggesting 4 individual FECs/year.

Ethnoveterinary pharmacology to control gastrointestinal nematodes in sheep: evaluation of three aqueous plant macerates

F CASTAGNA[1], E PALMA[1,2], C PIRAS[1], C CRINGOLI[3], L RINALDI[3], A BOSCO[3], V MUSOLINO[1], D BRITTI[1], V MUSELLA[1]

[1]Department of Health Sciences-University of Catanzaro Magna Græcia, CISVetSUA, Catanzaro, Italy; [2]Nutramed S.c.a.r.l. Complesso Ninì Barbieri, Roccelletta di Borgia, Catanzaro, Italy; [3]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, CREMOPAR, Regione Campania, Naples, Italy.

Keywords: Plant macerates, Gastrointestinal nematodes, Sheep, Ethnoveterinary pharmacology

INTRODUCTION. Resistance of gastrointestinal nematodes (GIN) to anthelmintic drugs is of high concern for the livestock productions in many European countries (Vineer et al., 2020 Parasite 27: 69) and is driving research towards the evaluation of ethnoveterinary remedies. In Calabria (southern Italy) many plants have been used in ethnoveterinary medicine for parasite control in animals (Passalacqua et al., 2006 J Ethnobiol Ethnomed 2: 52) and in the present study some of them were identified and tested as possible alternatives to control GIN in sheep.

MATERIALS AND METHODS. For the study, 105 sheep farms were examined, among those, 45 were managed by farmers according to the ancient tradition of the area. Most promising ethnoveterinary remedies for GIN control were discovered through the evaluation of field data analysis and interviews with the farmers. Subsequently, the *in vivo* anthelmintic efficacy (AE) of three ethno-veterinary plant-based remedies was evaluated in one sheep farm. The first extract was an aqueous macerate of bark and leaves of *Salix caprea*, the second and the third were the aqueous macerates of *Artemisia campestris* (whole plant) and the whole fruits of *Punica granatum,* respectively. AE was evaluated according to the Faecal Egg Count Reduction Test (FECRT) performed with FLOTAC (Cringoli et al., 2010 Nat Protoc. 5: 503) using the formula (FECR= 100×(1-[T2/C2])) recommended by the World Association for the Advancement of Veterinary Parasitology (Coles et al., 1992 Vet Parasitol. 44: 35-44).

RESULTS AND CONCLUSIONS. The results showed a FECR of 50.1% for the *P. granatum* macerate after 7 days (D7), such an effect was persisting after 14 days (44.3%) and still above 40% (40.4%) after 21 days from the administration; a low effectiveness of the *A. campestris* macerate (D7=20.4%; D14=9.7% and D21=4.3%) and an efficacy close to zero (D7=0.2%; D14=0 and D 21=0.3%) of *S. caprea* macerate. *P. granatum*-related effectiveness could be attributed to the synergistic action of the tannin-derivatives and phenolic acids. The used macerate was previously analyzed via LC / HRMS, ESI (-) highlighting a wide panel of possible effective compounds (Castagna et al., 2020 Pathogens. 9: 1063). The obtained results could represent a new way to approach anthelmintic resistance through a Green Veterinary Pharmacology.

Characterization of mitochondrial DNA of *Fasciola hepatica* from Algerian and Sardinian isolates and relationships with other areas of the world

M CHAOUADI[1], F SCARPA[2], I AZZENA[2,3], P COSSU[2], KH HARHOURA[4], M AISSI[4], F TAZEROUTI[1], G GARIPPA[2], P MERELLA[2], M CASU[2], D SANNA[3]

[1]Laboratory of Biodiversity and Environment: Interactions and Genomes, Faculty of Biological Sciences, University of Sciences and Technology Houari Boumediene, Alger, Algeria; [2]Department of Veterinary Medicine, University of Sassari, Sassari, Italy; [3]Department of Biomedical Sciences, University of Sassari, Sassari, Italy; [4]Laboratory of Animal Health and Production, Superior National Veterinary School Rabie Bouchama, Alger, Algeria.

Keywords: *Fasciola hepatica*, COI, Sardinia, Algeria, mtDNA

INTRODUCTION. Fasciolosis caused by *Fasciola* spp. is a parasitic disease affecting the liver of humans and herbivores. This parasitosis causes serious economic losses in livestock, and despite the impact of this parasite on the economy of Mediterranean countries, a few molecular studies have been conducted in Algeria and Sardinia, where only *Fasciola hepatica* is present.

MATERIALS AND METHODS. Twenty-four *Fasciola* flukes isolated from 12 Algerian cattle and 16 flukes collected from one cattle and one goat from Sardinia (Italy) were characterized using mitochondrial (COI) and nuclear (ITS) markers. Sequences of COI and ITS fragments were aligned using the package Clustal Omega. Phylogenetic analyses were performed onCOI and ITS sequences isolated in the present study and those of *F. Hepatica* available in GenBank.

RESULTS AND CONCLUSIONS. All samples isolated in the present study were identified as *F. hepatica* and all COI sequences belonged to the most frequent worldwide-diffused haplotype, with the only exception of two new haplotypes that were found in two flukes isolated from one Algerian and one Sardinian cattle. Analysis of variation among the COI sequences from Algeria, Sardinia and all over the world did not reveal high levels of genetic divergence. Phylogeographic analyses performed for Algerian COI sequences evidenced the presence of a very frequent haplotype, which was present in almost all isolates, with a few derived haplotypes that were found in single flukes. The phylogenetic analysis showed a genetic similarity between isolates from different parts of the world. The analysis of the ITS region showed a very low level of variability which prevented performing further phylogenetic and phylogeographic analyses. This study reports two new COI lineages for *F. Hepatica* identified in Algerian and Sardinian cattle. The presence among Algerian samples of a low number of COI haplotypes, distributed according to a typical founder effect distribution, is the possible consequence of the recent introduction of *F. hepatica* from Europe. The low levels of genetic variation among isolates from all continents are consistent with a common origin for the flukes worldwide. This pattern is likely mediated by a constant gene flow related to livestock trading across countries.

Occurrence of *Babesia* species in symptomatic dogs and co-infection with *Hepatozoon canis* in eastern Romania

L CIUCA[1], G MARTINESCU[2], L MIRON[2], C ROMAN[2], D ACATRINEI[2], G CRINGOLI[1], L RINALDI[1], MP MAURELLI[1]

[1]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, CREMOPAR, Naples, Italy; [2]Faculty of Veterinary Medicine, Department of Parasitic Diseases, University of Life Sciences "Ion Ionescu de la Brad" Iași, Romania.

Keywords: *Babesia rossi*, Dogs, Romania, *Babesia* species

INTRODUCTION. Although the distribution of *Babesia* spp. and *Hepatozoon canis* is well known in several parts of Romania (Ionita et al., 2011 Parasitol Res. 110: 1659-64; Hamel et al., 2012 Parasitol Res. 110: 1537-45), is still marked lack of information in many places of the country. Therefore, this study aims to investigate the occurrence of *Babesia* species and the co-infection with *H. canis* in dogs suspected of babesiosis and in their ticks in Iasi, eastern Romania.

MATERIALS AND METHODS. Whole blood samples were collected from 90 owned dogs at the Clinics of the Faculty of Veterinary Medicine of Iasi, showing clinical signs specific for babesiosis (55 had mild clinical signs and 35 expressed the acute form). All the dogs tested positive for the babesial parasites using stained blood smears. Species, stage, and sex of all the ticks (N=31) collected from the coat of the dogs (N=15) were determined under a stereomicroscope. Additionally, 69 ticks collected from the coat of other dogs previously diagnosed with babesiosis by stained blood smears, were included in the study. All the blood and tick samples were screened for identification of *Babesia* species and *H. canis* using a PCR protocol (Bajer et al., 2019 Ann Agric Environ Med. 26: 538-43) with some modifications.

RESULTS AND CONCLUSIONS. The main clinical signs displayed by the infected dogs were: anorexia, fever, haemoglobinuria, anaemia, constipation alternating with diarrhoea and splenomegaly. Prevalence rates were higher in dogs between 1-3 years (27%) and in males (63%); the most frequently affected breeds were crossbreed (27%), followed by Peking dog (9.8%) and German Sheperd (6.9%). Three *Babesia* species were found in dogs: *B. canis* (94.4%), *B. vogeli* (3.4%) and *B. rossi* (2.2%). All the dogs resulted negative for *H. canis.* The 100 ticks examined (32% males; 65% females; 97 adults, two nymphs and one larva) were identified as follow: *Ixodes ricinus* (64%), *Dermacentor reticulatus* (33%) and *Rhipicephalus sanguineus* group (3%). *B. canis canis* (83.7%) *Babesia canis vogeli* (13.5%) and *B. microti* (2.7%) were found in ticks. In addition, 15 ticks (9=*D. reticulatus* and 6=*I. ricinus*) were positive for *H. canis*, of which six were co-infected with *B. canis canis* and one with *B. microti*. The study revealed the first identification of *B. rossi* in two symptomatic dogs from Romania. Therefore, the genetic characterization of *Babesia* species, could be helpful for practitioners to select appropriate testing, treatments and for understanding risks for infection.

Life-threatening strongyloidosis in a puppy

M COLOMBO[1], **G CAPITANI**[2], **S MORELLI**[1], **A DI CESARE**[1]

[1]Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy; [2]Clinica Veterinaria Etiopia, Rome, Italy.

Keywords: *Strongyloides stercoralis*, Puppy, Italy, Baermann

INTRODUCTION. *Strongyloides stercoralis* is a worldwide-distributed intestinal nematode infecting humans, dogs and other animals including non-human primates and wild canids. Reports on *S. stercoralis* infections in dogs from Europe are limited (Basso et al., 2018 Parasitol. Res. 118:255-66). Dogs acquire strongyloidosis through active penetration or ingestion of infective larvae, or *via* lactation (Shoop et al., 2002 J Parasitol. 88:536-39). Auto-infections are frequently described, leading to long-lasting and to potentially life-threatening hyper-infection syndrome. Infected dogs show subclinical pictures to severe signs. Gastrointestinal and respiratory signs, along with anemia, lethargy, reduced growth and sudden death, are described in young dogs (Thamsborg et al., 2017 Parasitol. 144:274-84). This report describes a severe clinical case of *S. stercoralis* hyper-infection in a puppy.

MATERIAL AND METHODS. A 3-months-old miniature Pomeranian Spitz was brought to a veterinary practice for a sudden onset of cough and diarrhea. Blood count, chest radiographs and SNAP*Giardia*® were performed and the puppy was empirically treated using amoxicillin/clavulanic acid. Nevertheless, cough and general clinical conditions worsened in two days, and the dog was referred to veterinary clinic with lateral decubitus, drooling, hypoglycemia (38 mg/dL), anorexia, vomit and diarrhea with hematochezia. Thereafter the puppy was subjected to hemogasanalysis, SNAP Parvo®, fresh fecal smear, faecal floatation and Baermann's test.

RESULTS AND CONCLUSIONS. The puppy scored positive to the SNAP *Giardia*® and negative to the SNAP Parvo®. Floatation was negative, while rabdithoid larvae were detected at the fecal smear and in Baermann sediment. Larvae were microscopically identified as *S. stercoralis* (Inês et al., 2011 Acta Trop. 120:206-10). After clinical stabilization, the puppy was treated with maropitant, metoclopramide, probiotics and fenbendazole (50 mg/kg q12 for 7 days) (Paradies et al., 2017 Rev. Veterinary Clin. 51:55-9). The Baermann tests performed 5 days and 5 weeks post treatment (p.t.) were negative. The clinical condition of the dog rapidly improved until complete resolution of clinical signs. From the second day p.t. vomit and diarrhea ceased, while the cough disappeared 3 weeks p.t.. This case report shows the importance of routine Baermann examinations in puppies with respiratory and gastrointestinal signs, as strongyloidosisis often overlooked due to the low awareness on this potentially fatal parasitosis.

Bovine besnoitiosis: a case report in Sicily

M COLTRARO[1], VC NEVE[2], A STAMILLA[3], G CASCONE[1], F ANTOCI[1], F SALINA[1], M BARBAGALLO[4], F SPADOLA[2], R PULEIO[1], SA MIGNACCA[4,5]

[1]Istituto Zooprofilattico Sperimentale of Sicily, Italy; [2]Department of Veterinary Science, University of Messina, Italy; [3]Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A), University of Catania, Italy; [4]DVM freelance, Enna, Italy; [5]Current address: Animal and Plant Health Agency, Weybridge, United Kingdom.

Keywords: *Besnoitia besnoiti*, Bovine besnoitiosis, Protozoa, Sicily

INTRODUCTION. Bovine besnoitiosis is a parasitic disease caused by the tissue cyst-forming protozoan *Besnoitia besnoiti*. The European Food Safety Authority (EFSA) has reported that bovine besnoitiosis is re-emerging in Europe (EFSA Journal, 2010, EFSA Journal. 8: 1499-514). It represents a chronic and debilitating disease that is responsible for severe economic losses such as poor body condition, decreased milk production, infertility in bulls and occasional abortion (Gutiérrez-Expósito et al., 2017 Int J Parasitol. 47: 737-51). Autochthonous outbreaks of the disease were reported in Central Italy for the first time in 2009 (Gentile et al., 2012 Vet Parasitol. 184: 108-15). In Sicily, although the disease was observed in a heifer in 2014 (Di Marco et al., 2016 LXX SISVET. 430-31), no epidemiological data is known. This study describes an autochthonous outbreak of Bovine besnoitiosis in North-Western Sicily: a bull that shows typical clinical symptoms related to bovine besnoitiosis and moreover, the serological investigation of the herd, suspected of being infected by *B. besnoiti.*

MATERIALS AND METHODS. In a 16-month-old affected bull, clinical status was evaluated both in acute and chronic stage. After slaughtering, samples of skin, conjunctiva, tongue, and tendons were collected for histopathological examination. Furthermore, in the bull and in the Limousine herd of 72 animals, serological investigation using ID Screen® Besnoitia Indirect 2.0 ID.vet was performed.

RESULTS AND CONCLUSIONS. The bull showed typical clinical signs of besnoitiosis such as: high fever, diffuse oedema at thejoints of limbs and scrotum, hyperemia of muzzle and eyes, orchitis, thickening of the nasal planum and wheezing. About 10-12 days later, skin lesions (hyperkeratosis, alopecia, dandruff and scab) appeared. At the 58th day after the beginning of the first signs, the animal showed diffuse skin thickening, was emaciated and reluctant to move. Histological examination showed multifocal-coalescing thick-wall cysts surrounding parasitophorous vacuole containing thousands of banana-shaped bradyzoites all the collected samples. ELISA confirmed the seropositivity of the bull, moreover, 79,17% of the herd resulted positive. Finally, since all the animals were born in the farm or were bought from nearby farms this case confirms the presence of autochthonous *B. besnoiti* also in Sicily and may contribute to a further epidemiological study in this area.

Molecular detection of *Tritrichomonas foetus* in cats from Abruzzo region (central Italy)

PE CRISI[1], B PAOLETTI[1], S MORELLI[1], G SIMONATO[2], M COLOMBO[1], PG TISCAR[1], A BOARI[1]

[1]Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy; [2]Departmentof Animal Medicine, Production and Health, University of Padua, Legnaro, Italy.

Keywords: *Tritrichomonas foetus*, Cat, PCR, Central Italy

INTRODUCTION. *Tritrichomonas foetus* (Trichomonadida, Tritrichomonadidae), a protozoan parasite causing chronic diarrhea in domestic cats and transmitted through the fecal-oral route (Gookin et al., 2004J Clin Microbiol. 42: 2707-10), has been reported worldwide. The clinical manifestations caused by *T. foetus* are variable, ranging from subclinical to severe. The infection is more frequent in catteries and shelters, and in purebred and young cats.Only few studies have investigated the occurrence of *T. foetus* in catsfrom limited regions of Italy (Mancianti et al., 2015J Feline Med Surg. 17: 163-67; Veronesi et al., 2016 Vet Parasitol Reg Stud Reports. 6: 14-19). This study has investigated its prevalence in cats froma region of Central Italy.

MATERIALS AND METHODS. In 2019, individual faecal samples were collected from105 cats (37 privately owned, 68 colony-cats) referred at the University Veterinary Teaching Hospital of Teramo, Abruzzo region. The faecal samples were tested using aspecific PCR protocol (Gookin et al., 2002 J Clin Microbiol. 40: 4126-30). All samples were also subjected to concentration-floatation, sucrose gradient centrifugation and Baermann techniques for the detection of other endoparasites. A statistical analysis evaluating the prevalence of infection and possible risk factors (i.e. age, sex, breed, housing, presence of other parasites and chronic gastrointestinal signs) associated with *T. foetus* infection was performed using Fisher's exact test and the odds ratio (OR).

RESULTS AND CONCLUSIONS. *Tritrichomonas foetus* was detected in 18/105 (17.14%) cats. This infection rate is higher if compared to previous studies carried out in Italy (0.75 - 32.4%). In only two cats, *T. foetus* was found in association respectively with *Giardia* and *A. abstrusus*. The infection was detected more frequently in cats with chronic gastrointestinal signs (38.9% *vs* 14.9%; $p = 0.01$; 95% CI = 1.40-13.99; OR = 4.27) and no other statistically significant associations emerged. This study confirms the endemicity of *T. foetus* in cats from central Italy and its importance as a cause of chronic gastrointestinal signs. Moreover, the data here presented showed that the infection may also occur in adult and mixed-breed catsas previously reported (Doi et al., 2012J Vet Med Sci. 74: 413-41; Gruffydd-Jones et al., 2013 J Feline Med Surg. 15: 647-49).

Potential role of *Hepatozoon canis* in a fatal systemic disease in a puppy

A DE BONIS[1], **M COLOMBO**[1], **A GHIRALDELLI**[2], **R BACCI**[3], **S MORELLI**[1], **M VIGNOLI**[1]

[1]Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy; [2]Pet Care Veterinary Clinic, Bologna, Italy; [3]Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy.

Keywords: *Hepatozoon canis*, Dog, Skin, Liver

INTRODUCTION. Dogs become infected with the protozoan *Hepatozoon canis via* the ingestionof an infected tick harbouring the sporozoites. Clinical pictures range from subclinical infections to life-threatening signs, being nonspecific clinical-pathologic alterations and general signs predominantly reported (Baneth, 2011 Vet Parasitol. 181: 3-11).

MATERIALS AND METHODS. A 2-months-oldfemale, mixed-breed puppy originating from Southern Italy was referred for gastrointestinal signs, migrating lameness and pruritic dermatitis. The animal was subjected to different examinations. The dog tested negative to SNAP 4Dx® and to *Leishmania infantum* antibodies, and positive for *Hepatozoon* spp. gamonts at the blood smear. Thus, it wastreated with imidocarb dipropionateand doxycycline every two weeks, until negativization of the blood smear. After an initial improvement, gastrointestinal signs recurred 2 months later and diffuse superficial pyoderma appeared on the thoracolumbar region, along with fever, lethargy, and weight loss. The dog was subjected to complete haemato-biochemical examination, urine analysis, coagulation test, pre-/post-prandial bile acids test, complete copromicroscopic exams, abdominal ultrasonography, whole-body CT, gastrointestinal endoscopy with hepatic, gastric, duodenal and cutaneous biopsies. Skin biopsies and blood samples were subjected a PCR-coupled sequencing protocol for *Hepatozoon* spp. (Tabar et al., 2008 Vet Parasitol. 151: 332-36).

RESULTS AND CONCLUSIONS. Anaemia, hypoalbuminemia, hypocobalaminemia, increased liver enzymes andpre-/post-prandial bile acids, bilirubinuria were detected. Alterations were consistent with a pre-existing cholangiohepatitis and multiple acquired extrahepatic shunts secondary to portal hypertension. Liver and skin histopathology revealed hypoplasia and hyperplasia of hepatic veins and arterioles respectively, and pyoderma. Blood and skin samples were PCR positive for *H. canis*. The dog was euthanatized due to a clinical worsening two months later. The role of *H. canis* in the systemic disease here described is factually plausible as i) skin lesions, though rare, are described in dog hepatozoonosis (Little and Baneth, 2011 J Vet Diagn Invest. 23: 585-88), ii) a vertical transmission of *H. canis* could have led to pre-natal hepatic alterations, and/or iii) regardless of if pre- or post-natal, the infection could have triggered the worsening of a not-related hepatic disease. Further studies are warranted to investigate more in depth the pathogenic role of *H. canis* after vertical transmission and in young puppies.

Prevalence and molecular identification of *Cryptosporidium* spp. in sheep farms from Sardinia

G DESSÌ, C TAMPONI, G SANNA, AP PIPIA, A VARCASIA, L MELONI, S KNOLL, L CAVALLO, F PORCU, C PASINI, MF SINI, F NONNIS, A SCALA

Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Italy.

Keywords: *Cryptosporidium*, Sheep, Lambs, Zoonosis

INTRODUCTION. *Cryptosporidium* causes significant intestinal disease in both human and animals, especially in calves, lambs, and goat kids (Santin et al., 2020 Vet Clin North Am Food Anim Pract. 36: 223-38). Little is known about *Cryptosporidium* infections in apparently healthy sheep in Italy and the only data concern the prevalence in diarrhoeic lambs (Paoletti et al., 2009 Exp Parasitol. 122: 349-52). This study aims to report the prevalence and zoonotic potential of *Cryptosporidium* spp. in three different sheep categories in Sardinia, Italy.

MATERIALS AND METHODS. A total of 915 individual faecal samples were collected from 61 sheep farms of Sardinia. Five samples were collected from each animal group as follow: lambs aged 5-30 days (group 1), sheep from parturition to 30 days (group 2) and sheep within the 30 days before parturition (group 3). Each sample was examined for the presence of *Cryptosporidium* spp. oocysts by microscopic examination of faecal smears stained using the modified Ziehl-Neelsen technique as previously described (Angus, 1987 In Pract. 9:47–49). *Cryptosporidium* species were determined by nested PCR of the SSU rRNA gene and the subsequent restriction fragment length polymorphism analysis (Xiao et al., 1999 Appl Environ Microbiol. 65: 3386-91; Appl Environ Microbiol. 71: 4446-54).

RESULTS AND CONCLUSIONS. *Cryptosporidium* oocysts were detected in 10.1% (CI95%: 8.1–12.1) of the examined animals and in 34.4% (CI95%: 22.5–46.3) of the farms. The prevalence found in group 1 (16.4%) was significantly higher than those found in group 2 (6.6%) (χ^2 =14.52; P < 0.001) and group 3 (7.2%) (χ^2 = 12.35; P < 0.001). Instead, no significant differences were recorded between groups 2 and 3 (χ^2 = 0.10; P = 0.719). Fifteen samples belonging to group 1 were successfully genotyped as *Cryptosporidium parvum* (subtypes IIa15G2R1 and IIdA20G1) and *Cryptosporidium ubiquitum* (subtype XIIa). Results of the present study highlight the widespread presence of *Cryptosporidium* spp. in sheep farms of Sardinia. Adult sheep could be responsible for environmental contamination and transmission of the parasite to lambs (Ortega-Mora et al., 1999 Int J Parasitol 29: 1261-68). Furthermore, pre-weaned lambs are shedders of zoonotic and pathogenic *Cryptosporidium* species (Xiao, 2010 Exp Parasitol. 124: 80-89). Therefore, it is important establish adequate monitoring programs to control this infection in sheep farms, especially through coprological examination.

Preliminary epidemiological investigation on *Leishmania infantum* infection in captive ring-tailed lemurs (*Lemur catta*)

M DIAFERIA[1], C FRANCHETTO[3], M CAPASSO[2], L FERRETTI[3], G MORGANTI[1], L RINALDI[3]

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Perugia; [2]Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli Federico II; [3]DVM.

Keywords: *Leishmania infantum, Lemur catta*, Zoological gardens, Italy

INTRODUCTION. Leishmaniosis is a zoonosis endemic in several regions of the Mediterranean basin caused by the protozoan *Leishmania infantum*. Dogs are considered as the main reservoir of infection but this parasite can infect a wide spectrum of domestic and wild hosts. Zoo animals kept in captivity in endemic areas are susceptible to *L. infantum* infection (Souza et al., 2014 Vet Parasitol. 200: 233-41). However, little is known about the role of these species as reservoirs and data about clinical pictures are scant. Aim of the present study was to investigate the prevalence of *L. infantum* infection in lemurs housed in a zoological garden ("Zoo delle Maitine") located in a suburban area of the Benevento, Campania region, southern Italy.

MATERIALS AND METHODS. In March 2020, during mandatory health control clinical practices, an overall of twelve lemurs were submitted to general clinical examination, blood sampling and oculo-conjunctival swab. All animals were males. Three lemurs were younger than 6 years, 4 subjects were in the age range of 6-8 years, while 5 were older than 8 years. Seven animals were transferred from the "Zoo Safari Ravenna" (Emilia Romagna region) one month before, while five lemurs were housed permanently in the zoological garden. Anti-feeding products had been never used on animals as prophylactic treatments. Hematological and biochemical panel were performed. An aliquot of serum was tested by serological test (IFAT), using a dilution of 1:100 as cut off (Mirò et al., 2018 Parasit Vectors 11: 185-91). DNA was extracted from oculo-conjunctival swabs and subjected to PCRs targeting a 120bp kinetoplast DNA (Francino et al., 2006 Vet Parasitol. 137: 214-21).

RESULTS AND CONCLUSIONS. No serological or biomolecular evidence of *L. infantum* were detected in examined lemurs. Clinical examinations were normal and haematological and biochemical parameters recorded were in ranges. These preliminary data represent the first step of an epidemiological survey and suggests the implementation of the sample population with subjects coming from zoos located in areas with a different status of endemicity for *L. infantum*. In facts, zoo animals maintained in captivity in endemic urban environments could be under high risk of infection and therefore to define monitoring programs could be an important concern both for animal and human health.

Epidemiological survey on parasites of terrestrial tortoises from Sardinia

F DIANA[1], C TAMPONI[1], S PINNA[1], F NONNIS[1], L CAVALLO[1], S CARTA[1], S CAFIERO[1], G DESSI'[1], F PUDDA[2], A VARCASIA[1], A SCALA[1]

[1]Department of Veterinary Medicine, Institute of Parasitology and Parasitic Diseases; University of Sassari; [2]FORESTAS, Centro di recupero della Fauna Selvatica, Bonassai Olmedo, Italy.

Keywords: Cryptosporidiosis, Endoparasites, Tortoise, Sardinia

INTRODUCTION. Wild and captive tortoises are natural hosts to a wide variety of parasites, but broadly they may be divided into trematodes (flukes), cestodes (tapeworms), nematodes and protozoa. Although in Sardinia live three species of the genus *Testudo*, of which one (*Testudo marginata*) constitutes an endemic species typical of the island, at present little is known about their parasitological fauna. The aim of this study is to investigate the prevalence of parasites in tortoises in Sardinia, with particular regard to the detection of zoonotic species.

MATERIALS AND METHODS. From October 2013 to March 2021 a total of 192 turtles were examined, belonging to the three species present in Sardinia (*T. marginata, Testudo graeca, Testudo hermanni*). Individual faecal samples were collected and processed by flotation technique with a zinc sulfate solution (specific gravity = 1.2) for endoparasites and Ziehl-Neelsen modified by Angus (1987) technique for *Cryptosporidium* spp. oocysts detection.

RESULTS AND CONCLUSIONS. Out of 192 tortoises analyzed (118 *T. hermanni,* 63 *T. marginata* and 11 *T. graeca)* an overall prevalence of 80.2% was found at the copromicroscopic investigation. *Testudo marginata* scored a prevalence of 71.4% for endoparasites, while *T. hermanni and T. graeca* of 83.9% and 90.9%, respectively (x2=. The most recovered parasites were Oxyurids (75%), followed by the ciliate *Nyctoterus* spp. (16.7%) and Ascarids (3.1%). The 1% of prevalence was found for hookworms, coccidian and *Balantidium* spp., and 0.5% for tapeworms and *Giardia* spp.

The Ziehl–Neelsen staining revealed the presence of *Cryptosporidium* spp. oocysts in 10.4% of tortoises. Molecular investigation is currently underway for the genetic characterization of the *Cryptosporidium* isolates found in Sardinian tortoises. The results of this survey show oxyurids as the main parasites affecting testudo, isolated in almost all native tortoises, often with high prevalence. Another interesting aspect of this study is the finding in 10.4% of the examined animals of *Cryptosporidium* spp., species of potential zoonotic interest. In conclusion, we can say that the Sardinian tortoises have high incidence of parasites, although the examined animals show no apparent clinical manifestations.

Microscopic and histological investigations show that *Onchocerca dukei* is widespread in Tanzanian cattle

FM DINI[1], **G POGLAYEN**[1], **C BENAZZI**[1], **A GENTILE**[1], **B MORANDI**[2], **R GALUPPI**[1]

[1] Department of Veterinary Medical Sciences, University of Bologna, 40064 Ozzano Emilia (BO), Italy; [2] Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", via G. Salvemini1, Perugia, 06126, Italy.

Keywords: Meat safety, Parasitic diseases, Onchocerciasis, Tanzania

INTRODUCTION. Cattle is the most widely raised animal species in Tanzania, where livestock is one of the main agricultural activities. Slaughtering takes place mainly in rural slaughter slabs and municipal slaughterhouses, and according to local legislation it must be supervised by Official Veterinarians (VOs), who report any organs to be discarded and, after stamping the carcass, authorize its free consumption. The abstract aims to describe the results of laboratory analyses on yellowish nodular formations on the surface of the thoracic muscle attributed to cysticercosis during the VO inspection at the Songea slaughterhouse (Ruvuma Region, Tanzania).

MATERIALS AND METHODS. According to slaughtering records, nodules ascribed to *Cysticercus bovis* were found in 21 cattle over 614 slaughtered animals (3.4%). Eighteen muscular nodules were collected from different animals: seventeen samples were stored in 70% alcohol for parasitological examinations and one in formalin for histopathology. The collected samples were processed at the Department of Veterinary Medical Sciences, University of Bologna.

RESULTS AND CONCLUSIONS. Adult nematodes (male and female) and larvae mixed with necrotic material were observed during nodule dissection. Based on morphological characteristics the nematodes were identified as *Onchocerca dukei*, according to Bain et al. (1976 Ann Parassit. 51: 461-71; 1982 Ann Parassit. 57: 587-91) and Whal et al. (1994 Vet Parasitol. 52: 297-311). Histologically the muscle nodule appeared as a typical parasitic granuloma with a central area showing transverse and longitudinal sections of nematodes surrounded by necrotic cellular debris, inflammatory cells, and limited by concentric fibrous reaction. *O. dukei* has been described in several African countries (Hira et al., 1978 Ann Parassit. 53: 309-10; Vuong et al.,1994 Revue Élev Méd Vét Pays Trop. 47: 47-51), but knowledges about its presence in Tanzania are lacking. Our report will therefore be useful in considering muscular onchocerciasis in differential diagnosis of cysticercosis in Tanzania. The possibility of having basic, very simple and inexpensive laboratory diagnostics as an aid to visual inspection may be helpful, decreasing the quantity of condemned meat at the slaughterhouse.

Influence of management measures on gastrointestinal parasites in dairy cattle herds in northern Italy

AL GAZZONIS, SA ZANZANI, L VILLA, G ALOISIO, E MIGLIORATI, MT MANFREDI

Department of Veterinary Medicine, Università degli Studi di Milano, Milan, Italy.

Keywords: Gastrointestinal nematodes, *Eimeria* spp., Dairy cattle, Biosecurity measures

INTRODUCTION. Among endoparasites of dairy cattle, gastrointestinal nematodes (GIN) and *Eimeria* spp. have a significant impact affecting animal health, welfare and productivity. Since several risk factors related to management and biosecurity measures implemented in farms may influence parasites dynamics and spread even within intensive farms, an epidemiological survey focusing on GIN and *Eimeria* was planned with the aim of evaluate the impact of rearing system on parasitic infections in dairy cattle.

MATERIALS AND METHODS. Fecal samples of 495 animals from twenty dairy cattle farms in Lombardy region (northern Italy) were collected and analyzed by FLOTAC DUAL TECHNIQUE® (flotation solutions: NaCl and $ZnSO_4$, specific weight 1200 and 1350) (Cringoli et al., 2010 Nature Protocols. 5: 503-15). Besides individual and herd information, data on management measures (comprising facilities, sanitary and biosafety procedures) were collected through a questionnaire including 29 dichotomic questions. On the basis of obtained data, for each farm a management measures score (MMS) was calculated and analyzed through generalized linear models (GLMs) to evaluate its effect on GIN and *Eimeria* egg/oocysts excretion.

RESULTS AND CONCLUSIONS. Both GIN and*Eimeria* were found to be widely spread at individual (16.6% and 45.9%, mean EPG/OPG=3.1 and 43.6) and farm level (63.1% and 89.5%). GLMs showed that MMS had a different impact on GIN and *Eimeria* considering production categories (two-way interaction between productive category × MMS: p=0.21 and p=0.0001). Dry cows, showing prevalence and parasitic load values similar to those of heifers (P=28.6%, mean EPG=5.1, and P=26.5%, mean EPG=3.8, respectively), were most at risk of GIN infection in low-scoring farms, while lactating cows were the least risk category in high-scoring farms. A high MMS represented a protective factor against *Eimeria* in calves aged 1 to 6 months (P=52.1%, mean OPG=91.3) more than in heifers (P=90.9%, mean OPG=95.6). The application of appropriate managerial and sanitary measures, especially targeting heifers and dry cows, may therefore reduce the risk of parasitic infections also in other categories, such as lactating cows and calves, having a positive impact on the whole herd.

Coccidiostatic effects of two condensed and hydrolysable tannin mixes used as feed additives in rabbits

A GRECO, S MINIERI, S MANCINI, I ALTOMONTE, G ROCCHIGIANI, D GATTA, G PACI, S PERRUCCI

Dipartimento di Scienze Veterinarie- Università di Pisa, Viale delle Piagge n. 2, 56124 Pisa, Italy.

Keywords: Rabbit, *Eimeria* spp., Tannins, Coccidiostatic effects

INTRODUCTION. Coccidiosis is one of the most important rabbit diseases. Due to anticoccidial resistance issues and drug use limitations in farm rabbits, alternative strategies for the control of rabbit coccidiosis are needed. This study was aimed to evaluate the coccidiostatic effects of two condensed and hydrolysable tannin mix concentrations in rabbits.

MATERIALS AND METHODS. Sixteen rabbits of 35 days in age and naturally infected by coccidia, were used in the study. Rabbits were single caged and randomly divided into four different groups (C, TC, T 0.3%, and T 0.6%) of four animals each that were fed with different diets for a period of nine weeks. Group C (untreated control) received a commercial pelleted feed. Group TC (treated control) was fed with the pelleted feed supplemented with 1 ppm diclazuril. T 0.3% and T 0.6% groups were fed with the pelleted feed supplemented with 0.3% and 0.6% tannin mix, respectively. For the evaluation of the anticoccidial effects, individual faecal samples were collected weekly from each rabbit and quantitatively analysed for counting *Eimeria* spp. oocysts (oocyst number per gram of faeces, OPG). Involved *Eimeria* species were identified based on their morphological, metrical, and molecular features. Data were statistically evaluated.

RESULTS AND CONCLUSIONS. Overall, a lower mean *Eimeria* OPG number was observed in T 0.3% and T 0.6% groups compared to controls. Moreover, the mean *Eimeria* OPG number recorded in T 0.6% group at the 5 sampling was significantly lower ($p < 0.01$) than that of all other groups. No significant differences in food digestibility were observed among groups. *E. intestinalis*, *E. exigua, E. magna, E. coecicola, E. perforans, E. media, E. stiedae, E. flavescens, E. vejdoskyi* and *E. nagpurensis*, were identified. Obtained results confirm the anticoccidian properties previously reported for tannins and are promising about the use of the 0.6% tannin mix evaluated in this study as a coccidiostatic in rabbits.

Molecular approach for contemporary detection of *Hepatozoon* spp. and *Cytauxzoon* spp. in felids: preliminary data

M GRILLINI, G SIMONATO, G DOTTO, C TESSARIN, A FRANGIPANE DI REGALBONO

Department of Animal Medicine, Productions and Health, University of Padova, Legnaro, Italy.

Keywords: *Cytauxzoon*, *Hepatozoon*, Cat, PCR

INTRODUCTION. Cytauxzoonosis and hepatozoonosis are emerging vector-borne protozoan diseases affecting both domestic and wild felids. Usually, felids are asymptomatic but with persistent blood parasitaemia suggesting their potential role as reservoir (Díaz-Regañón et al.,2017 Parasit Vectors. 10:112). No molecular procedure detecting both protozoa is nowadays available, thus the aim of this study was to define a rapid molecular assay able to simultaneously detect these protozoa.

MATERIALS AND METHODS. K_3EDTA blood samples of owned and free-ranging cats were included for assay validation. Primers were designed to amplify a ~300 bp region of the 18S-rRNA gene of Piroplasmida order by SYBR green real time PCR. Results were achieved through the melting curve analysis. Specificity was verified using different strains of protozoa (ATCC isolates or field samples positive to *Cytauxzoon* spp., *Hepatozoon* spp., *Babesia/Theileria* spp., *Leishmania* spp., *Toxoplasma* spp.) and bacterial isolates. Sensitivity tests are still ongoing.

RESULTS AND CONCLUSIONS. This assay showed high specificity, accuracy and absolute repeatability. No cross-reactivity was observed with bacteria or other protozoa belonging to the same order. Moreover, different Genera can be distinguished by melting curve analyses: *Babesia* spp./*Theileria* spp. showed a melting temperature (Tm) of 76°C, *Hepatozoon* spp. of 78°C and *Cytauxzoon* spp. of 81.5°C (Fig. 1). However, since most of the blood samples/isolates used for validation were not certificated (i.e. due to the lack of ATCC strains) validation tests are ongoing to increase the number of blood samples and strains tested. If preliminary data will be confirmed, this procedure could represent an innovative and useful method, for both screening and diagnostic confirmation of Piroplasmida infections in felids.

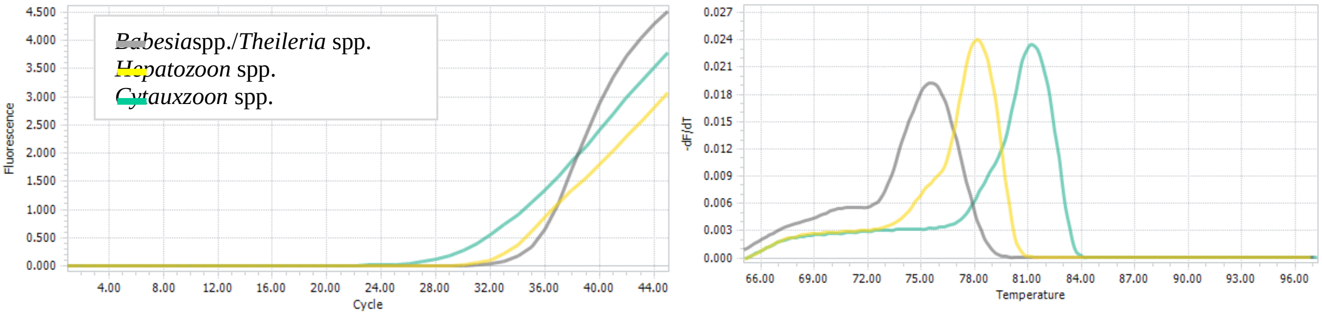

Figure 1: Amplification curves and melting peaks of positive controls.

A retrospective analysis of hookworm infection in dogs from southern Italy

S ILLIANO, L CIUCA, S PENNACCHIO, A BOSCO, MP MAURELLI, G CRINGOLI, L RINALDI

Department of Veterinary Medicine and Animal Production, University of Naples Federico II, CREMOPAR, Naples, Italy.

Keywords: Hookworms, Dogs, Southern Italy

INTRODUCTION. The aim of this retrospective study was to estimate the prevalence of hookworm infection in owned dogs and examine trends with age, gender and breed size by reviewing the data from routine diagnostic activity atthe Laboratory of Parasitology and Parasitic Diseases, Department of Veterinary Medicine and Animal Production, University of Napoli Federico II (PAR-UNINA).

MATERIALS AND METHODS. The retrospective study included data (January 2009-March 2021) from the copromicroscopical examinations (total samples=5,644) of owned dogs referred by veterinary practitioners to the PAR-UNINA labs. All the samples were tested by the FLOTAC technique (Cringoli et al., 2010 Nat Protoc. 5: 503-15) using NaCl (specific gravity 1200) as flotation solution and a detection limit of 2 eggs per gram (EPG) of faeces. The statistical analysis included Chi-Square tests and cross-tabulation to evaluate the association between the positivity for Ancylostomatidae and the anamnestic data regarding: gender (females=2519; males=3125); age, considering three age groups, i.e. 1-24 months (63.4%); 25-72 months (24.1%) and ≥73 months (12.5%); and breed size, i.e. small (26.9%), medium (55.9%) and large (17.2%).

RESULTS AND CONCLUSIONS. The results showed an overall prevalence of 14.3% (95%CI =13.4-15.2) for Ancylostomatidae with a mean eggs per gram of faeces (EPG) of 29.6 (min=2; max=556). Dogs in the age group 1-24 months (13.8%; 95%CI=12.7-15.0) showed significantly higher infection rates ($p< 0.05$) compared to older dogs. Moreover, there was a significant association ($p< 0.05$) between the positive dogs and the medium breed size group. No significant differences were found between the positive dogs and the gender ($p>0.05$). Although the study revealed the presence of hookworm infection in dogs from southern Italy, further molecular studies are needed to evaluate the occurrence of the hookworm species *Ancylostoma caninum* in single and/or mixed infections with *Uncinaria stenocephala*.

Development of a qPCR for the detection of *Chorioptes bovis* in equine skin scrapings

M ISAKSSON[1], G GRANDI[1,2], G GRÖNDAHL[1], S BORNSTEIN[1], I SKAAR[3], E OSTERMAN-LIND[1], K BERGVALL[2]

[1]National Veterinary Institute (SVA), Uppsala, Sweden; [2]Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden; [3]Norwegian Veterinary Institute, Oslo, Norway.

Keywords: *Chorioptes bovis*, Chorioptic mange, Horse, PCR

INTRODUCTION. Equine leg mange caused by *Chorioptes bovis*, often associated to pruritic pastern dermatitis, is a recognized welfare problem in horses. Diagnosis by direct microscopy can be challenging in field practice. Our aim was to develop a reliable, molecular tool for the detection of *C. bovis* DNA in skin scrapings.

MATERIALS AND METHODS. A new set of primers and Taqman probe were designed within the mithochondrial COI-gene sequence of *C. bovis*. DNA was extracted from four *C. bovis* positive and eight *C. bovis*-negative horses at microscopy sampled with different techniques. PCR analysis was run using 400 nM of each primer and 133 nM of the hydrolysis probe labelled with FAM-MGBNFQ (2 minutes of initial denaturation at 95ºC followed by 45 cycles of 95ºC for 5 seconds and 60ºC for 30 seconds). Samples were considered positive if the reaction curve exhibited the characteristic exponential shape and reached above the selected threshold based on the baselines in the samples and controls. To test the specificity of the assay, nine species of environmental mites were tested, all with negative results.

RESULTS AND CONCLUSIONS. The results from microscopy of the samples used for optimization of sampling were in general congruent with the PCR results, except in one case, when after acaricidal treatment a horse was still positive at PCR but negative at microscopy. While our PCR method showed a good specificity and sensitivity, it still needs to be tested on larger sample batches and the timing for post treatment follow-up analyses has to be set up.

Source of funding: Swedish-Norwegian Foundation for Equine Research

Multiple drug resistance in hookworms infecting greyhounds in the USA

PD JIMENEZ CASTRO[1], A VENKATESAN[2], E REDMAN[2], R CHEN[2], DA ZULUAGA SALAZAR, R AVRAMENKO, A MALATESTA[3], H HUFF[1], JS GILLEARD[2], RM KAPLAN

[1]Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA; [2]Department of Comparative Biology and Experimental Medicine, Faculty of Veterinary Medicine, Host-Parasite Interactions Program, University of Calgary, Alberta, Canada; [3]Laboratorio de Parasitología Veterinaria, Corporación Universitaria Santa Rosa de Cabal-UNISARC, Santa Rosa de Cabal, Risaralda, Colombia; [4]College of Veterinary Medicine, Tuskegee University, USA.

Keywords: *Ancylostoma caninum*, Multiple drug resistance, Greyhounds, Zoonosis

INTRODUCTION. The hookworm *Ancylostoma caninum* is the most prevalent nematode parasite of dogs. Recently, we confirmed multiple-drug resistance (MDR) in several *A. caninum* isolates to all anthelmintic drug classes approved for the treatment of hookworms in dogs in the United States (USA). Cases of MDR hookworms are highly overrepresented in greyhounds, suggesting that the MDR worms evolved on racing greyhound farms/kennels. The aims of this study were to evaluate the range of drug-resistant phenotypes and to investigate the molecular epidemiology of the *A. caninum* infecting greyhounds.

MATERIALS AND METHODS. Fecal samples from recently retired greyhounds originating from geographically diverse areas of the USA were acquired from three greyhound adoption kennels and three veterinary practices. The egg hatch assay (EHA) and the larval development assay (LDA) were used to measure resistance to benzimidazoles and macrocyclic lactones, respectively. The deep amplicon sequencing assay was used to identify and determine the frequency of non-synonymous SNPs at codons 167, 198, and 200 of the isotype 1-beta tubulin gene.

RESULTS AND CONCLUSIONS. 219 fecal samples were examined, with a mean FEC of 822.4 eggs per gram (EPG). We performed 23 EHA and 22 LDA on either individual or pooled feces, representing 81 animals. Mean and median IC_{50} and IC_{95} values for the EHA were 5.3 uM, 3.6 uM, and 24.5 uM, 23.4 uM, respectively. For the LDA, mean and median IC_{50} values were 749.8 nM, >1000 nM, respectively. These range 62 to 68 times higher than our susceptible laboratory isolates. For samples collected <10 days post-treatment with albendazole, moxidectin, or a combination of febantel-pyrantel-moxidectin, the mean FEC were 349.3, 333.3, and 834.8 EPG, respectively. We obtained DNA from 70 fecal samples, and deep sequencing of the isotype 1 beta-tubulin gene revealed the presence of the F167Y resistance polymorphism in 99% of these samples, with 69% having ≥75% resistant allele frequency.

These clinical, *in vitro,* and genetic data provide strong evidence that racing and recently retired greyhound dogs in the USA are infected with MDR *A. caninum* at very high levels in terms of both prevalence and intensity.

Animal welfare and parasite infections in organic and conventional dairy farms in central Italy: a pilot study

L LANZONI, M CHINCARINI, J DI PASQUALE, S MORELLI, G VIGNOLA, B PAOLETTI, A DI CESARE

University of Teramo, Faculty of Veterinary Medicine, Teramo, Italy.

Keywords: Sustainability, Dairy cattle, Organic farming, Gastrointestinal nematodes

INTRODUCTION. The 2021-2027 European Green Deal contains an “organic farming action plan”, thought to lead to more sustainable and adaptable farming. Low animal welfarestandardsand helminth infections have a heavy negative impact on the production efficiency (Orjales et al., 2017 Vet Parasitol. 243: 115-18; Dawkins et al., 2017 Anim Prod Sci. 2: 201-8).

The control of gastrointestinal (GI) helminthes in conventional (CONV) dairy farmsis primarily based on theuse of chemical compounds. This approach is increasingly unsustainable as anthelmintic resistance is spreading (Vercruysse et al., 2018 Parasitol, 145: 1655-64). The aim of the present pilot study was to evaluate the animal welfare and parasite prevalence in organic (ORG) and CONV dairy farms in order to achieve preliminary data useful to improve the system’s resilience.

MATERIALS AND METHODS. An on-farm welfare assessment (“AssureWel” protocol) and a parasitological investigations (flotation, sedimentation and McMaster techniques) were performed on a cluster of adult animals (Total: ORG-n=148; CONV-n=165) from 5 ORG and 5 CONV dairy farms in central Italy.A Chi-squared test was used to evaluate statistically significant associations ($p \leq 0.05$) using R statistical software (R Core Team 2020).

RESULTS AND CONCLUSIONS. The most significant result concerning animal welfare was the difference in the animals showing rear leg lesions between CONV (26.7%) and ORG farms (10.1%), ($p<0.01$). Anthelmintic treatments were used regularly in 4/5 CONV farms and in none of the ORGs. In 2 CONV farms (40%) and 4 ORGs (80%) at least one animal tested positive for GI parasites. No significant differences in parasites prevalence were identified between the two farming systems (ORG=10.9%; CONV=7.3%). Specifically, *Eimeria* spp. oocysts (ORG=7.5%; CONV=4.9%) and Strongylidae eggs (ORG=4.1%; CONV=4.9%) were found in ORG and CONV farms. A significant influence of cow’s parity on parasite prevalence was found in ORG farms, with a reduction of positive cases with the increase of age ($p=0.03$). Tolerance to GI parasite infections may be higher in livestock reared on ORG farms due to the continuous exposure to parasite infections that may lead to improved resilience (Orjales et al., 2017 Vet Parasitol. 243: 115-8). Further studies on welfare and sustainable approaches to parasite control, such as integrated control measures and targeted selective treatmentsare truly warranted.

Intestinal parasites of *Lepus corsicanus* (DE WINTON, 1898) in Sicily

F MACCHIONI[1], G ROMEO[2], V TROCCHI[3], F CECCHI[1], E ROMI[1], V SERGI[1], A COLI[1], C LENZI[1], G MONNI[1], L STANCAMPIANO[4]

[1]Department of Veterinary Science, University of Pisa; [2]Office for Hunting and Fishing Activities of Grosseto Regional Administration; [3]Federazione Italiana della Caccia (FIDC) Roma; [4]Department of Veterinary Medical Sciences, *Alma Mater Studiorum* – University of Bologna.

Keywords: Hare, Nematoda, Cestode, Coccidia

INTRODUCTION. The Italian hare, also known as Appenine hare, *Lepus corsicanus* (DE WINTON, 1898) is an endemic species. In Sicily, Italian hare is widespread with medium-low densities. Little knowledge is available to date about the parasitosis of this endemic species. The first and the only study on *L. corsicanus* parasites in Italy dates back to 2012 (Usaiet al., 2012 Helminthologia. 49: 71-77).

MATERIALS AND METHODS. Twenty-seven individuals of *L. corsicanus* (18 females and 9 males) were collected in the provinces of Messina, Catania and Agrigento (Sicily) in 2017 and 2018. The intestine was analyzed with the sedimentation and counting technique (Eckert et al., 2001WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern). Adult nematodes were isolated, counted, separated by gender, and microscopically examined. Specific identification of male nematodes was conducted according to taxonomical literature (Tenoraet al., 1986 Parasitol Hung. 19: 43-75). Cestodes were isolated and identification is still in progress. Coprological analyzes were carried out for all individuals (Mini-FLOTAC technique). Prevalence data were analyzed using Fisher exact test; abundance data of male parasites were analyzed using multivariate negative binomial regression with Stata 12.1.

RESULTS AND CONCLUSIONS. Two macroparasites were found: *Trichostrongylus retortaeformis* with a prevalence of 66.7% (95% CI 47.7-85.7), abundance 42, range 0-273 and Anoplocephalidae with a prevalence of 11.1%. No difference in *T. retortaeformis* prevalence was highlighted between adult and young hares, while males were almost significantly more infected than females (Fisher exact test, p=0.052). *T. retortaeformis* distribution was aggregated (Negative binomial k parameter 0.31) and its abundance was positively related to higher hare weight, although the relation was not fully statistically significant (p=0.054) and higher in males (p=0.009) while no significant differences was highlighted between young and adult hares (p=0.245). Aggregated parasites distribution suggests balanced *T. retortaeformis* host-relationship. Coprological analysis revealed 77.7% Coccidia prevalence and 48.1% strongyles-eggs prevalence. Further analysis is going to be performed, with special attention on parasite ecology.

Prevalence and risk factors of gastrointestinal parasite infections in show jumping horses in the district of Pisa, Italy

M MAESTRINI[1], G POLITANO[1], S MANCINI[1], MB MOLENTO[2], M LONGINOTTI[3], S PERRUCCI[1]

[1]Department of Veterinary Sciences, University of Pisa, Italy; [2]Department of Veterinary Medicine, University of Paraná, Brazil; [3]Veterinary practitioner, Horse Farm FM del B, Pisa, Italy.

Keywords: Horses, Gastrointestinal parasites, Nematodes, Protozoa, Anthelmintic treatment

INTRODUCTION. Gastrointestinal parasites are one of the major threats to equid health and welfare worldwide. Moreover, anthelmintic resistance is frequently observed in horse nematodes.

MATERIALS AND METHODS. This study was aimed to evaluate the prevalence and risk factors of gastrointestinal parasites in 110 show jumping horses from the district of Pisa. Individual fecal samples were examined by quali-quantitative parasitological techniques (Flotation and McMaster) and a commercial rapid immunoassay for the detection of *Giardia duodenalis* and *Cryptosporidium* spp. faecal antigens. Gastrointestinal strongyles were identified at the genus/species level. Differences about species composition and prevalence according to sex, type of housing, and age were statistically evaluated. Nematode-positive animals were treated with different anthelmintic protocols and examined after the treatment by using the Fecal Egg Count Reduction Test.

RESULTS AND CONCLUSIONS. 60 out of 110 horses (54.54%) scored positive for single or mixed infections. Identified parasites included cyathostomins (54/110, 49.09%), *Parascaris* spp. (7/110, 6.36%), *G. duodenalis* (2/110, 1.81%) and *Cryptosporidium* spp. (2/110, 1.81%). Nematode infections were found significantly prevalent ($p<0.05$) in 1-3 years old foals (6/7 positive animals). However, while *Parascaris* spp. was equally distributed among positive horses, cyathostomins were found prevalent ($p<0.05$) in horses maintained outdoor. Finally, 16 nematode-positive horses were found still positive (five horses for *Parascaris* spp. and 11 horses for cyathostomins) after the treatment with ivermectin or pyrantel pamoate. Results obtained underline the need of effective measures for the control of horse nematode infections in horse farms. Moreover, the lack of proper drug efficacyobserved in some treated horses may suggest nematode resistance.

Serological and molecular detection of *Babesia caballi* and *Theileria equi* in horses from Latium, between July 2018 and April 2021: a retrospective study

R MAGGI[1], **AG TADDEO**[1], **M COLOMBO**[2]

[1]Equine practitioner, Rome, Italy; [2]University of Teramo, Faculty of Veterinary Medicine, Teramo, Italy.

Keywords: Piroplasmosis, Ixodid ticks, qPCR, ELISA

INTRODUCTION. *Babesia caballi* and *Theileria equi* are Ixodid tick-transmitted protozoans, causative agents of equine piroplasmosis (EP), which has a worldwide economic impact on the equine industry. Although *B. caballi* and *T. equi* can cause similar clinical pictures, *T. equi* infection has a higher mortality rate compared to *B. caballi* (Butler et al., 2005 Tijdschr Diergeneeskd. 130: 726-31). Horses surviving clinical infection may remain inapparent carriers. The aim of this retrospective study is to document the prevalence of equine piroplasmosis (EP) in Latium using molecular and serological methods.

MATERIALS AND METHODS. A total of 95 horses living in Latium (Central Italy), sampled between July 2018 and April 2021, were included in the study. Out of 95 horses examined, 79 were subjected to both Enzyme-linked Immunosorbent Assay (ELISA) and to Real time PCR (qPCR) for both pathogens (Group A), 9 horses were examined using only ELISA (Group B) and 3 horses using only qPCR (Group C), 4 horses were subjected only to qPCR for *B. caballi* and ELISA for both pathogens (Group D).

RESULTS AND CONCLUSIONS. In Group A 26 horses (32.9%) had antibodies towards *T. equi*, while 22 horses (27.8%) were molecularly positive to *T. equi* and 3 (3.8 %) to *B. caballi*. In Group B 3 horses (33.3%) resulted positive to *T. equi*, in Group C 2 (66.7%) scored positive to *T. equi* and 1 (33.3%) to *B. caballi*. Within Group D 1 horse (25%) showed antibodies for *B. caballi*. In one horse a mixed infection by both pathogens was detected using qPCR. Total qPCR positivity was 29% for *T. equi* (A, C) and 4.6 % for *B. caballi* (A, C, D). Total seropositivity (A, B, D) was 31.5% for *T. equi* and 1.1% for *B. caballi*. These results show the higher presence of *T. equi* compared to *B. caballi*. Tick control and detection of horses acting as healthy carriers may help preventing horses to be infected, lowering the risk of clinical disease in endemic areas (Tirosh-Levy et al., 2020, Ticks Tick Borne Dis. 11: 1-12) and minimizing economical losses, as international movimentation often requires horses to be serologically and molecularly negative for EP. Few data are available in literature regarding EP epidemiology in Italy (Moretti et al., 2010 Vet. J. 184: 346-50; Bartolomè Del Pino et al., 2016 Ticks Tick Borne Dis. 7: 462-69), thus further studies are needed to better understand the distribution of EP in a larger population of horses from endemic and non-endemic areas to implement the control strategies.

Survey on the presence of *Leishmania infantum* in peridomestic rodents from the Emilia-Romagna region

A MAGRI[1], R GALUPPI[1], ML FIORAVANTI[1], M CAFFARA[1]

[1]Department of Veterinary Medical Sciences, Alma Mater StudiorumUniversitàdi Bologna, Ozzano dell'Emilia, 40064 Bologna, Italy.

Keywords: *Leishmania infantum*, Rodents, Epidemiology

INTRODUCTION. In the Mediterranean area *L. infantum* is the causative agent of the Zoonotic Visceral Leishmaniasis (ZVL), having the dog astraditionally recognized reservoir. Starting from the '70s (Pampiglione et al., 1982 G Mal Infett Parassit. 11: 1475-80) recurrent outbreaks of human leishmaniosis have been reported in the Emilia-Romagna (ER) region, where recent studies have found some human strains genetically different from those isolated in dogs of the same area (Rugna et al., 2017 Vector Borne Zoonotic Dis.17: 409-15). In this study the preliminary results on a survey ongoing on peridomestic rodentsarereported, to evaluate their possible role in the transmission of *L. infantum* in ER.

MATERIALS AND METHODS. Ninety-seven peridomestic rodents were collected during pest control programsfrom the provinces of Ravenna and Forlì-Cesena (ER): 27 brown rats (*Rattus norvegicus*), 29 black rats (*R. rattus*) and 41 mice (*Mus musculus*). When possible, according to the carcass condition, 25 mg of tissue were collected from ear lobes, prescapular lymph nodes and liver and 10 mg from spleen. DNA was extracted with commercial kit. A real-time PCR was performed targetingthe a 71bp conservative region ofkinetoplast minicircle DNA, as described by Tsakmakidis et al. (2017 Vet Parasitol Reg Stud Reports. 16: 100279).

RESULTS AND CONCLUSIONS. 11.34% of the rodents examinedtested positive for *L. infantum*, in particular 3 brown rats (11.11%), 3 black rats (10.34%), and 5 mice (12.19%). These values are lower than the ones found in other surveys from endemic Countries (Di Bella et al., 2003 J Mt Ecol. 7: 125-29; Helhazar et al., 2013 Parasites Vectors. 6: 88; Galáan-Puchades et al., 2019 Emerg Infect Dis. 25: 1222-24), but comparable to the one described in Montecristo Island (Zanet et al., 2014 Vet Parasitol. 199: 247-49), in absence of carnivore hosts. Most of the surveys from literature were conducted only on the spleen. In the present study 6 subjects were positive only in different tissues: liver or ear lobes or lymph nodes, suggesting that also other organs in addition to spleen should be examined during epidemiological surveys. Although some of the positive animals were from an area where, according to 2020 ER surveillance plan, phlebotomines and human cases are scant, these preliminary findings indicate the opportunity to further investigate the possible role of peridomestic rodents in *L. Infantum* epidemiology in the Emilia-Romagna region.

Comparing pooled with individual faecal samples for the assessment of gastrointestinal strongyles infection burden in goats

A MAURIZIO[1], C TESSARIN[1], L STANCAMPIANO[2], C ASTI[3], E MARCHIORI[1], R CASSINI[1]

[1]Department of Animal Medicine, Productions and Health, University of Padova; [2]Department of Veterinary Medical Sciences, University of Bologna; [3]Department of Parasitology, Ankara University, Turkey.

Keywords: Gastrointestinal strongyles, Goats, Sampling method, Faecal egg count

INTRODUCTION. Gastrointestinal strongyles (GIS) are a well-known problem for goats and their burden is typically monitored by faecal egg counts (FECs). Pooled samples can be used to reduce time and cost of the analyses, but they are less informative than individual FECs. This study aimed to compare the results obtained with pooled and individual FECs in goats, in order to evaluate whether significant differences exist between the two approaches.

MATERIALS AND METHODS. The study involved 11 farms located in Veneto and Friuli-Venezia Giulia. A total of 285 individual faecal samples were collected between November 2018 and September 2019 from the rectum of goats and 50 pools were composed thoroughly mixing 5 grams of faeces from 3-8 animals, according to the housing partition of the flock. FECs were performed for GIS on both individual and pooled samples by a modified McMaster method. A permutation test (100.000 iterations) based on Pearson correlation was performed in the statistical software R to verify the agreement between FECs from pools and average FECs from corresponding individual samples. For the latter it was also possible to estimate a 95% confidence interval, calculated as in Maurizio et al., 2021 Vet Sci. 8: 69.

RESULTS AND CONCLUSIONS. Pooled FECs were highly and positively correlated to individual counts, with a correlation coefficient r=0.88 ($p<0.001$). However, a tendency of pools to overestimate the abundance for high emission levels was recorded, in contrast with previous findings in sheep (Rinaldi et al., 2014 Vet Parasitol. 205: 216-23), as well as a tendency to underestimate it for lower egg outputs (Fig. 1). Furthermore, individual samples provided information about the heterogeneity of the sample, which significantly affected, at times (wide intervals), the interpretation of the egg output. In conclusion, while this study confirmed the validity of analysis based on pooled samples, it also highlighted they hold intrinsic limits that should not be ignored nor overlooked.

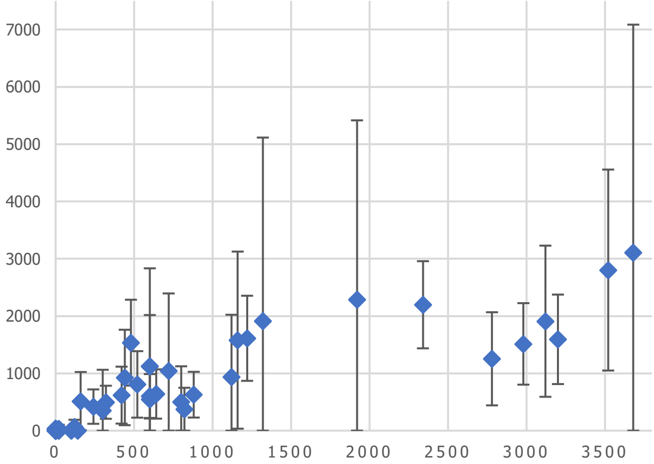

Figure 1. Correlation between eggs per gram of faeces (EPG) of pooled (x-axis) and individual (y-axis) FECs. Error bars represent 95% confidence intervals. The line of equality is indicated in blue.

Trials of oral treatments against gill monogenean parasites of *Sparus aurata* L.

P MERELLA[1], C BURREDDU[1], FE MONTERO ROYO[2], G GARIPPA[1]

[1]Dipartimento di Medicina Veterinaria, Università di Sassari, Italy; [2]ICBiBE, University of Valencia, Paterna, Spain.

Keywords: Aquaculture, Fish, Monogenea, Treatment

INTRODUCTION. The hematophagous gill parasite *Sparicotyle chrysophrii* (Monogenea: Microcotylidae) has become one of the main harmful pathogens of the gilthead sea bream *Sparus aurata* in cage culture, causing important economic losses. The control of monogeneans in sea cages with tarpaulin baths is a common practice, but its useis complex and implies environmental impact, health risks for workers and high cost of resources and personnel. On the other hand, oral treatments are practical and have limited environmental implications. In-feed treatments against monogeneans have been poorly tested for the gilt head sea bream. In this study, the efficacy of feed supplemented with chemical (praziquantel and fenbendazole) and natural (garlic and caprylic acid) compounds against *S. chrysophrii* on *S. aurata* is evaluated.

MATERIALS AND METHODS. To test the efficacy of oral treatments, from November 2018 to September 2019 five trials were carried out. For each trial, 75 infected fish (30-70 g total weight) were divided equally into three groups (one control and two treated) and kept four weeks in independent 142 L aquaria. During trials, fish were fed six days a week, with a 2% ration. The control group was fed with Vita 2 Veronesi, the others with the same feed supplemented with naturals/chemicals, in order to administer: garlic, 400 mg/kg BW/day; caprylic acid, 400 mg/kg BW/day; praziquantel, 50 mg/kg BW/day; fenbendazole, 75 mg/kg BW/day. Not ingested feed was removed to assess the effectiveration and estimating the actual dose of compound administered. Weekly, the gills of five specimens per group were examined for monogeneansat thebeginning of the experiment (T0), and at 7, 14, 21 and 28 days.

RESULTS AND CONCLUSIONS. Results showed the efficacy offenbendazole against *S. chrysophrii*, and that a two-doses treatment of 75 mg/kg BW/day allowed to eliminate all the parasites. Instead, praziquantel had a variable efficacy, mainly related to its poor palatability. Regarding natural treatments, garlic did not contribute to a reduction of the infection, while caprylic acid showed possible long-term efficacy. Further investigations will allow the development of tools for the control of monogeneans in aquaculture, crucial to increase performance and improve fish quality.

ACKNOWLEDGMENTS. Thanks to: Bayer Vietnam Co, Animal Health, Aquaculture Business Unit (Ho Chi Minh City); Veronesi Aqua Italia (Verona); Roha Italy (Verona); Compagnie Ittiche Riunite (Golfo Aranci), Acquacoltura Palma D'Oro (Stintino); Acquario di Cala Gonone (Dorgali).

Work supported by the resources of Fondazione di Sardegna - Call 2015

Acaricidal treatment with formic acid shortens honey bee pupal lenght: aComputed Tomography-based study

G MESZAROS, R RIZZI, J BASSI, M DI GIANCAMILLO, M MORTARINO

Department of Veterinary Medicine, Università degli Studi di Milano, Lodi, Italy.

Keywords: Honey bee pupal length, *Varroa destructor*, Formic acid, Computed Tomography

INTRODUCTION. Formic acid (FA) when used against the mite *Varroa destructor* in honey bee coloniesis reported to have deleterious, acute effects on brood development, detectable in most cases by visual inspection of the brood combs (Pietropaoli et al, 2019 J Apic Res 58: 824-30). Moreover, possible subacute effects of FA on honey bee brood are not sufficiently known, due to the difficulties of carrying out in-vivo investigations inside the capped brood cells. In this study, a non-invasive Computed Tomography (CT) technique was used to assess a subclinical effect of formic acid miticide treatment on honey bee brood.

MATERIALS AND METHODS. The study was performed on six honey bee colonies housed at the Centro Zootecnico Didattico Sperimentale of the University of Milan, starting from 5th June 2019. In each colony, a comb hosting eggs within a range of maximum two days age difference was obtained by temporary queen caging. On day 9th, these combs were taken from the corresponding colonies and submitted to the first CT examination with a GE Brightspeed™16-slices CT scanner. Immediately after the scans, each comb was returned to its colony of origin. Then, three colonies were treated with FA using two MAQS® gel pads (NOD Apiary Products Ltd) for each hive, and the remaining three colonies were left as untreated controls. On day 19th, the same combs as above underwent the second CT scanning. The CT scans were visualized by Weasis version 2.0.5 image viewer. For each comb, a library of approx. 1700 images was obtained, and a set of regularly spanned images (one out of 100 scans) was analyzed to assess the length of each pupa using the measuring tool provided by the program viewer. The data were analyzed by the GLM procedure of SAS® 9.4 program for the effect of treatment group and comb side on pupal length.

RESULTS AND CONCLUSIONS. A total of 521 pupae (386 in the control colonies, and 135 in the treated colonies) were measured. Pupae in the treated combs were significantly ($p<0.05$) shorter (9.92 ± 0.07 mm) than those one in the control group (10.71 ± 0.04 mm). This study supports the evaluation of hidden tolerability issues provided by miticidal treatments on honey bee brood by applying a non-invasive CT imaging technology.

Taenia multiceps coenurosis in adult sheep with neurological disorders

SA MIGNACCA[1,2], C TAMPONI[3], L MELONI[3], A VARCASIA[3], S KNOLL[3], F NONNIS[3], L CAVALLO[1], F PORCU[3], C PASINI[3], MF SINI[3], G DESSÌ[3], L MANUNTA[3], A SCALA[3]

[1]DVM freelance, Enna, Italy; [2]Current address: Animal and Plant Health Agency, Weybridge, UK;[3]Department of Veterinary Medicine, Universityof Sassari, Italy.

Keywords: *Coenurus cerebralis*, Coenurosis, Sheep, *Taenia multiceps*

INTRODUCTION. Coenurosis (Gid)is a worldwide metacestodosis caused by the larval stage of *Taenia multiceps* (Leske, 1780 Leipzig, Octavo, 52). In endemic areas, the chronic form in young animals under 18-24 months of age have often been described. However, outbreaks in adultsappear extremely rare due to the immunocompetence after continual contact with the parasite (Gauci et al., 2008 Int J Parasitol. 38: 1041-50). The authors herein describe outbreaks of coenurosis in adult animals reared in endemic areas.

MATERIALS AND METHODS. The present study involved 3 sheep dairy farms (Fs 1 and 2intensives; F3 semi-extensive), located in the main islands in Italy (Fs 1 and 2 in Sardinia and F3 in Sicily), in which Gid was suspected. Clinical and pathological investigations were performed, and epidemiological data were collected. Appropriate drug (Praziquantel) on selected heads of F3 was administered. On-farm health management measures were taken.

RESULTS AND CONCLUSIONS. On average, 4% of adults (2,5-6y-o), and 5% of young (4-18 m-o - only F2 was exposed to Gid) animals showed classical neurologic signs of Gid (apathy, ataxia, circling, head turn, and unilateral or bilateral amaurosis). On average, 3% of adults, and 2% of young animals died. Imaging technology (MR, TAC) and pathological exams performed on 5 adults, and on 2 young animals confirmed the presence of single or multiple cysts of different sizes within the brain. Survivors of Fs 1 and 2 were regularly slaughtered; in contrast, 5 out of 6 of the F3'streated animals were successfully recovered. A lack of immunocompetence, because of a previous absence of *T. multiceps* in the farms (associated with the indoor rearingin the Fs 1 and 2, and the rarity of definitive hosts and other flocks within the area of theF3), was hypothesized. This allowed the infection in a large number of animals since the parasites started to spread within the flocks, although Gid is endemic on both islands. This permitted the disease also in adult ewes between 2 and 6 years of age. In conclusion, investigation in small ruminants presenting neurological disorders should be encouraged, especially considering that some of them can have zoonotic relevance (Varcasia et al., 2013 Parasitol Res. 112: 2079-82).

Retrospective study on the parasites frequency of dogs referred to the Veterinary Teaching Hospital (VTH)-University of Bologna, combined to haematological results

B MORANDI[1,2], G ORLANDI[1], G POGLAYEN[1], M PIETRA[1], R GALUPPI[1]

[1]Department of Veterinary Medical Sciences, University of Bologna, 40064 Ozzano Emilia (BO), Italy; [2]Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", via G. Salvemini1, Perugia, 06126, Italy.

Keywords: Veterinary teaching hospital, Endoparasites, Dog, Epidemiology

INTRODUCTION. Although many studies on the frequency of endoparasites in dogs, in Italy, have been reported, no correlation with haematological results has been recently made. Parasitic infections are endemic worldwide and have been described as constituting the greatest single worldwide cause of illness and disease in animals and humans (Steketee, 2003 J Nutr. 133: 1661-67). Frequency and putative risk factors of endoparasite infections diagnosed through fecal examination of dogs submitted to the Veterinary Teaching Hospital (VTH) of the Department of Veterinary Medical Sciences of the University of Bologna were determined, combined to haematological profiles.

MATERIALS AND METHODS. Data were retrospectively obtained (January 1, 2011 - July 31, 2019) through management software Fenice® used by the VTH. Three specific datasets were built: one with the total of the fecal samples examined within the study period; and two were subsets of the first one, including just those which had a complete blood count (CBC) and serum chemistry, respectively. To be included in these two datasets haematological exams dated back no more than ten days respect to fecal examination. Investigated predictors of endoparasitism included sex, age, seasonality, eosinophilia, serum albumin, albumin-globulin ratio.

RESULTS AND CONCLUSIONS. A total of 1,972 dogs were evaluated for endoparasite status using specific diagnostic tests: direct smear, Baermann, flotation solution in a standardized centrifugal flotation method. The overall proportional infection was 10.4 %. The most frequent parasites recovered were coccidia around 3%, *Toxocara canis* (2.8%) and *Giardia duodenalis* (1.6%). Diagnoses occurred more often in winter months compared to the others (P<0.001). Age appears as the most relevant risk factors, where dogs younger than one year-old had 6 times higher probability of being diagnosed positive compared to older ones (OR=6.09; P<0.001). No significant association was detected analysing coprological examination and CBC parameters. On average, serum albumin was lower in parasitized animals compared to negative ones (t=4.105; P<0.001). These results are of value to estimate parasite impact and to assist researchers, veterinarians, and pet-owners with suitable information to control parasites.

Development of *Aelurostrongylus abstrusus* in hibernated *vs* non-hibernated *Cornu aspersum* snails

S MORELLI[1], M COLOMBO[1], A DIAKOU[2], D TRAVERSA[1], M GRILLINI[3], A DI CESARE[1]

[1]Faculty of Veterinary Medicine, University of Teramo, Italy; [2]School of Veterinary Medicine, Aristotle University of Thessaloniki, Greece; [3]Department of Animal Medicine, Production and Health, University of Padua, Legnaro, Italy.

Keywords: *Aelurostrongylus abstrusus*, *Cornu aspersum*, Larval developmental rate, L3

INTRODUCTION. The angiostrongylid *Aelurostrongylus abstrusus* inhabits the alveoli/alveolar ducts and bronchioles of domestic cats worldwide. First stage larvae (L1) are shed *via* the faeces in the environment, where they develop to the infectious third larval stage (L3) in gastropod intermediate hosts, i.e. slugs and snails (Morelli et al., 2021 Pathogens 10:454). Knowledge on factors influencing the larval developmental rate (LDR) inside mollusks is poor. Recent experiments have suggested that temperature has an impact on survival, vitality, infectivity and/or LDR to L3 of feline lungworms inside mollusks (Morelli et al., 2020 Vet. Parasitol. 282:109123; Napoli et al., 2020 J. Helminthol. 94: e113). The present study has investigated the effect of temperature on the LDR of *A. abstrusus* inside the land snail *C. aspersum*. The results are compared with those of a recent similar study on the crenosomatid *Troglostrongylus brevior*, which has shown that *T. brevior* increases its LDR in hibernated snails (Morelli et al., 2020 Vet. Parasitol. 282:109123).

MATERIALS AND METHODS. Overall, 300 farm-bred snails were infected with 500 L1 of *A. abstrusus* and kept in *vivaria* at 25 ± 2 °C. On day 15 post infection (D15), 20 snails were digested to assess the overall LDR to L3 (0.8%) and randomly divided in 2 groups, i.e. 180 kept in *vivaria* at 25 ± 2 °C (G1) and 100 hibernated at 4 ± 2 °C (G2). On D30 i) 20 snails from each group were digested to evaluate the LDR to L3 and ii) another batch of 80 snails were selected from those living in the *vivaria* and hibernated at 4 ± 2 °C, setting up the group 3 (G3), i.e. snails hibernated on D30. The LDR was determined digesting 20 snails from each group on D45, D60 and D75.

RESULTS AND CONCLUSIONS. An overall higher LDR of *A. abstrusus* was detected in G1, i.e. 2.8%, 1.4%, 6.0%, 5.0% (mean 3.8%), compared to G2, i.e. 3.2%, 2.8% 1.0% and 0.6% (mean 1.9%) on D30, D45, D60 and D75, respectively, and to G3, i.e. 2.0%, 3.5%, 1.5% (mean 2.3%) on D45, D60 and D75. These results indicate that the LDR of *A. abstrusus* is positively influenced by the increase of temperature. The larvae may also develop at low temperatures, though less efficiently than *T. brevior*. Overall, this study further corroborates the hypothesis that angiostrongylids may have lower cold-resistance abilities than crenosomatids (Morelli et al., 2020 Vet. Parasitol. 282:109123).

Molecular detection of *Leishmania infantum* in cutaneous lesions of horses from endemic CanL areas of northern and central Italy: preliminary results

G MORGANTI[1], AL GAZZONIS[2], P ROCCABIANCA[2], G AVALLONE[3], G RIGAMONTI[1], C BRACHELENTE[1], M DIAFERIA[1], F VERONESI[1]

[1]Department of Veterinary Medicine, University of Perugia, Italy; [2]Department of Veterinary Medicine, University of Milan, Italy; [3]Department of Veterinary Medical Sciences, University of Bologna, Italy.

Keywords: *Leishmania infantum*, Horse, Skin biopsies, Italy

INTRODUCTION. *Leishmania infantum* is a protozoan causing human zoonotic visceral leishmaniasis (ZVL) and visceral-cutaneous canine leishmaniosis (CanL) in the Mediterranean Basin. Besides dogs and cats, *L. infantum* may infect a large number of wild and livestock species, including horses, which could contribute as secondary hosts, reservoirs or just as good phlebotomine blood sources to the epidemiological scenario of the disease (Gazzonis et al., 2020 Parasit Vectors. 13). Since the nineties, both in the Old and New world, clinical cases of Equine Leishmaniasis (EL), typically characterized by papular and nodular skin lesions (Limeira et al., 2019 Rev Bras Parasitol Vet. 28: 574-81), have been increasingly reported, and low to moderate sero-prevalence levels have been found in different regions of the Mediterranean area (including Italy). Aim of the present study was to evaluate the presence of lesions possibly associated to EL in endemic CanL areas of northern and central Italy as these areas were previously investigated by an extended serological survey (Gazzonis et al., 2020 Parasit Vectors. 13).

MATERIALS AND METHODS. A retrospective study on overall 42 cutaneous biopsies (n. 20 from Perugia, n. 6 from Bologna, n. 16 from Milan) from horses with papular and/or nodular lesions, compatible with EL, was conducted. DNA was extracted from formalin-fixed paraffin-embedded samples and subjected to PCRs targeting a 120bp kinetoplast DNA and the internal trascribed spacer-1 (ITS-1) of ss-rRNA (Francino et al., 2006 Vet Parasitol. 137: 214-21; El Tai et al., 2000 Trans R Soc Trop Med Hyg. 94: 575-79). The amplified products were sequenced.

RESULTS AND CONCLUSIONS. A single positive *L. infantum* case for the kinetoplast DNA target was found from the Bologna caseload. The horse had a nodular lesion on the face, near the right ear. The histological examination revealed a lymphoplasmacytic dermatitis.Pathological and healthy skin samples (the latter collected at necropsy from animals died from unrelated causes) should be investigated on a larger scale, also by using immunohistochemistryanalysis, to provide information on the potential clinical impact of EL in the practice and the role of horses in the epidemiological ZVL and CanL scenario.

Mapping the spatial distribution of *Calicophoron daubneyi* infection in a Mediterranean area

M NOCERINO, A BOSCO, M SANTANIELLO, G CRINGOLI, L RINALDI

Department of Veterinary Medicine and Animal Production, University of Naples Federico II, CREMOPAR, Naples, Italy.

Keywords: *Calicophoron daubneyi*, Spatial modelling, GIS, Predictive model

INTRODUCTION. Rumen flukes, more specifically *Calicophoron daubneyi*, represent a growing threat to the health of small ruminants (Cringoli et al., 2004 Vet Parasitol. 122: 15-26; Sargison et al., 2016 Vet Parasitol. 219: 7-16). Geographical information systems (GIS) are configured as ideal technologies to determine environmental risk factors and to predict and delimitate the areas at risk of parasitic infection. The aim of this study was to assess the spatial distribution of *C. daubneyi* infection in sheep and goats in a Mediterranean area and to develop a predictive model of the geographical distribution of rumen flukes.

MATERIALS AND METHODS. A cross-sectional coprological survey was conducted in 682 sheep and 73 goat farms located in the Basilicata region (southern Italy). The faecal samples were analysed using the FLOTAC technique witha detection limit of 6 eggs per gram of feces (EPG) utilizing a zinc sulphate flotation solution (specific gravity = 1.35) (Cringoli et al., 2017 Nat Protoc. 12: 1723-32). The land use, the presence of streams and brooks, the soil texture, the elevation, the slope and the aspect of the study area, were the environmental variables investigated using the Arc-GIS Pro 2.7 software (ESRI). Univariate statistical analysis was conducted to assess the strength of association between environmental variables and farm positivity to *C. daubneyi*; the variables that showed significance were used as predictors in a stepwise logistic regression analysis using the SPSS software (Version 17). Furthermore, in order to develop a spatial predictive model of *C. daubneyi* distribution in Basilicata, an adaptation of the Random Forest algorithm was used to predict the presence/absence of the infection in study area.

RESULTS AND CONCLUSIONS. The results showed that 7.9% of sheep farms and 2.7% of goat farms were infected by *C. daubneyi*. The statistical analysis revealed altitude, soil texture and presence of streams and brooks as variables significantly ($P<0.05$) associated to the *C. daubneyi* distribution in study area. The findings demonstrated the efficacy of GIS for monitoring the spatial distribution or rumen flukes, providing useful tools to predict the parasitic infection in the study area and at other locations characterized by the same environmental explanatory variables.

Strongylid nematodes shared between domestic and wild ruminants in the Maiella National Park (Abruzzo region, central Italy)

B PAOLETTI[1], S ANGELUCCI[2], S MORELLI[1], C SMOGLICA[1], A BARLAAM[1], A DI CESARE[1]

[1]Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy; [2]Maiella National Park, Via Badia 28, Sulmona, Italy.

Keywords: Strongyles, Ruminant, Maiella National Park, Central Italy

INTRODUCTION. Strongylid nematodes (SN) are recognized as a major concern to livestock production. As the control of SN relies heavily on the use of parasiticides, anthelminthic resistance (AR) is increasing (Barone et al., 2020 Vet Parasitol. 279: 109041). It is known that wild ruminants may harbor several SN that typically infect livestock andit is assumed that they couldalso act as reservoirs in the translocation of anthelmintic resistance (Chintoan-Uta et al., 2014 Proc Biol Sci. 281: 20132985). This risk is even increased where domestic and wild ruminants graze on the same pastures. The aim of the present study was to obtain novel information on the distribution of SN in sheep and wild ruminants (i.e. red deer, chamois) sharing the same pasture in the Maiella National Park.

MATERIALS AND METHODS. A total of 20 faecal pool samples were collected from sheep (n. 7) and wild ruminants (n. 13) and examined by classic copromicroscopic tests. Gastrointestinal strongyle (GIS) positive pools were subjected to coprocolture. L3s were morphologically and molecularly identified (Gasser et al., 1993 Nucleic Acids Research. 21: 2525-26; van Wyk et al., 2013 Onderstepoort J Vet Res. 80: 539).

RESULTS AND CONCLUSIONS. Thirteen faecal pools were positive for GIS and 16 pools for bronchopulmonary strongyles (BPS) (Table 1). The present data confirm that wild ruminants may harbor strongyle species that usually affect sheep (Zaffaroni et al., 2000 Vet Parasitol. 90: 221-30). Further studies are required to investigate the pattern of transmission of strongyles between domestic and wild ruminants and the role of wildlife in the spread of anthelmintic-resistant nematodes.

Parasite	Sheep		Wild ruminants	
	MorphId	MolecId	MorphId	MolecId
Haemonchus contortus	yes	yes	yes	yes
Teladorsagia circumcincta	yes	yes	yes	yes
Trichostrongylus colubriformis	yes	yes	yes	yes
Oesophagostomum spp.	yes	yes	yes	no
Oesophagostomum venulosum	no	yes	no	yes
Chabertia ovina	yes	yes	no	no
Ostertagia-type group	no	n.d.	yes	n.d.
Ostertagia leptospicularis	no	no	no	yes
Muellerius capillaris	yes	n.d.	yes	n.d.
Neostrongylus linearis	no	n.d.	yes	n.d.

Table 1. Results of larval identification of L3 (GIS) and L1 (PBS) found in at least one faecal pool. MorphId: Morphologic Identification; MolecId: Molecular Identification; n.d.: not determined.

The study was carried out in the framework of the Project "Demetra" [Dipartimenti di Eccellenza 2018-2022, CUP_C46C18000530001], funded by the Italian Ministry for Education, University and Research.

Preliminary study on proventricular parasites of birds of prey in Italy: anatomo-histopathological findings

S PAVONE[1], A BAZZUCCHI[1], M GOBBI[1,2], A FRATTO[1], B CAPONI[1], R RINOLDO[1], T MARCHETTI[3], F VERONESI[4]

[1]Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Diagnostica Generale Umbria; [2]Associazione WildUmbria, Perugia (Italy); [3]Dipartimento di Medicina Veterinaria, Ospedale Veterinario Universitario Didattico (OVUD), Università degli Studi di Perugia; [4]Dipartimento di Medicina Veterinaria, Laboratorio di Parassitologia, Università degli Studi di Perugia.

Keywords: Birds of prey, Histology, Proventricular parasites

INTRODUCTION. Parasite survey of birds of prey have been conducted in Asiatic countries (Okulewicz 1997 PTP, Warsawa 1-43) as well as in many countries in Europe (Borgsteede et al., 2003 Acta Paras. 48: 200-07); however, little is known about the helminth fauna of birds of prey in Italy and lesions related to proventricular parasites. Objective of the present work is to contribute to the knowledge of the proventricular parasites.

MATERIALS AND METHODS. From 2020 to 2021, 41 birds of prey (Accipitriformes, Falconiformes and Strigiformes) rescued by a local wildlife rescue center (WildUmbria), have been investigated after death. The diagnosis of proventricular parasitosis was assessed on the presence of parasites detected free in the lumen of proventriculus in postmortem examination or histologically. Localization within the mucosa, sub-mucosa, deep gastric gland (DGG), tunica muscolaris and sierosa, was defined and a semi-quantitative (mild, moderate, severe) evaluation of lesions and parasitic burden were performed.

RESULTS AND CONCLUSIONS. Forty-one (73%) of the birds showed proventricular parasitosis. No correlation was found between histological lesions and body condition score (BCS) as well as age and gender ($p > 0.05$). Twenty-one animals (71%) showed mild, 8 (26%) moderate, and 1 (3%) severe parasitic burdens, respectively. The deep gastric gland seems to be the most involved area, and granulomatous lesions were often associated with parasites. The helminths were detected also in the *interstitium* and serosa, supporting a vascular involvement trough migration. Proventricular parasitosis appear to be a common problem of birds of prey of Italy. The parasitic burden is statistically related to the severity of the histological lesions. The vascular involvement (migration) seems to be a common event. A damage of secretory and contractile activity due to the parasitic destructive inflammatory lesions could be suggested. These injuries could be at the base of impairment of digestion, pellet formation and egestion ("casting") and therefore represent a predisposing factor to develop gastrointestinal problems.

Endoparasites in goat breedings of Sardinia, Italy

F PORCU, F FIORE, C TAMPONI, L MELONI, C PASINI, S KNOLL, MF SINI, A VARCASIA, L CAVALLO, G DESSÌ, A SCALA

Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Italy.

Keywords: Endoparasites, Goat, Sardinia, Copromicroscopic examination

INTRODUCTION. This study aimed to assess the prevalence of endoparasitic infections in dairy goat farms of Sardinia and to evaluate differences in the parasitic fauna of different age categories.

MATERIALS AND METHODS. The study was carried out in Sardinia from March 2019 to April 2021 and involved 217 dairy goats farms, that mainly rear Sarda breed and Saanen. The survey included a total of 352 fecal pooled samples, 256 from adult goats and 96 from replacement goats (<18 months). The samples were examined using the FLOTAC® technique with a zinc sulphate solution ($ZnSO_4$), specific gravity 1350, and eggs per gram (EPG) were determined according to Cringoli et al. (Cringoli et al., 2010 Nat Protoc. 5: 503-15).

RESULTS AND CONCLUSIONS. Gastrointestinalnematodes (GIN)were detected in 75.8% of adult goats and in 51% of replacement goats ($\chi 2=19.98$; $P<0.0001$). Bronco-pulmonary nematodes (BPN) were foundin 51.2% of adults and 14.6% of replacement goats ($\chi 2=38.58$; $P<0.0001$), and *Nematodirus* spp. in 22.3% of adults and 7.7% of replacement goat ($\chi 2=0.87$; P=0.35); *Strongyloides papillosus* in 3.5% of adults and 2.1% of replacement goats ($\chi 2=0.11$; P=0.73) and *Trichuris* spp. in 51.2% of adults and 46.9% of replacement goats ($\chi 2=0.51$; P=0.47). *Skrjabinema ovis*was found in 26.2% of adults and 18.8% of replacement goats ($\chi 2=0.09$; P=0.14); *Fasciola hepatica* in 0.8% of adults and 1% of replacement goats ($\chi 2=0.00$; P=1.00) and *Dicrocoelium dendriticum* in 12.9% of adults and 5.2% of replacement goats ($\chi 2=3.51$; P=0.06); Paramphistomatidaein 9.8% of adults and 2.1% of replacement goats ($\chi 2=4.78$; P=0.028). *Eimeria* spp. was detected in 92.2% of adults and 95.8% of replacement goats ($\chi 2=0.94$; P=0.33) and *Moniezia* spp.in 14.5% of adults and 10.4% of replacement goats ($\chi 2=0.98$; P=0.32). According to the results of the present study, genus *Eimeria* spp. appear to be the most widespread parasite, as well as GIN and *Trichuris* spp. in adults and replacement goats. Adults appear to be the age group most affected by endoparasites, although significant differences were found only for GIN, BPN and Paramphistomatidae.

Concurrent cutaneous mast cell tumor and *Leishmania* sp. infection in a dog

V RINALDI, PE CRISI, A BOARI, G MARRUCHELLA

Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy.

Keywords: Dog, *Leishmania* infection, Mast cell tumor

INTRODUCTION. Cutaneous mast cell tumor (cMCT) is the most common canine skin neoplasm. In particular, Shar-Pei dogs are highly predisposed to the development of poorly differentiated and very aggressive cMCT (White CRet al., 2011 J Am Anim Hosp Assoc. 47: 210–16). We describe herein the main pathological features of cMCT with concurrent *Leishmania* sp. infection, which has been recently observed in a dog.

MATERIALS AND METHODS. An 8-year-old, spayed Shar-Pei developed a large skin lesion in the axilla region. Considering cytological findings, a poorly differentiated cMCT was diagnosed. Thereafter, the neoplasm was surgically excised and routinely processed for microscopic investigations. In addition, immunohistochemistry for mast cell (CD117) and histiocytic (MAC387) markers was carried out on seriate tissue sections.

RESULTS AND CONCLUSIONS. Histopathology allowed to classify this neoplasm as a high and 3rd grade cMCT, according to Kiupel and Patnaik grading systems, respectively (Blackwood et al., 2012 VCO. 10: e1-e29). Notably, a huge number of *Leishmania* amastigotes were seen, almost exclusively in the cytoplasm of CD117/pos-MAC387/neg neoplastic cells. *Leishmania* infection was then confirmed by serological methods. The presence of *Leishmania* amastigotes has been rarely reported in non-histiocytic tumors and never documented in cMCT (Foglia Manzillo et al., 2008 Vet Clin Pathol. 37: 298-301; Ferro et al., 2013 Vet Pathol. 50: 749-52). Actually, *in vitro* and *in vivo* studies indicate that mast cells play a role in the immune response to *Leishmania* sp. (Naqvi et al., 2017 Nature. 7: 13240), being also able to uptake such protozoa. Therefore, we consider that the present case could raise a number of intriguing issues, which should be particularly addressed in areas of endemic leishmaniosis. On one side, our findings suggest that neoplastic mast cells might be suitable for infection, further widening the tissue tropism of *Leishmania* sp. From the host point of view, *Leishmania* infection might contribute to the onset and/or affect the prognosis of cMCT. Finally, the effect of antineoplastic therapy on leishmaniosis and vice versa, should be also carefully considered. Overall, the present case highlights the need to investigate and better understand whether and how *Leishmania* infection and cMCT could affect each other.

Absence of *Leishmania infantum* in cats with dermatological signs in an endemic area

L SALVATI[1], S MORELLI[1], A DI CESARE[1], E DI GIULIO[2], M COLOMBO[1], D TRAVERSA[1]

[1]Faculty of Veterinary Medicine, University of Teramo, Italy; [2]Veterinary Clinic "James Herriot", Roseto degli Abruzzi, Italy.

Keywords: *Leishmania infantum*, Feline leishmaniosis, Dermatology

INTRODUCTION. *Leishmania infantum* is increasingly reported in cats living in endemic areas (Morelli et al., 2020 Front Vet Sci. 7: 616566). Nevertheless, reports of clinical illness due to feline leishmaniosis (FeL) are still rare. The most common clinical signs compatible with FeL include mucocutaneous or skin lesions (e.g. ulcerative, crusting, exfoliative or nodular dermatitis) and lymph nodes enlargement (Pennisi et al., 2015 Parasit Vectors. 8: 302). However, FeL remains poorly characterized (Savioli et al., 2021 Parasit Vectors. 14: 217) and the role of *L. infantum* in the etiopathogenesis of feline dermatopathies is still unclear. This study evaluated the exposure to *L. infantum* in cats with dermatological lesions in an endemic area of Italy (Adriatic coast, Abruzzo region).

MATERIALS AND METHODS. Thirty-nine cats with dermatological clinical signs were included in the study. All cats were clinically examined and sampled for blood and sera. Sera were subjected to an immunofluorescent antibody test (IFAT) for the detection of specific IgG against *L. infantum*, with a dilution of 1:80, as previously described (Morelli et al., 2020 Front Vet Sci. 7: 616566). Cytological examinations from skin lesions were also performed.

RESULTS AND CONCLUSIONS. Alopecia, crusting dermatitis, nodular skin lesions and furfuraceous dermatitis were reported in 16 (41.02%), 14 (35.89%), 7 (17.94%) and 4 (10.25%) cats, respectively. Only one cat was seropositive for *L. infantum* (2.56%). This was a 14-years-old female cat showing a single ulcerated nodule on the thorax. Amastigotes were not found at cytological examinations of the nodule while the histopatology was compatible with a squamous cell carcinoma (SCC). The cat died after the diagnosis without the possibility of more in-depth studies. FeL is commonly associated with impaired immunocompetence as in case of retroviral co-infections and malignant neoplasia (Pennisi et al., 2015 Parasit Vectors. 8: 302). Indeed, amastigotes of *Leishmania* were previously found in neoplastic tissues of two cats with SCC (Grevot et al., 2005 Parasite. 12: 271-75). Although the sample size is limited, the present study further demonstrates that cats living in endemic areas are at risk to be infected by *L. infantum*. Nonetheless, it is still not clear to what extent FeL should be considered in the differential diagnosis of feline dermatopathies.

The influence of melatonin on the Faecal Egg Count of gastrointestinal nematodes in sheep

A SCALA[1], A SPEZZIGU[2], C TAMPONI[1], G DESSÌ[1], L MELONI[1], S KNOLL[1], F NONNIS[1], G COSSO[1], S NAITANA[1], V CARCANGIU[1]

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Italy; [2]Embryo Sardegna, Technology, Reproduction and Fertility, Perfugas, Sardegna, Italy.

Keywords: Sheep, Melatonin, Gastrointestinal nematodes, Fecal egg count

INTRODUCTION. High levels of melatonin in sheep result in a positive signal which activates the hypothalamic-pituitary-gonadal axis and lead to the resumption of the reproductive activity. In order to improve fertility indices and concentrate the birth/parturition, we proceeded with the subcutaneous melatonin implants treatments (SMI). Given the influence of melatonin also on the pathogenic trend of several bacterial and protozoal infections, and fluke infections (es. *Schistosoma* spp.) (Aline do Carmo França-Botelho, 2020 Clin Microbiol Infect Dis. 5: 1-2; El-Sokkary et al., 2002 Free Radic Biol Med. 32: 319-32), we aimed to evaluate the possible effects of this hormone on the Faecal Egg Count (FEC) of gastrointestinal nematodes (GIN) in sheep.

MATERIALS AND METHODS. In June 2020, 240 Sarda breed sheep, between 7 and 9 months of age and belonging to four different flocks of northern Sardinia, were divided into two groups (consisting of 120 animals each) uniform for EPG levels: at D1 the first group was treated (SMI) with 18 mg of melatonin (Melovine®, Ceva Salute Animale) while the other one represented the control group (NSMI). Faecal samples were collected from each sheep at D0, D30 and D60 and a qualitative-quantitative copromicroscopic exam (McMaster) was performed using a saturated NaCl solution (specific gravity = 1.2) in order to evaluate the FECs of the GIN.

RESULTS AND CONCLUSIONS. Data analysis revealed no significant difference between EPG means observed in the two groups of sheep at D 30 (377.5 ±330.2 SMI e 334.6±335.8 NSMI – Mann-Whitney=15213; P=0.162) and D60 (362.1±390.2 SMI e 303.1±339.8 NSMI – Mann-Whitney=15120.5; P=0.220). Data stratification for each farm did not show any significant difference at D30 and D60 among EPG means in three farms (P>0.05), whereas a significantly higher EPG mean was detected in one farm in sheep of the treated group at D30 (P=0.004) and D60 (P=0.007). In conclusion, the SMI did not result in any significant reduction of EPG means of GIN in Sarda breed sheep.

The research was funded by Regione Autonoma della Sardegna, Legge 7/2007, 2019 (RIPROGENOV).

Large-scale epidemiological survey on *Parascaris* spp. egg shedding in horses of Italy and conditioning factors

C TAMPONI[1], L MELONI[1], A VARCASIA[1], G DESSÌ[1], G SANNA[1], SEDDA G[1], S KNOLL[1], L CAVALLO[1], F PORCU[1], C PASINI[1], MF SINI[1], G PREDIERI[2], A SCALA[1]

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Italy; [2]ACME s.r.l., Reggio Emilia, Italy.

Keywords: Horses, Ascariosis, *Parascaris*, Italy

INTRODUCTION. The present study aimed to describe the spread of *Parascaris* spp. in Italy, through a large-scale epidemiological survey and further to identify risk factors associated with ascarid egg shedding.

MATERIALS AND METHODS. The sampling was carried out between 2015 and 2016, and a total of 6,896 horses, from 6 months to 36 years old, from 548 different stables located throughout all regions and islands of Italy were included. Each faecal sample was accompanied by a form filled in with the horse's information. Coprological examination was carried out through the modified McMaster method as described by Raynaud (Raynaud, 1970 Ann Parasitol. 45: 321-42), using a sodium chloride (NaCl) supersaturated solution (specific gravity = 1.2) for flotation and an egg detection limit of 15 eggs per gram (EPG) of faeces. Data were collected on a spreadsheet and subsequently analyzed to highlight any correlation between *Parascaris* spp. egg shedding and the animal's age, sex, housing conditions, geographic provenance as well as the respective sampling season.

RESULTS AND CONCLUSIONS. An overall prevalence of 6.3% (95% CI 5.8-6.9) was found and among the examined stables, 35.8% (95% CI 31.9–39.9) showed at least one horse to be positive for *Parascaris* spp. eggs. Proportionally, 80.0% of the recorded *Parascaris* spp. eggs were found in 0.7% of the examined animals (high shedders). Ascariosis prevalence rates tended to decrease significantly with age ($\chi 2=386.202$, df=6, $P<0.001$). Statistically significant differences among prevalence rates were found between the different geographic areas of provenance, increasing from south to north ($\chi 2=14.155$; df=2; $P=0.0008$) and prevalence was found to be higher in horses reared outdoors compared to those raised indoors ($\chi 2=23.119$; df=2; $P<0.001$). Analysis of data based on sex and season did not show any significant differences. The results of the present survey show, despite the low *Parascaris* spp. prevalence found, ascariosis to represent a health issue for horses reared in Italy. Age (especially foals and yearlings) and housing type (specifically outdoor rearing) were identified to be significant risk factors for *Parascaris* spp. egg shedding.

This research was partially funded by "Fondo di Ateneo per la ricerca 2019" of Prof. Antonio Scala of the University of Sassari, Italy.

First report of *Ktariella polyorchis* Vala & Euzet, 1977 (Monogenea: Calceostomatidae) infection in farmed meagre *Argyrosomus regius* (Asso, 1801) (Actinopterygii: Sciaenidae)

P TEDESCO[1], M KOLEGA[2], S ČOLAK[2], A GUSTINELLI[1], D MEJDANDŽIĆ[2], M CAFFARA[1], R BARIC[2], ML FIORAVANTI[1]

[1]Department of Veterinary Medical Sciences, Alma Mater Studiorum Università di Bologna, 40064 Ozzano Emilia (BO), Italy; [2]CROMARIS d.d., 23000 Zadar, Croatia.

Keywords: Aquaculture, *Argyrosomus regius*, Monogeneans, *Ktariella polyorchis*

INTRODUCTION. Monogeneans of the Family Calceostomatidae Parona & Perugia, 1890 parasitize different species of Sciaenids worldwide: among these, the calceostomatid *Ktariella polyorchis* Vala & Euzet, 1977 has been described infecting the gills of wild meagre *Argyrosomus regius* (Asso, 1801) caught off Tunisia and Egypt. The present investigation reports for the first time the occurrence of *K. polyorchis* in broodstock of *A. regius* from a Croatian farm.

MATERIALS AND METHODS. At the farm, gills samples and skin scrapings were collected from heavily infected fish and preserved in 70% ethanol and 10% buffered formalin. Ethanol-fixed parasites were subjected to morphological analysis following clarification in Amman's lactophenol. Before clearing the parasites, a section of the body without diagnostic characters was excised with a sterile scalpel and processed for molecular analysis, amplifying and sequencing the complete ITS and partial 28S rDNA. For observation in Scanning Electron Microscopy (SEM), formalin-fixed specimens were dehydrated in a graded ethanol series, critical point dried and sputter coated with gold-palladium.

RESULTS AND CONCLUSIONS. Although being reported mainly from the gills, in our samples *K. polyorchis* were mainly found on the fins and skin, while the gills were infected with another monogenean species, the diplectanid *Diplectanum sciaenae* Van Beneden & Hesse, 1863; this finding may indicate a possible displacement of *K. polyorchis* in concurrent heavy infections with *D. sciaenae*. Originally assigned to the genus *Calceostoma* Van Beneden, 1958 based on the morphology of the anterior region and of the armature in the haptor, *K. polyorchis* was subsequently assigned to a separate genus based on the morphology of the male reproductive system. Our study adds further morphological information, providing the first detailed description of the parasite surface by SEM analysis, and the first molecular data, useful in future taxonomical and phylogenetic studies.

Acute hemorrhagic meningoencephalomyelitis due to erratic migration of *Aelurostrongylus abstrusus* in a kitten

FV TINOCO[1], S MORELLI[2], MF BRITO[3], GO PEREIRA[3], MC OLIVEIRA[3], D TRAVERSA[2], M COLOMBO[2], M GRILLINI[4], A DI CESARE[2]

[1]Veterinária Cães & Gatos, Barra do Piraí, 27113-150, Brazil; [2]Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy; [3]Department of Epidemiology and Public Health, UFRRJ, Seropédica, RJ, 23897-000, Brazil; [4]Department of Animal Medicine, Production and Health, University of Padova, Legnaro, Padova, Italy.

Keywords: *Aelurostrongylus abstrusus*, Brain, Neurological signs, Aelurostrongylosis

INTRODUCTION. The cat lungworm *Aelurostrongylus abstrusus* occurs worldwide, including in Brazil (da Silva Lima et al., 2021 Pathogens 10:595). Although clinical aelurostrongylosis is a typical respiratory disease, sporadic cases of non-respiratory signs, e.g. life-threatening diarrhea caused by intestinal larval invasion, have been documented (Philbey et al., 2014 J. Comp. Pathol. 150:357-60). This study describes the first case of a fatal neurological disease due to *A. abstrusus* in a kitten.

MATERIALS AND METHODS. A 3 months-old kitten was presented to a veterinary clinic in Barra do Piraí, Brazil, with dehydration, pale mucous membranes, abasia and posterior bilateral myoclonus. The clinical picture of the kitten dramatically worsened, with generalized flaccid paralysis and death few days after hospitalization. As the histological examination revealed the presence of nematode stages, samples from lung, cerebellum and spinal cord were subjected to PCRs-coupled sequencing protocols specific for *Gurltia paralysans*, *Troglostrongylus brevior* and *A. abstrusus* (López-Contreras 2020, Animals 10:1169; Morelli et al., 2021 Pathogens 10:454).

RESULTS AND CONCLUSIONS. Areas of hemorrhagic necrosis in the left frontal telencephalic cortex, cerebellum and brainstem were found at necropsy. Subarachnoid hemorrhages were present in the cervical and lumbar tract of spinal cord. Lung histology revealed embryonated eggs and nematode sections in the alveolar lumen, atelectatic and emphysematous areas, mild congestion and lymphocytic/histiocytic inflammation. Larval nematodes were also present in the subarachnoid space of the brain and the spinal cord; these tissues had hemorrhages/congestion and cerebral lymphocytic, eosinophilic and neutrophilic infiltrates. Samples were PCR-positive with 100% identity with *A. abstrusus* from Colombia (Accession Number MH779453). This study shows that aelurostrongylosis may be fatal in young animals and describes for the first time a fatal neurological aelurostrongylosis due to larval embolization in the central nervous system, causing an acute hemorrhagic meningoencephalomyelitis. Similar neurological phenomena have been documented in dogs infected with the closely related angiostrongylid *Angiostrongylus vasorum* (Bourque et al., 2008 Can. Vet. J. 43:876-9; Wessman et al., 2006 Vet. Rec. 158:858-63). Hence, further studies are necessary to elucidate if unusual localizations and migration of *A. abstrusus* are more frequent than expected.

Importance of prophylaxis on parasitic diseases in fish used for experimental trials

M TOMASONI, V MENCONI, P PASTORINO, D MUGETTI, G ESPOSITO, M PREARO

Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino.

Keywords: Amoebic gill disease, *Hexamita* sp., *Ichthyophtirius multifillis*, Laboratory fish

INTRODUCTION. Many pathogens are responsible for diseases outbreak in tests animal species and can impair the result of research and lead to economic losses. Therefore, the health surveillance is a keypoint in experimental animal health (Lipman and Perkins, 2002 Laboratory Animal Medicine Second Edition p. 1143-84). The study of diseases in experimental fish has been developed only in recent years (Iaria et al., 2019, Lab Anim. 53: 619-29). This study is aimed to report episodes of parasitic infestation in rainbow trout (*Oncorhynchus mykiss*) used in nutritional trials, stressing the importance of good sanitary prevention.

MATERIALS AND METHODS. During 2020 a sanitary survey was performed on an experimental facility. Fishes were seasonally monitored (10 fish/month by each tank). Each fish was subjected to anatomo-pathological, bacteriological, parasitological and histopathological examination.

RESULTS AND CONCLUSIONS. The parasitological exams showed the following results: mass infestation of *Saprolegnia* sp., *Gyrodactylus* sp. and *Ichthyophtihrius multifiliis*. Severe mortality outbreaks were caused by *Hexamita* sp. gut infestation and a severe Amoebic Gill Disease (AGD), confirmed by histopathological exam. Parasitic diseases could manifest a subclinical course, which hamper sanitary control and leading to under diagnose the presence of parasites. This can lead to episodes with low mortality and at worst high mortality, compromising the results of the trial. Nowadays, most experimental fish species come from farms, and the absence of pathogens is no tensured (Barton et. al., 2016, Zebrafish. 13: S-39). To reduce the introduction of pathogens, fish should come from certified farms that applying continuous health monitoring. Before the introduction in the experimental trials, fishes shall be subjected to a prior quarantine. Reporting the occurrence of etiological agents contribute to improve the knowledge needed to develop effective sanitary procedures. This study highlights the potential impact of parasitic diseases on experimental fishes. In this context, future studies should be focused to improve the welfare of the test animals.

Looking for dog blood donors in low and high endemic areas for vector-borne infections of North and Central Italy

F TONIOLO[1], S RAVAGNAN[1], E PORCELLATO[1], S SGUBIN[1], A MIGLIO[2], A NATALE[1], A CARMINATO[1], M VASCELLARI[1], G CAPELLI[1], MT ANTOGNONI[2]

[1]Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell'Università,10, 35020 Legnaro, Padua, Italy; [2]Department of Veterinary Medicine, University of Perugia.

Keywords: Dog blood donors, Vector-borne infections, North and central Italy

INTRODUCTION. Vector-borne pathogens (VBP) can be transmitted by blood transfusion. The safety of donated blood with respect to VBPs is guaranteed by serological and molecular screening of donors. In this study, we assessed exposure to and circulation of relevant VBPs in selected dogs, donor candidates from northern (NI) and central Italy (CI).

MATERIALS AND METHODS. Two hundred eighty-seven dogs, 126 from NI and 161 from CI, were included in the study following these inclusion criteria: age 2-8 years, body weight ≥25 kg, regularly vaccinated and treated against endo/ectoparasites. All the dogs underwent a clinical examination and were screened by IF test for *Leishmania infantum*, *Ehrlichia canis* and *Anaplasma phagocytophilum*. Real time PCR assays were applied to blood for *L. infantum*, *E. canis*, *A. phagocytophilum*, *Babesia/Theileria* spp. and *Rickettsia* spp..

RESULTS AND CONCLUSIONS. The results are reported in Tab.1. CI dogs were more exposed to *L. infantum* (12.5% vs 4.8%), while dogs from NI were more exposed to *A. phagocytophilum* (27.3% vs 1.6%). No difference was found for *E. canis*. The majority of the dogs (94.5%) was clinically healthy. Only seven CI dogs showed mild clinical signs, six with lymph node enlargement and one with mild anemia. Four of these latter and other two apparently clinically healthy were positives to PCR and serology for *L. infantum*. PCRs for all the other pathogens were negative. The difference of positivity found for *Leishmania* and *Anaplasma* is consistent with the higher abundance of sandflies in CI and of *Ixodes ricinus* in NI. This study confirms that *L. infantum* is one of the pathogens most likely to be transmitted by transfusion, and a very strict screening of donor candidate is recommended.

	Leishmania infantum			***Ehrlichia canis***		***Anaplasma phagocytophilum***	
IF Title	NI	CI Ab	PCR	NI	CI	NI	CI
1:40	-	-		**2** (1.2%)	**2** (1.6%)	-	-
1:80	**4** (2.5%)	**5** (4.0%)	**2** (1.6%)	**2** (1.2%)	**2** (1.6%)	**25** (15.5%)	-
1:160	**1** (0.6%)	**2** (1.6%)	-	**1** (0.6%)	**1** (0.8%)	**13** (8.1%)	-
1:320	-	4 (3.2%)	**3** (2.4%)	-	-	**3** (1.9%)	**2** (1.6%)
1:640	-	-	-	-	-	**1** (0.6%)	-
1:1280	-	**1** (0.8%)	**1** (0.8%)	-	-	**2** (1.2%)	-
Tot	**5** (3.1%)	**12** (9.5%)^{a}	**6** (4.8%)^{a}	**5** (3.1%)	**5** (4.0%)	**44** (27.3%)^{A}	**2** (1.6%)^{A}

Table 1 – Number and percentage of dogs from Northern and Central Italy positive to serology and PCR. Significant differences in prevalence are marked with equal superscript letters (lowercase= $p<0.05$; uppercase= $p<0.01$)

Enhance the immunomodulatory capability of the bacterium *Asaia* with the expression of a *Wolbachia* protein: implication for the control of leishmaniases

I VAROTTO-BOCCAZZI[1], I ARNOLDI[1,2], P GABRIELI[1], R NODARI[1], B BISAGLIA[1], A NEGRI[1], M GRAMICCIA[3], L GRADONI[3], V TRANQUILLO[4], C BANDI[1], S EPIS[1]

[1]Department of Biosciences and Pediatric Clinical Research Center "Romeo and Enrica Invernizzi", University of Milan, Milan, Italy; [2]Department of Biology and Biotechnology, University of Pavia, Pavia, Italy; [3]Istituto Superiore di Sanità, Department of Infectious Diseases, Unit of Vector-Borne Diseases, Rome, Italy; [4]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Bergamo, Italy.

Keywords: M1/Th1 immune response, Leishmaniases, *Wolbachia* surface protein

INTRODUCTION. Leishmaniases are severe vector-borne diseases commonly spread in the tropical, subtropical and temperate zones whose outcome is strongly impaired by the type of immune polarisation. In fact, it is known that hosts polarized on the M1/Th1 side are normally protected, while those displaying a M2/Th2 response tend to develop chronic infection associated with parasite survival. The exploitation of molecules with immunotherapeutic properties in association with conventional drugs appears as a promising strategy for the control of this parasitic disease. In this respect, we focused on the bacterium of the genus *Asaia,* expressing the *Wolbachia* surface protein (*Asaia*^{WSP}), considering the evidence about the role of this *Wolbachia* protein as a pro-M1 immunomodulator. Accordingly, the aims of the present study were: i) to verify if the chimeric bacterium *Asaia*^{WSP} could induce a M1 macrophage polarisation; ii) to determine if this immune polarisation could influence the vitality of *Leishmania* parasites *in vitro*.

MATERIALS AND METHODS. Typical markers of M1/Th1 macrophage polarisation were selected and analysed using an immortalized macrophage cell line. Particularly, phagocytosis, M1/Th1-cytokines, nitrites, costimulatory molecules and the expression of iNOS enzyme were analyzed. Then, the leishmanicidal activity was evaluated using the same macrophage cell line and the parasite *Leishmania infantum,* as parasite model.

RESULTS AND CONCLUSIONS. The results of the assays on phagocytosis, on M1/M2 marker production and on co-stimulatory molecules expression, were all coherent in indicating that *Asaia*^{WSP} possesses a higher capability to induce a classic M1 activation compared to the control bacterium *Asaia*^{pHM4}. Moreover, this type of immunological activation was suitable to inhibit *L. infantum* development inducing a reduction of the number of amastigotes in challenged macrophages, with evidence for an increase in amastigote killing.

Parasitological monitoring of mammals endoparasites in an Italian faunistic park: effect of two prophylactic treatments with ivermectin on nematodes infections

L VILLA[1], AL GAZZONIS[1], D CARTAGENA[2], M MORTARINO[1], E BONACINA[2], D GUADAGNINI[2], C ALLIEVI[1], MT MANFREDI[1], SA ZANZANI[1]

[1]Department of Veterinary Medicine, Università degli Studi di Milano, Lodi, Italy; [2]Parco faunistico "Le Cornelle", Valbrembo, Italy.

Keywords: Endoparasites, Zoo mammals, Ivermectin, Control

INTRODUCTION. Parasite infections in zoo mammals are generally asymptomatic; however, these infections could also determine serious diseases, and some species are also zoonotic. This study aimed to evaluate the efficacy of two prophylactic treatments with ivermectin for the control of nematode infections in two groups of mammals housed in a faunistic park sited in northern Italy.

MATERIALS AND METHODS. The study included 21 species of herbivores treated twice/year (in March and November), daily for 30 days, with ad libitum administration of in-feed formulation of ivermectin (1.7 kg/ton). Besides, 13 carnivores and primates' species treated once a month, from March to November, with oral or subcutaneous administrations of ivermectin (200 µg/kg b.w.) were also sampled. For both groups, a "late spring-early summer" and an "autumn" sampling were performed. Overall, 52 (46 pooled and six individual) and 32 (28 pooled and four individual) fecal samples from 153 herbivorous mammals and 28 carnivores and primates were collected, respectively. FLOTAC® dual technique was performed using the flotation solutions FS2 (NaCl; s.g.=1200) and FS7 ($ZnSO_4$; s.g.=1350).

RESULTS AND CONCLUSIONS. Out of 84 fecal samples, 23 tested positive for at least one parasitic taxon (27.4%), with higher prevalence in the "autumn" (14/42, 33.3%) than in "late spring/early summer" sampling (9/42, 21.4%). Parasite infections were only evidenced in herbivores mammals: nematode eggs, i.e., *Nematodirus* spp. (9/52, 17.3%), *Capillaria* spp. (8/52, 15.4%), *Trichuris* spp. (8/52, 15.4%), *Parascaris* spp. (3/52, 5.8%) and Strongylida (2/52, 3.8%) were frequently observed (21/52, 40.4%); these results may indicate a lower effectiveness of the adopted prophylaxis in controlling genera such as *Nematodirus* spp., *Capillaria* spp. and *Trichuris* spp. Coccidian oocysts of *Eimeria* spp. were also detected (7/52, 13.5%). None of the samples from carnivores and primates were positive, indicating the effectiveness of the adopted monthly prophylactic treatment. Parasitological monitoring of mammals housed in faunistic parks could both provide information on the efficacy of prophylactic treatments, and indications to limit parasite circulation.

Condensed tannins in adult grazing goats: effect on gastrointestinal nematodes

SA ZANZANI[1], **AL GAZZONIS**[1], **L VILLA**[1], **M VILLA**[2], **G ZANATTA**[3], **MT MANFREDI**[1]

[1]Department of Veterinary Medicine, Università degli Studi di Milano, Lodi, Italy; [2]Veterinarian Pratictioner, Varese, Italy;

[3]Zootechnician, Varese, Italy.

Keywords: Goats, Condensed tannins, Gastrointestinal nematodes

INTRODUCTION. Gastrointestinal nematodes (GIN) infections are largely widespread in grazing goats. Among the alternative methods to conventional anthelmintic drugs for integrated control of GIN infections, condensed tannins (CT) have found various application in ruminants. This study aimed to evaluate the efficacy of a treatment protocol with Quebracho tannins (QT), a type of CT obtained from hardwood trees belonging to the Anacardiaceae family, to control natural GIN infections in a goat flock.

MATERIALS AND METHODS. The study included 28 adult Alpine goats from a single grazing flock in northern Italy (45°54'30" N;8°39'22" E). A group of 14 animals (treated group, T) received 200 gr/daily for three weeks of commercially available QT (Silvafeed BYPRO Q; Silvateam, San Michele Mondovi, Piedmont, Italy). The remaining 14 goats were allocated in the control group (C). Four individual faecal samples from the rectum of each enrolled animal were collected weekly and analyzed by FLOTAC® double technique, using the flotation solutions FS2 (NaCl; s.g.=1200), to determine the number of eggs per gram of faeces (EPG) at each sampling point (T0, T1, T2 and T3). Generalized linear mixed models (GLMM) were implemented by SPSS v. 20.0 (IBM, Chicago, IL).

RESULTS AND CONCLUSIONS. Mean EPG values of C goats at T0 and T3 were 2716 and 2316, respectively, while those recorded in T were 3813 and 825. A GLMM (categoric predictors: sampling point, C *versus* T, and their interaction) showed that in C, T3 logarithmically transformed EPG values (LogEPG) did not differ from T0, T1, and T2 LogEPG values. On the contrary, in T goats, T3 LogEPG values significantly differed from T0, T1, and T2 LogEPG values (p-values < 0.001), thus showing a significant reduction of faecal eggs output after three weeks of QT daily administration. In T, the mean body weight (bw) of enrolled animals was 60.8 kg, ranging from 49.1 to 72.9 kg; administered grams of QT/kg bw/day ranged from 4.1 to 2.7 in the lightest and the heaviest animal, respectively. A GLMM including only treated animals (predictors: sampling point (categorical), grams of QT/kg bw/day (continuous) and their interaction) showed that the most significant differences between T3 logEPG values and T0, T1 and T2 logEPG values were obtained in animals that received almost 3.2 grams of QT/kg bw/day.

POSTER SOIPA

PARASSITOLOGIA UMANA

What's in a child's ear? The first case of otomyiasis by *Sarcophaga argyrostoma* (Diptera, Sarcophagidae) and review of the literature

A BARLAAM[1,2], L PUTIGNANI[3], S PANE[3], RA PAPINI[4], A GIANGASPERO[1]

[1]Department of Agriculture, Food, Natural Resources and Engineering, University of Foggia, Foggia, Italy; [2]Faculty of Veterinary Medicine, University of Teramo, Italy; [3]Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; [4]Veterinary Teaching Hospital "Mario Modenato", Department of Veterinary Sciences, University of Pisa.

Keywords: Myiasis, *Sarcophaga argyrostoma*, Acute otitis media, Child

INTRODUCTION. Within the family Sarcophagidae, myiases-causing larvae of the species belonging to the genus *Sarcophaga* are rarely identified at the species level as identification only by microscopy can be challenging or even impossible. Therefore, cross-checking of the morphological and molecular data is highly recommended (Giangaspero et al. 2017, Sci World J. 2017: 9064531). We describe a rare case of aural myiasis in a child and review the literature on otomyiases caused by members of the Sarcophagidae family in humans and animals.

MATERIALS AND METHODS. A 1-year-old girl living in the countryside of Cerveteri town (Roma) was presented to the Emergency Room of Bambino Gesù Pediatric Hospital in Rome after experiencing nocturnal crying, pruritus and serosanguinous discharge from the right ear for about four days. The girl was subjected to ear cleaning with physiological solution, during which a larva was recovered. Ear examination revealed acute otitis mediain the left auditory canal. The larva was sent for identification to the Parasitology Unit of the University of Foggia, Italy, where it was microscopically observed and tested using a PCR protocol targeting a 710-bp gene fragment of the *cox1* gene-based DNA barcode with sequencing. The patient was treated with an antibiotic (Cefixime) for five days, and fifteen days later, the otitis had successfully resolved. Relevant research papers were searched and selected through the most common electronic databases.

RESULTS AND CONCLUSIONS. Morphological features only enabled us to suspect *Sarcophaga* (*Liopygia*) *argyrostoma*. Given the overlapping characters with *S.* (*L.*) *crassipalpis* and *S.* (*L.*) *cultellata*, molecular investigation was needed for confirmation. The BLAST analysis showed 100% homology with *Sarcophaga argyrostoma* (Diptera: Sarcophagidae) (AC: MZ189703). Worldwide, 34 (33 in humans, one in a goose) previous cases of otomyiasis caused by Sarcophagidae members are recorded: 18/34 (52.9%) by *Wohlfahrtia magnifica* and 7/34 (20.6%) by three species of *Sarcophaga*. In 9/34 (26.5%) cases, the larvae were only identified at the genus or family level. In 23/33 (69.7%) human cases, children were involved. This is the first report of human otomyiasis caused by *S. argyrostoma* in a child and the first supported by molecular analysis for identification.

Bioinformatically informed peptide microarray for the development of a specific and sensitive test for the diagnosis of hepatic cystic echinococcosis

G BATISTI BIFFIGNANDI[1], F TAMAROZZI[2], A VOLA[3], M MARICONTI[3], E OLIVIERI[4], L PETRONE[5], D SASSERA[4]

[1]Department of Clinical-Surgical, Diagnostic and Pediatric Sciences, University of Pavia - Pavia (Italy); [2]Department of Infectious-Tropical Diseases and Microbiology IRCCS Sacro Cuore Don Calabria Hospital, Negrar - Verona (Italy); [3]Unit of Microbiology and Virology, IRCCS San Matteo Hospital Foundation - Pavia (Italy); [4]Department of Biology and Biotechnology, University of Pavia - Pavia (Italy); [5]Translational Research Unit, National Institute for Infectious Diseases "Lazzaro Spallanzani" - IRCCS - Roma (Italy).

Keywords: Echinococcosis, Diagnosis, Bioinformatic

INTRODUCTION. Echinococcal cysts mostly develop in the liver, and are classified in active, inactive and transitional on the basis of imaging. Available serological tests for Cystic echinococcosis (CE) have unsatisfactory performances and do not reliably support the diagnosis and follow-up of CE when imaging is inconclusive or not available. This can result in CE misdiagnosis, mismanagement, and dispersion of health care resources. We developed a bioinformatic pipeline to identify new target antigens to improve the serodiagnosis of hepatic CE, both for parasite detection and discrimination of cyst stages.

MATERIALS AND METHODS. All proteins of the *E. granulosus* genome were retrieved and analyzed with a two steps bioinformatic workflow: 1) inference of the recognition by the immune system, integrating the results of multiple softwares for prediction of their cellular localization and function (TMHMM2, SignalP, predGPI and DeepLOC); 2) prediction of B-cell antigenic epitopes presence (Bepipred, SVMTrip and LBTOPE). Regions predicted to be highly antigenic regions were then synthesized and tested through a peptide microarray challenged with sera from patients with hepatic CE in different stages. Generalized linear model was used to assess sensitivity and specificity of each peptide.

RESULTS AND CONCLUSIONS. The bioinformatic pipeline allowed to predict the most immunogenic peptides from the 11,319 predicted proteins of *E. granulosus*. Manual integration of the results of multiple softwares allowed obtaining a set of 950 exposed proteins in step 1. These proteins were screened for presence of B-cell epitopes in step 2, obtaining 2,380 antigenic epitopes, then synthesized on a peptide microarray and tested for humoral response. Statistical analysis of the microarray results highlighted 4 candidates putatively useful for the diagnosis of CE infection (sensitivity≥76%, specificity≥60%), and 4 others to discriminate between active vs inactive cysts (sensitivity≥70%, specificity≥70%).

Development of a new molecular tool to study the transmission of *Plasmodium falciparum* parasite inside its main vector, the mosquito *Anopheles coluzzii*

G BEVIVINO[1], P AVELLINO[2], F SANTOLAMAZZA[3], F LOMBARDO[1], D MODIANO[1]

[1]Dept of Public Health and Infectious Diseases, Sapienza University of Rome, Italy; [2]Sant'Andrea Hospital, Rome, Italy; [3]Dept of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy.

Keywords: Malaria transmission, Human genetics, *Anopheles coluzzii*, *Plasmodium falciparum* ookinete

INTRODUCTION. Malaria transmission from human to mosquito strictly depends on the presence of *P. falciparum* gametocytes in the peripheral human blood. Human carriers of hemoglobin genetic variants (HbC and HbS) are less susceptible to malaria and simultaneously show an infectious capacity higher than wild type genotypes, contributing to malaria circulation as super-spreaders. Methodological approaches currently used to study malaria transmission dynamics involve: i) microscopic/molecular identification of gametocyte stages in the human peripheral blood and ii) identification of parasite's oocysts inside the midgut basal lamina of infected mosquitoes. However, these approaches are hampered by ethical, logistic and practical issues related to field and laboratory work. This project aims developing a new diagnostic molecular method, based on RTqPCR analysis of both genomic DNA and RNA extracted from single infected *An. coluzzii* mosquitoes, to investigate malaria transmission.

MATERIALS AND METHODS. Nucleic acids are extracted from single mosquitoes both to characterize the human genotype (genomic DNA) and to quantify *P. falciparum* ookinetes (RNA). Using this approach, we aim correlating the load of Plasmodium parasite with the human genetic background. The setting up of this protocol relies on i) the identification of suitable ookinetespecific markers and ii) the employment of blood fed mosquitoes infected with *P. falciparum*, collected at different time points spanning the formation and maturation of the ookinete stage inside mosquito midgut.

RESULTS AND DISCUSSION. Here we propose the use of infected mosquito as biological container of genetic information of all malaria cycle components (human, parasite and mosquito). Preliminary data revealed that human genetic polymorphisms are detectable by employing RTqPCR of genomic DNA extracted form single blood fed mosquitoes up to 24 hours post blood-feeding, corresponding to ookinete invasion of midgut. From diverse transcriptomic data, we have selected 4 associate-microneme genes (encoding for CTRP, WARP, SOAP and CHT1) showing ookinete-specific expression profile. We attempt to use these genes as molecular markers for ookinete identification and quantification inside infected *An. coluzzii* mosquitoes.

Analysis of IgG responses to the *Aedes albopictus* 34k2 salivary protein in individuals from Dengue and Chikungunya endemic areas

S BUEZO MONTERO[1], P GABRIELI[2], A POINSIGNON[3], BZ HUBERT ZAMBLE[3], F REMOUE[3], B ARCÀ[1]

[1]Department of Public Health and Infectious Diseases, Division of Parasitology, Sapienza University of Rome, Rome, Italy; [2]Department of Biosciences, University of Milan; [3]MIVEGEC, University of Montpellier, IRD, CNRS - Montpellier, France.

Keywords: *Aedes albopictus*, Dengue, Chikungunya

INTRODUCTION. The rapid world-wide spread of *Aedes albopictus*, a vector of arboviral diseases like chikungunya, dengueor Zika, points up the need for better vector surveillance and control. Human antibody response to mosquito salivary antigens is emerging as a relevant additional tool to directly assess vector-human contact, monitor efficacy of control interventions and evaluate the risk of arboviral transmission. Recently, we showed that IgG responses to the *Ae. albopictus* 34k2 salivary protein (al34k2) appear suitable to evaluate seasonal and spatial variations of human exposure to *Ae. albopictus* in conditions of natural exposure in a non-endemic area of Italy. The aim of this study was the validation of the al34k2 antigen in epidemiological settings with ongoing arboviral transmission maintained by *Ae. albopictus.*

MATERIALS AND METHODS. ELISA were used to measure human IgG responses to the al34k2 in adults from an area of Reunion Island where *Ae. Aegypti* is absent and *Ae. albopictus* represents the unique vector of chikungunya. In addition, to check the specificity and/or cross- reactivity of this biomarker, we also analyzed the IgG responses i) of the same individuals from Reunion Island to the orthologous 34k2 salivary protein from *Ae. aegypti* (ae34k2) and ii) of Bolivian subjects, only exposed to *Ae. aegypti*, to both al34k2 and ae34k2. A group of French individuals, not exposed to either *Ae. albopictus* and *Ae. aegypti*, was used as control group.

RESULTS AND CONCLUSIONS. Individuals from Reunion showed significantly higher IgG responses to al34k2 than to ae34k2 validating this antigen as a good and specific marker of human exposure to *Ae. Albopictus* in an endemic area. In contrast, IgG responses to the ae34k2 showed in both areas a low specificity and yielded a relatively high background, even in unexposed controls. These results provided a clear evidence that IgG responses to al34k2may represent a suitable marker of human exposure to *Ae. albopictus*. On the contrary, the *Ae. aegypti* orthologue ae34k2 does not appear suitable as marker of human exposure to *Ae. aegypti* due to the unspecific IgG response and a high background.

Leishmania infantum dampens neurotoxic NO release by infected macrophages in the presence of amyloid β

E CALVO-ALVAREZ[1], F PEREGO[1], D TARAMELLI[2], N BASILICO[1]

[1]Dipartimento di Scienze Biomediche, Chirurgiche e Odontoiatriche, Università degli Studi di Milano, Milano (Italy); [2]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milano (Italy).

Keywords: *Leishmania infantum*, Alzheimer's disease, Amyloid β, Nitric oxide

INTRODUCTION. The innate immune response and chronic neuroinflammation are at the forefront of Alzheimer's disease (AD) pathology, the most frequent cause of dementia worldwide (Heneka et al., 2015 Lancet Neurol. 14:388-405). Amyloid β (Aβ) deposition in the brain activates microglia (the macrophages of the CNS), which in turn release pro-inflammatory cytokines and neurotoxic free radicals, including nitric oxide (NO) which is toxic to neurons, exacerbating the neuroinflammatory response (Akama et al., 2000 J Biol Chem. 275:7918–24). Thus, interventions that downregulate the release of neurotoxic NO could be beneficial in AD. Interestingly, our group has recently demonstrated that *L. infantum* parasites inhibit the NLRP3 inflammasome in Aβ-treated human macrophages, which plays a major role in the neuroinflammation of AD (Saresella et al., 2020 Brain Behav Immun. 88:597–605). These parasites have evolved sophisticated mechanisms to subvert macrophage functions and innate inflammatory responses, including the release of microbicidal molecules such as deadly NO (Olivier et al., 2005 Clin Microbiol Rev 18:293–305). Here we have investigated whether *L. infantum* could also suppress toxic macrophage functions even in the presence of Aβ.

MATERIALS AND METHODS. Immortalized bone marrow derived macrophages (BMDM) (Hornung et al., 2008 Nat Immunol. 9:847) and murine microglia (Halle et al., 2008 Nat Immunol. 9:857–865), were infected with *L. infantum* parasites (1:10 cell:parasite ratio) and treated with bacterial LPS, IFN-γ and Aβ as inflammatory stimuli. After 24 h, the production of NO and the pro-inflammatory cytokine TNFα was assessed by the Griess assay and ELISA, respectively. The intensity of the infection was studied by Giemsa staining.

RESULTS AND CONCLUSIONS. Preliminary data indicate that *L. infantum* parasites are phagocytosed by both macrophage types, although microglial phagocytes exhibited a lower infection rate. In addition, *L. infantum* significantly reduced the production of both NO and TNFα in Aβ-treated macrophages without impacting cell viability, suggesting that the parasites and/or parasite-derived compounds might be potentially exploited as immunomodulatory molecules against inflammation and neurotoxicity in AD. Further investigations aiming at deciphering the exact role of *Leishmania* interfering with the NO signaling pathway in microglia will be undoubtedly needed.

Whole-genome sequencing analysis of populations with an uncertain species status at the Far-West of *Anopheles gambiae* range

B CAPUTO[1], CM DE MARCO[1], A MILES[2], A DELLA TORRE[1]

[1]Dipartimento di Sanità Pubblica e Malattie Infettive, Università Sapienza, Rome, Italy; [2]Wellcome Sanger Institute, UK.

Keywords: Mosquitoes, Malaria vectors, Genomics

INTRODUCTION. *Anopheles coluzzii* and *An. gambiae* are the main malaria vectors in sub-Saharan Africa. The two species have recently diverged and are strongly reproductively isolated throughout Africa, but stable frequencies of putative hybrids >20% have been reported from the far-west of the species range. Whole genome sequencing data by the*Anopheles gambiae* 1000 genomes project (Ag1000G; www.malariagen.net/projects/ag1000g) have attributed an “uncertain species status” to far-west populations.

MATERIALS AND METHODS. We carried out population genomic analyses (i.e. PCA, Admixture, Tess, Fst, F3 statistics, Patterson’s D statistics, TreeMix) on a dataset including 112 *An. coluzzii* and *An. gambiae*individuals from 7 far-west populations and 1,069 individuals from 20 populations from west and central Africa sequenced by Illumina technology within Ag1000G. We focused on the euchromatic regions of chromosome-3 (3L region 15-41 Mbp; 3R region 1-24 Mbp) which are considered neutral regions not affected by low recombination due to vicinity to centromere or presence of paracentric inversions.

RESULTS AND CONCLUSIONS. Results show evidence of a putative “far-west taxon” characterized by i) polymorphic chromosome-X IGS diagnostic markers; ii) *An. gambiae*-like chromosome-X pericentromeric region of interspecific divergence; iii) admixture of specie-specific autosomal ancestry informative markers. In addition, the putative “far-west taxon” shows i) a chromosome-3 unique gene-pool highly differentiated from west-African *An. coluzzii* and *An. gambiae* populations (PCA, Admixture, Tess, Fst, F3); ii) an origin not due to admixture between *An. coluzzii* and *An. gambiae* (Patterson’s D statistics, TreeMix); iii) evidence of gene-flow with west-African *An. coluzzii* populations, predating the insurgence of *kdr* resistant mutation conferring resistance to insecticides in this species (F3-statistics and *kdr* genotyping). Further demographic/phylogenetic and ecological/malariologial characterizations are needed to better clarify the actual taxonomic status of the “far-west taxon” and its role as malaria vector.

Intestinal parasitosis in Italy: results of the third AMCLI-CoSP national survey

D CROTTI[1,5], R GARGIULO[2,5], A RAGLIO[3,5], L PETRULLO[4,5], AMCLI-COSP WORKING GROUP[5]

[1]MD LP, Perugia; [2]UOC Microbiology and Virology, AUSL Modena, Italy; [3]ASST PAPA GIOVANNI XXIII Bergamo, Italy; [4]UOS Parasitology, AORN dei Colli, Cotugno Hospital, Napoli, Italy; [5]Working Group - Association of Italian Clinical Microbiologists-Study Committee for Parasitology: F Bernieri, MG Coppola, V Besutti, E Oliva, S Varani.

INTRODUCTION. The epidemiology of intestinal parasites in Italy is scarcely known. The objective of the third national epidemiological AMCLI-CoSP survey was to assess the prevalence of intestinal parasitosis and to verify the quality of the diagnostic methods adopted in relation to the recommendations indicated by AMCLI-CoSP.

MATERIALS AND METHODS. A dedicated form was distributed to all participants, on which statistical surveys were carried out on the frequency of protozoa and helminths. 29 diagnostic laboratories participate in this survey. The data collection instructions concerned the performance of the standard parasitological examination and the specific detection of *Enterobius vermicularis* (Ev), *Strongyloides stercoralis* (Ss), *Dientamoeba fragilis* (Df), *Entamoeba histolytica/dispar* (Ehd) and *Cryptosporidium* spp. (Cspp). The O&P was carried out on 57024 patients, the scotch test on 7985, the specific detection for Ss, Df, Cspp and Ehd on 511, 21263, 6850, 9453, respectively. Only 18 laboratories performed routinely Giemsa or Trichomic stain, 10 culture for Ss, 15 antigen test or culture for Ehd, 21 stain or antigen test for Cspp, and only 6 laboratories examined 3 or more samples from the same patient.

RESULTS AND CONCLUSIONS. According to the O&P, 4251 (7.5%) subjects were infected with one or more parasites. Pathogenic protozoa were detected in 1432 cases (2.5%), and helminths in 407 cases (0.7%). *G. duodenalis* was identified by O&P in 483 (0.8%) patients, Df in 776 (1.4%), and *C. belli* in 5 (<0.1%). The patients with O&P positive for helminths were: 188 (0.3%) for Ev; 42 (<0.1%) for Ss; 31 (<0.1%) for Hookworm; 19 (<0.1%) for *T. trichiura*, 11 (<0.1%) for *A. lumbricoides*; 68 (0.1%) for *Taenia* spp.; 17 (<0.1%) for *H. nana*; 10 (<0.1%) for *D. latum*; 18 (<0.1%) for *S. mansoni*; 1 (<0.1%) for *F. hepatica* and 3 (<0.1%) for *D. dentriticum*. The specific search for Ev revealed 1239 (15.5%) positive among 7985 patients, for Ss 30 (5.9%) in 511, for Ehd 98 (1.0%) in 9453, for Cspp 21 (0.3%) in 6850, for Df 933 (4.4%) in 21263. Helminths, excluding Ev, seem to be rare in Italy, whereas protozoa are relatively more frequent. Since few laboratories apply the recommended methods and analyse the necessary number of samples per subject, it is likely that detection rates are underestimated. There is the need to improve the knowledge of the correct diagnostic procedures for intestinal parasites and/or implement the use of new technology without losing the expertise in parasitology.

Hsp-70 polymorphism characterizes *Leishmania donovani* complex strains from Mediterranean area

T DI MUCCIO[1], D TONANZI[2], G LA ROSA[2], M GRAMICCIA[1]

[1]Vector-Borne Diseases Unit, Infectious Diseases Department, Istituto Superiore di Sanità, Rome, Italy; [2]European Union Reference Laboratory for Parasites, Infectious Diseases Department, Istituto Superiore di Sanità, Rome, Italy.

Keywords: *Leishmania donovani* complex, *Leishmania infantum*, Hsp70-RFLP, Genotyping

INTRODUCTION. *Leishmania donovani* complex (cx) includes *L. donovani* sp. and *L. infantum* sp., the aetiological agents of visceral (VL) and cutaneous (CL) leishmaniasis, species very close but characterized by different ecology and clinic. *L. infantum* is the main species causing leishmaniasisin Mediterranean area where it shows wide genetic polymorphism.Over the past few decades, several molecular targets have been proposed for *Leishmania* diagnosis and species typing, but not international guidelines were accepted so far.We analysed a large and geographically representative set of *L.donovani* cx strains by Hsp70 gene, according to suggestion of LeishMan consortium strategy for validation of this locus for typing (van der Awera et al., 2016 Euro Surveill. 21:49).

MATERIALS AND METHODS. Eighty-nine *L. infantum* strains (15 zymodemes, ZMONs) from Mediterranean area and five *L. donovani* strains (3 ZMONs) from Africa and India were submitted to Hsp70 sequencing and RFLP assays. The diagnostic results were compared with those obtained by ITS and cpB molecular targets already widely recognized as tool for molecular identification at taxonomic level.

RESULTS AND DISCUSSION. The results showed a good agreement between Hsp70, ITS and cpB typing. In addition, Hsp70 showed an interesting polymorphism dividing *L. infantum* ZMON24 strains in two clusters which correlated with clinical features rather than geographical origin. *L. infantum* ZMON24 VL strains resulted genetically similar to *L. infantum* sp, whereas ZMON24 CL strains showed hybrid traits *L. infantum/L. donovani* according to cpB analysis that showed the presence of both cpB copies, cpBE (*L. infantum* specific) and cpBF (*L. donovani* specific). We supported the validity of Hsp70assay for species typing. In addition, we found a reproducible discriminating Hsp70 PCR-RFLP typing method into *L. infantum* sp able to identify *L. infantum* ZMON24 CL strains that showed *L. infantum/L. donovani* traits. A putative hybrid event between *L. infantum* and *L. donovani* spp populations, widespread from Africa out over the Mediterranean area, could be supposed.

Evaluation of a multiplex Real-Time PCR assay for the diagnosis of human intestinal protozoa in Azienda Ospedaliero Universitaria Pisana

R FONNESU[1], R FAIS[1], G TRILLOCCO[1], E BALESTRI[1], A CARA[1], M PRATO[1], A LUPETTI[1,2], V MANGANO[1,2]

[1]Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia, Università di Pisa; [2]SD Microbiologia Universitaria, AOUP.

Keywords: Intestinal protozoa, Diagnosis, Multiplex Real Time PCR

INTRODUCTION. The gold standard for diagnosis of human infection with intestinal protozoa is microscopic examination of stool specimens following concentration. However, this method has limited sensitivity at low parasite densities, requiring observation of multiple specimens and specific staining. Furthermore, morphologically identical taxa with different pathogenicity such as species of the *Entamoeba histolytica* complex cannot be identified. This study aims to evaluate a new multiplex Real-Time PCR assay (Allplex™ GI-Parasite Assay, Seegene) for the detection of DNA of *Giardia duodenalis, E. histolytica* sensu stricto*, Cryptosporidium* spp.*, Cyclospora cayetanensis, Diantamoeba fragilis* and *Blastocystis hominis.*

MATERIALS AND METHODS. Multiplex Real-Time PCR was performed on: i) positive controls stool samples (N=9) to evaluate performance in alternative storage conditions (RT, 4°C, -20 °C, EtOH, formalin and Ecofix) from the validated one; ii) stool samples from patients (N=100) previously tested with an immunochromatographic test (ImmunoCard STAT! CGE, Meridian Bioscience) for antigen detection of *G. duodenalis, E. histolytica* complex and *Cryptosporidium* spp. to compare results of the two methods; iii) stool samples from patients (N=54) following introduction of the new method in the diagnostic routine to assess prevalence of infection.

RESULTS AND CONCLUSIONS. Preservation in EtOH showed 100% sensitivity andwas the best alternative to freezing, allowing storage at RT and thereby avoidance of the cold chain. Compared to ICT, multiplex Real-Time PCR was equally sensitive but allowed to discriminate between *E. dispar/moskovskii* and *E. histolytica* ss., as well as detection of *B. hominis* e *D. fragilis*. Prevalence of infection was: 0% for *E. histolytica* ss. and *C. cayetanensis*, 2% for *G. duodenalis* and *Cryptosporidium* spp., 13% for *D. fragilis* and 20% for *B. hominis*. The observed prevalence of *B. hominis* and *D. fragilis* is in line with data from Italy and other European countries reported in the literature. Detection of these protozoa is fundamental to gain new insights into their uncertain pathogenetic role, making multiplex Real-Time PCR a useful diagnostic and investigation tool.

Seroprevalence of *Trypanosoma cruzi* infection among school-age children in the Bolivian Chaco

S GABRIELLI[1], **M SPINICCI**[2], **F MACCHIONI**[3], **M STROHMEYER**[2], **M ROSELLI**[2], **A BARTOLONI**[2]

[1]Dipartimento di Sanità Pubblica e Malattie infettive, Roma, Italia; [2]Dipartimento Medicina Sperimentale e Clinica, Università degli Studi di Firenze, Firenze, Italia; [3]Dipartimento di Scienze Veterinarie, Università degli Studi di Pisa, Italia.

Keywords: Chagas disease, Bolivia, Seroprevalence, Children

INTRODUCTION. Chagas Disease is a neglected tropical vector-borne disease caused by the protozoan *Trypanosoma cruzi*, with some of the most serious manifestations affecting the cardiovascular system. It is a chronic, stigmatizing condition, closely associated with poverty, affecting close to 6 million people globally and endemic in 21 Latin American countries. Regional intergovernmental control programs, launched during the 1990s, allowed the interruption of the vector transmission in several Latin American countries (Gürtler 2009, Mem Inst Oswaldo Cruz. 4: 52-59). By contrast, such interventions obtained a limited success in the Bolivian Chaco, where the risk for *Triatomina infestans* transmission continued to be high, and the house's infection risk is still above 3% (Ministerio de Salud Estado Plurinacional de Bolivia 2017). Since the seroprevalence for *T. cruzi* increased with age, its evaluation in younger age classes is considered a reliable indicator of the impact of vector control interventions. Thus, in 2017, 2018 and 2019 we conducted a cross-sectional serosurvey for *T. cruzi* in school-aged children living in rural communities of the Cordillera and Gran Chaco Provinces to evaluate the active vector-borne transmission of CD in the Bolivian Chaco.

MATERIALS AND METHODS. Cross-sectional surveys were conducted in 9 primary schools, randomly selected as sentinel sites, involving N=500 children (8-9 y.o.). Serum samples extracted from dried blood spots collected from each child, were submitted to 2 serological assays to detect anti-*Trypanosoma cruzi* antibodies.

RESULTS AND CONCLUSIONS. Serological tests performed on a sub-sample of 120 subjects continuously recruited in all the 3 years, detected antibodies in 5.8%, 6.6%, 9.2% of the children during 2017, 2018 and 2019, respectively, showing an overall seroprevalence of 5.8%. Positive seroconversion was observed in 2.5% of the children. Collected data suggest that vector control intervention carried out in the studied areas didn't produce a drastic reduction of *T. cruzi* prevalence in children below 15 years of age, which still remains constantly about 6% (Spinicci et al., 2020J Infect Dev Ctries. 14: 199-206). Such unvaried prevalence value jointly with the detection of 2.5% of new infections in our limited sampling, suggests that other factors affecting *T. cruzi* transmission are also operating and control programs so far ongoing should be changed or increased.

Preliminary evaluation of a new Schistosoma Immunochromatographic Test

SS LONGONI[1], C PIUBELLI[1], F PERANDIN[1], E RIZZI[1], N LUCHETTA[1], M DEGANI[1], S TAIS[1], A MORI[1,2], Z BISOFFI[1,2]

[1]Department of Infectious-Tropical Diseases and Microbiology, IRCCS Sacro Cuore Don Calabria Hospital, Negrar di Valpolicella, Verona, Italy; [2]Department of Diagnostics and Public Health, University of Verona, Verona, Italy.

Keywords: *Schistosoma*, Antibodies, Diagnosis

INTRODUCTION. More than 90% of schistosomiasis infections occur in sub-Saharan Africa. Europe faced a huge wave of migration from low-middle income countries (LMIC), and the environmental changes could possibly promote the spread of infectious diseases worldwide. Temperate and tropical strains of *Schistosoma* can survive to the cold Mediterranean winter and permanently establish autochthonous *foci*. Most of *Schistosoma* infections are clinically silent for a long time and hence not routinely screened in at risk populations. A rapid ICT test would be an easy and cheap tool that could be implemented also in the field.

MATERIALS AND METHODS. Commercially available IVD-CE Pink-ICT (LDBIO Diagnostics) can be used only with serum or plasma, while the new Schistosoma black-latex based IgG-IgM ICT (Black-ICT), presenting a black latex matrix instead of pink, should avoid this limitation due to possible colour interferenceswith haematic samples. We evaluated the performances of the new Black-ICT, testing serum samples from *Schistosoma* infected patients. Positivity was determined by direct detection of *Schistosoma* sp. eggs in stools/urine and/or at least 2 concordant positive out of 4 different immunological tests. On this basis, 50 positive and 50 negative banked serum samples were analyzed with the new test. In order to evaluate the signal intensity of the Black-ICT, we developed a reference colorimetric signal scale. For each serum aliquot, Pink and Black-ICT tests were carried out following the manufacturer's instruction. Two experienced lab technicians, blinded to the specimen classification and to the respective results, independently read and interpreted the ICTs.

RESULTS AND CONCLUSIONS. The results of the new Black-ICT indicate a high sensitivity (49/50 or 98.0% for both technicians) while specificity was dependent on the application of the colorimetric scale, thus reaching 87.8%. In contrast to Pink-ICT, we observed that Black-ICT presented a weak background signal, which could mislead even a highly trained operator. In view of a possible use of this test on blood collected from finger prick, results are promising, providing that a background control is developed. A further prospective study evaluating the performance of the Black-ICT on whole blood from fingerprick should be planned.

Microscopic examination of indoor dust: a useful survey tool for diagnosis and verify of environmental contamination of human scabies mite *Sarcoptes scabiei*

P MASINI[1], S ZAMPETTI[2], F BIANCOLINI[2], G MIÑÓN LLERA[3]

[1]Cani Anti Cimici ©, Perugia, Italy, www.canianticimici.com; [2]Ecotrade Solution Srl, Roma, Italy, www.glispecialistidelladisinfestazione.com; [3]Biologist freelancer, Oviedo, Spain.

Keywords: *Sarcoptes scabiei*, Scabies, Scabies diagnosis, Microscopic examination of indoor dust

INTRODUCTION. The human scabies mite *Sarcoptes scabiei* var. *hominis* (Linnaeus, 1758) (Acarina: Sarcoptidae) is an obligate pararasite of humans that cause the condition called scabies (Arlian et al., 2017 Parasit Vectors 10(1): 297). An entire family (four adults) living in an apartment in Roma, Italy, was afflicted by itch and recurrent erythematous papular lesions. Since the dermatologist has suspected scabies, a microscopic examination of indoor dust was performed (Stingeni et al., 2017 JEADV 31(9): 1526-1533), in order to diagnose this parasitosis with certainty and verify the environmental contamination.

MATERIALS AND METHODS. In June 2018, a manual collection of 7 indoor dust samples was carried out by the patients themselves from every room/area. The dust samples were sifted and examined under a stereo-microscope (Motic SMZ168, Milan, Italy). *S. scabiei* mites were identified taxonomically placing them on slides in Berlese solution and observed by optical microscope (Motic BA310, Milan, Italy) (Fain A. 1968 Acta Zool Pathol Antverp 47(1):1–196).

RESULTS AND DISCUSSION. In 3 of the 7 samples it was possible to identify various adult specimens, nymphs, larvae and eggs of *S. scabiei*. All mites were non-viable. The microscopic examination of the indoor dust allowed to diagnose scabies, without using complex and invasive techniques, such as skin scraping. Based on these results, contextually with the topical therapy on all members of the family (5% permethrin cream), the three areas contaminated with *S. scabiei* were sanitized with liquid nitrogen spray method (Criopest, Ecotrade Solution srl, Rome, Italy). Daily disinfection operations were also adopted: used clothes, bed linen, towels and other fabrics were periodically washed at 60°C. After cryogenic environmental treatment of the contaminated areas, daily disinfection operations and continuation of local therapy, the skin lesions disappeared in about 5 weeks. The subsequent 6-month follow-up was negative.

Outbreak of occupational dermatitis in a cereal and legume trading company caused by mite *Pyemotes ventricosus*

P MASINI[1], S ZAMPETTI[1], F BIANCOLINI[2], G MIÑÓN LLERA[3]

[1]Cani Anti Cimici©, Perugia, Italy, www.canianticimici.com; [2]Ecotrade Solution Srl, Roma, Italy, www.glispecialistidelladisinfestazione.com; [3]Biologist freelancer, Oviedo, Spain.

Keywords: *Pyemotes ventricosus*, Occupational dermatitis, Microscopic examination of indoor dust

INTRODUCTION. *Pyemotes ventricosus* (Newport, 1850) (Acarina: Pyemotidae) is an ectoparasitoid mite that attacks and kills immature stages of a number of insect species infesting wood, cereals and other vegetable materials (Ibarra, 1993 Springer Science + Business Media Ed). This mite can be responsible for temporary ectoparasitosis in humans when an individual comes in contact with infested material. In this work we reported the outbreak of occupational dermatitis in a cereal and legume trading company associated with *P. ventricosus.*

MATERIALS AND METHODS. In a cereal and legume trading company in Umbria, Italy, some of the employees involved in the direct handling of cereals grain manifested itching and erythematous, edematous and papular skin lesions. An ectoparasitosis has been suspected and 9 indoor dust and grain samples were collected from various areas of the company where the cereal grains were processed. A microscopic examination of samples was performed (Stingeni et al., 2017 JEADV 31(9): 1526-1533), in order to detect the arthropod responsible for clinical symptoms. Arthropods were observed with a stereomicroscope (Motic SMZ-168, Milan Italy) and identified taxonomically placing them on slides in Berlese solution by optical microscope (Motic BA310, Milan, Italy) (Krantz et al., 2009, Texas Tech University Press) (Newport., 1850 Annals and Magazine of Natural History Series 2. 6, 395–396).

RESULTS AND CONCLUSIONS. In 1 (chickpea sample) of the 9 samples, the mites *P. ventricosus* were found. The mites were vital and with high densities. The removal of the infested chickpea batch determined the resolution of the dermatitis. One-year follow-up was negative. *P. ventricosus* was therefore the causative agent of the outbreak of occupational dermatitis in the company's employees. Since all the samples were not infested with insect pests, our hypothesis is that there was a contamination by *P. ventricosus* of chickpea batch. This contamination occurred in the farm that cultivated chickpeas and supplied the trading company. The chickpeas were probably been stored or processed in an environment of the farm heavily infested with *P. ventricosus*.

A case of aetiological diagnosis of ectoparasitosis by crab louse *Pthirus pubis* through a microscopic examination of the indoor dust

P MASINI[1], S ZAMPETTI[2], F BIANCOLINI[2], R ROMANI[3], G MIÑÓN LLERA[4]

[1]Cani Anti Cimici©, Perugia, Italy, www.canianticimici.com; [2]Ecotrade Solution Srl, Roma, Italy, www.glispecialistidelladisinfestazione.com; [3]University of Perugia, Perugia, Italy, Department of Agricultural, Food and Environmental Sciences, University of Perugia, Perugia, Italy; [4]Biologist freelancer, Oviedo, Spain.

Keywords: *Pthirus pubis*, Pubic louse, Indoor dermatitis, Microscopic examination of environmental dust

INTRODUCTION. *Pthirus pubis* Linnaeus, 1758 (Anoplura: Pthiridae), also known as pubic louse o crab louse, is an obligate haematophagous ectoparasite of humans (Mullen et al, 2009 Elsevier Ed. 59-82). In an apartment in the city of Rome, Italy, a man of 45 years old, with widespread body hair, had recurrent itching and papular lesions on the arms, legs, abdomen and pubic region. After a dermatological examination, the physician suspected an ectoparasitosis by *Cimex lectularius* (Linnaeus, 1758) (Hemiptera: Cimicidae). An olfactory inspection with bedbug detection dogs was performed (Masini et al., 2017 Int J Dermatol 56: 1-3) (Masini et al., 2020 JEADV 34(1): 28-30).

MATERIALS AND METHODS. Since the olfactory inspection did not detect the presence of the bedbugs, in November 2019, a collection of indoor dust was carried out from every room of apartment, using a vacuum cleaner modified (BuTure CR20, Shenzhen, Cina) according to the Sercombe method (Sercombe et al., 2005 Allergy 60: 515-520). A microscopic examination of samples was performed (Stingeni et al., 2017 JEADV 31(9): 1526-1533), in order to detect the arthropod responsible for clinical symptoms. The crab louse was identified taxonomically by a stereomicroscope (Motic SMZ-168, Milan, Italy) (Lane., 1993 Springer Ed).

RESULTS AND CONCLUSIONS. The microscopic examination of the indoor dust revealed the presence, in all the rooms examined, of dead specimens, body fragments and eggs, both empty eggshells and eggs embryonated still vital of *P. pubis*. The examination has allowed, in this case, to detect the environmental contamination by *P. pubis* and thus to reveal the ectoparasitosis in progress. The incorrect initial aetiological diagnosis may have been caused by the extensive body hair that covered the man's body. Crab lousecan infests, not only the pubis, groin, buttocks and perianal region, but in particular in hairy males, thighs, abdomen, chest, axillae and beard (Veraldi et al., 2018 Korean J Parasitol 56(5): 487-489). The skin lesions spread in various regions of the body, and not only in the pubic region, would have misled the physician in the differential diagnosis.

The pathogenic potential of *Sclerodermus domesticus* and *Cephalonomia gallicola*, two flat wasps that sting man in indoor environments

P MASINI[1], **S ZAMPETTI**[2], **F BIANCOLINI**[2], **R ROMANI**[3], **G MIÑÓN LLERA**[4]

[1]Cani Anti Cimici©, Perugia, Italy, www.canianticimici.com; [2]Ecotrade Solution Srl, Rome, Italy, www.glispecialistidelladisinfestazione.com; [3]University of Perugia, Perugia, Italy, Department of Agricultural, Food and Environmental Sciences, University of Perugia, Perugia, Italy; [4]Biologist freelancer, Oviedo, Spain.

Keywords: *Sclerodermus domesticus*, *Cephalonomia gallicola*, Flat wasps, Pathogenic potential

INTRODUCTION. *Sclerodermus domesticus* Klug, 1809 and *Cephalonomia gallicola* Ashmead, 1887 (Hymenoptera: Bethylidae) are two aculeate flat wasps, 2-4 mm long, ecto-parasitoids of the immature stages of some Coleoptera and Lepidoptera. These bethylid insects can also sting humans causing dermatitis characterized itch and erythematous papular lesions (Azevedo CO, et al. 2018, Zootaxa 4489 (1): 001–294). In this work we evaluated their pathogenic potential towards humans.

MATERIALS AND METHODS. Between 2018 and 2019, we selected 19 houses, all infested by common furniture beetles belonging to the genus *Oligomerus* Redtenbacher, 1849 and *Anobium* Fabricius, 1775 (Coleoptera: Ptinidae), in which the only pathogenic arthropods were *S. domesticus* and *C. gallicola*, in order to attribute only to them the skin lesions. Their presence and the absence of other arthropods potentially able of causing dermatitis in humans was confirmed through a microscopic examination of environmental dust (Stingeni et al., 2017 JEADV 31(9):1526-1533). Skin lesions in patients, when present, were examined by a dermatologist to confirm that they were caused by the bite or sting of arthropods and not by other medical conditions. The collected data were processed statistically.

RESULTS AND CONCLUSIONS. In 5 houses on 19 (26,31%) *S. domesticus* and *C. gallicola* had not caused skin lesions. One of these houses, however, was inhabited very sporadically and for short periods. Of the 36 persons that lived in the 19 houses infested with these bethylid hymenopterans, 23 (63,88%) were stung and showed recurrent skin lesions, whereas 13 (36,11%) did not show lesions. In some cases (21,42%), not all the members of the family showed skin lesions. Most of the time (78,57%), instead, all the people of the infested house were stung. These data indicate that not all people living in an environment infested with these flat wasps are stung and have skin lesions. This data should not mislead the physician in the differential diagnosis, but it can be considered normal.

Outbreaks of occupational dermatitis caused by *Pyemotes ventricosus*

I MORETTA[1], L STINGENI[2], MA PRINCIPATO[1,3]

[1]Dip. di Medicina Veterinaria, Università degli Studi di Perugia; [2]Dip. di Medicina, Sez. di Dermatologia Clinica, Allergologica e Venereologica, Università degli Studi di Perugia; [3]Centro di Ricerca Urania, Perugia.

Keywords: *Pyemotes ventricosus*, Occupational dermatitis, E.D.P.A.®

INTRODUCTION. *Pyemotes ventricosus* Newport, 1850 (Acarina: Pyemotidae) is a mite known in Italy for its parasitic association with the furniture worm, generally *Oligomerus ptilinoides* (Coleoptera: Anobiidae) and, above all, as a cause of dermatitis in humans inside homes with worm-eaten furniture. The episodes of entomodermatosis that we report were related to the professional activity of the patients and had recurred periodically, for some years, only in the spring-summer period, during their work.

MATERIALS AND METHODS. In the period 2018-2019, on the recommendation of the Dermatological Clinic of Perugia, we carried out an environmental parasitological examination (E.D.P.A.® or Direct Indoor Dust Examination) of the working environments of some patients who presented sting lesions of the "strophulus" type (erythematous-edemato-vesicular), distributed mainly to the trunk and arms, referable to bites of arthropod (Stingeni et al., 2017 J Eur Acad Dermatol Venereol. 31: 1526-33). They were united by the manipulation, during the working activity, of woody material or objects and they were antique dealers (n° 3), carpenters (n° 2), restorers (n° 3) and florists who used woody material for ornamental purposes (n° 2).

RESULTS AND CONCLUSIONS. The E.D.P.A.® has detected the presence of females of *Pyemotes ventricosus* in all environments. The mites came out of the galleries of woodworms present in the wood of frames, antique furniture, beams, boards and various wooden objects and, even, from reeds used for ornamental purposes. The resolution of the infestation was obtained by eliminating the worm-eaten material or by treating it with woodworm or with heat (65° C). The environment was then treated with a common residual pyrethroid in order to eliminate any free mites. The complete resolution of the infestation is evidenced by the absence of new bites starting from two days post-treatment. Following the environmental remediation, the complete recovery of the patients, treated exclusively with a symptomatic therapy (corticosteroid ointments and oral antihistamines), was obtained in about two weeks, and no relapses were observed during the following 1-year follow-up.

Investigating the impact of *Schistosoma haematobium* infection on immunity to *Plasmodium falciparum* malaria in populations from Burkina Faso

M OUEDRAOGO[1,2], **I NEBIE**[3], **Y KABORE**[2], **SB SIRIMA**[3], **F BRUSCHI**[4], **D MODIANO**[5], **VD MANGANO**[4]

[1]PhD School in Microbiology, Infectious Diseases and Public Health, Sapienza University of Rome, Italy; [2]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougu, Burkina Faso; [3]Groupe de Recherche Action en Santé, Ouagadougou, Burkina Faso; [4]Dep. of Translational Research, University of Pisa, Italy; [5] Dep. of Public Health and Infectious Diseases, Sapienza University of Rome, Italy.

Keywords: Urinary schistosomiasis, Malaria, Immunity, Burkina Faso

INTRODUCTION. Several studies indicate that infection with helminths might modulate the immune response towards intracellular pathogens including *Plasmodium* in areas of co-endemicity (Salgame et al., 2013 Nat Immunol. 11: 1118-26). We recently reported that in rural villages of Burkina Faso the seroprevalence of *Strongyloides stercoralis*, *Wuchereria bancrofti* and *Schistosoma haematobium* was 5%, 16% and 63% respectively, in line with estimates of infection prevalence in the region for the three parasites (Mangano et al., 2020 Acta Tropica. 205: 105381). The aim of the present investigation was to assess the impact of serological markers of *S. hematobium* infection on the prospective risk of *Plasmodium falciparum* parasitaemia among study populations, using a repeated cross-sectional surveys design (Mangano et al., 2015 J Inf Dis. 4:626-34).

MATERIALS AND METHODS. IgM and IgG against *S. haematobium* Soluble Worm Antigen Protein (SWAP) and Soluble Egg Antigen (SEA) were measured by an in-house ELISA protocol (Mutapi et al., 1997 Parasite Immunol 19:255-63) in plasma samples collected from N=452 subjects. Statistical analysis was performed using STATAv13. Association analysis between the presence of *S. haematobium*-specific antibodies at baseline and the number of *P. falciparum* infections determined by microscopy over 5 surveys was conducted using Poisson regression adjusting for age, sex, ethnicity and haemoglobin genotype.

RESULTS AND CONCLUSIONS. Association analysis showed a significant increase in the incidence of *P. falciparum* infections among anti-*S. haematobium* seropositive subjects (IRR=1.30, 95%CI= 1.09-1.57, p-value=0.004). These observations are in line with those of a systematic review and meta-analysis indicating an association between *S. haematobium* infection and increased prevalence of *P. falciparum* asymptomatic/uncomplicated malaria (Degarage et al., 2016 Plos Neg Trop Dis. 10: 1-18). To support interpretation of these results a parasitological survey will be conducted in the study area to identify serological correlates of prevalence and intensity of *S. haematobium* infection.

In vitro antimalarial activity of inhibitors of human Rac1: a possible new way to fight *Plasmodium*?

S PARAPINI[1], S PAONE[2,3], M POURSHABAN[2], E ERBA[4], A CONTINI[4], S D'ALESSANDRO[5], A OLIVIERI[3]

[1]Departmentof Biomedical Sciences for Health, University of Milan, Milan, Italy; [2]Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy; [3]Department of infectious Diseases, Italian National Institute of Health (ISS) Rome, Italy; [4]Department of Pharmaceutical Sciences, University of Milan, Milan, Italy; [5]Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy.

Keywords: Antimalarials, RAC1, *Plasmodium falciparum*

INTRODUCTION. Malaria accounts for millions of cases and thousands of deaths every year. In the absence of an effective vaccine, drugs are still the most important tool in the fight against the disease. *Plasmodium* parasites developed resistance for all the classes of known antimalarial drugs. Thus, the search for antimalarial drugs with novel mechanisms of action is compelling. The human GTPase Rac1 plays a role in invasion of the host cells by many intracellular pathogens, including *Leishmania* and *Toxoplasma*. Also in *Plasmodium falciparum*, it was suggested an involvement of this host GTPase both during the invasion process and parasite intracellular development.

MATERIALS AND METHODS. *Plasmodium* viability was measured by pLDH assay; parasite invasion efficiency was measured by FACS analysis of fluorescent labelled parasites; parasite intraerythrocytic development was measured by microscopic observation of Giemsa-stained smears; cell toxicity was evaluated by MTT assay on human microvascular endothelial cells.

RESULTS AND CONCLUSIONS. Fourteen commercially available or newly synthetized Rac1 inhibitors were tested for their potential antimalarial activity. Among these, three inhibitors had an IC_{50} lower than 1 µM, only two were inactive (IC_{50}> 50 µM), whereas the remaining showed intermediate IC50s. EHop-016 was the most effective compound, with an IC_{50} of 138.8 ± 16.0 nM and Selectivity Index of 37.8. EHop-016 did not inhibit parasite invasion of red blood cells but affected parasite intraerythrocytic growth. EHop-016 showed a promising activity that raises attention on this class of molecules as potential antimalarials and deserves further investigation.

Neglected parasitic infections in Campania, South Italy region, from 2015 to 2019

L PETRULLO, A FIORETTI, M ASCIERTO, L ATRIPALDI, MG COPPOLA

UOC Microbiologia e Virologia, AORN Dei Colli, Napoli.

Keywords: Neglected diseases

INTRODUCTION. Neglected Diseases (NTDs) occur more often in Western Nations with a high economic standard due to the growing phenomenon of globalization. The goal of the hospital UOS Parasitology team "D. Cotugno” of Naples is to diagnose NTDs parasitic to highlight and report the data to the authorities in charge.

MATERIALS AND METHODS. Concentration techniques, extemporary and permanent colorings and culture were used for copro-urological parasites; instead, indirect serological techniques and direct techniques, such as smears, thick drops and culture were used for parasites of organs and tissues.

RESULTS AND CONCLUSION. The copro-parasitological samples were 3674, whose 9% were from non-EU patients and 91% from autochthonous patients. 128 of these samples showed positive results: 6 *Ascaris lumbricoides*, 13 *Ancylostoma duodenale*, 4 *Strongyloides stercoralis*, 5 *Schistosoma mansoni* and 1 *Trichuristrichiura* and 39 *Giardia duodenalis*, 55 *Entamoeba histolytica*, 5 *Taenia* spp. 151 samples were received for the diagnosis of *S. haematobium*: 68% autochthonous and 32% non-EU, 27 (18%) were positive, 38 samples were analysed for microfilariae, whose 2 (5%) were positive for *Dirofilaria* spp., 88 samples were received for Chagas: 89% native and 11% non-EU, whose only 3 (4%) were positive, all belonging to non-EU group. 543 samples were analyzed for suspicion of Leishmaniosis, 91% autochthonous and 9% non-EU, whose 26 (5%) were positive. 223 samples were analyzed for *Echinococcus* spp*.,* 82% autochthonous and 18% non-EU, whose 32 (18%) were positive. Our data show that of these 4717 biological samples analyzed, 209 NTDs were highlighted and treated, whose 122 (58.4%) are from autochthonous patients who travelled to endemic countries to work or to study, while 87 (41.6%) are from non-EU patients.

Gene expression profiles and temperature in *Anisakis simplex* ss, *Anisakis pegreffii* and *Hysterothylacium aduncum*

A PIZZARELLI, S CAVALLERO, I BELLINI, S D'AMELIO

Department of Public Health and Infectious Diseases, Sapienza University of Rome, Italy.

Keywords: Gene expression, *Anisakis, Hysterothylacium,* Pathogenicity

INTRODUCTION. Anisakiasis is a zoonotic fish-borne disease caused by the ingestion of marine food infected with larvae of the genera *Anisakis* and to less extent of the genus *Hysterothylacium,* considered less pathogenic, despite sharing several known antigens with *Anisakis* (Valero et al., 2003 J Investig Allergol Clin Immunol. 13: 94-98). Temperature is a crucial factor for parasite survival and host interaction, as demonstrated in *A. simplex* sensu stricto (AS), which increases the production of excreted/secreted proteins according to increasing temperature (Bahlool et al., 2013 Fish Shellfish Immunol 35: 734-739), while *H. aduncum* (HA) survival rate appears improved at lower temperature (Iglesias et al., 2002 Parasitology. 125: 467-75). The aim of the study was to investigate the gene expression profile of different transcripts in two temperature conditions in *A. pegreffii* (AP), AS and HA, selected among those found enriched in the pharynx, such as: the major allergen Anis1, the pathogenic molecules belonging to CRISPs family, the metallopeptidase astacin AST, the toxin ShK and two isoforms of the heat shock protein (1Hsp90 and 2 Hsp90). These gene products are involved in many biological processes crucial for these parasitic nematodes, playing a role in the pathogenesis (Cavallero et al., 2020 Genes. 11: 321).

MATERIAL AND METHODS. Six AS, 24 AP and 30 HA third-stage larvae were incubated for 24h at two different temperature conditions (4°C and 37°C). Total RNA was isolated and used for cDNA synthesis and qRT-PCR. A reference gene was included in each plate to perform the relative quantification of transcripts.

RESULTS AND CONCLUSION. Preliminary results show a higher Anis1 and ShKt expression level in AS at 37°C (corresponding to the human body temperature) and an opposite trend for CRISPs. In AP Anis1 is more expressed at 37°C, while an opposite trend is observed for AST and CRISPs. The increase of Anis1 at 37°C could suggest that the production of this protein increase when the larva meets the definitive host. As regards to HA, no significant differences in the relative expression of 1Hsp90 and 2Hsp90 were detected at the two temperature conditions. Given the low reliability of gene prediction for HA based on AS genome, the coding genomic region and expression pattern of Anis1 in *Hysterothylacium* needs further investigation. The orthologous Anis1 region has been partially characterized in HA and, so far, a sequence of around 400bp was obtained.

An organotypic model for the study of retinal *Toxoplasma gondii* infection

<u>V RODRIGUEZ FERNANDEZ</u>[1,2], MG ROSSINO[3], R AMATO[3], S PIAGGI[1], B PINTO[1], G CASINI[3], F BRUSCHI[1]

[1]Department of Translational Research, School of Medicine, University of Pisa, Pisa, Italy; [2]PhD School in Infectious Diseases, Microbiology and Public Health, La Sapienza University, Rome, Italy; [3]Department of Biology, University of Pisa, Pisa, Italy.

Keywords: Retina, Ocular toxoplasmosis, Retinal infection, Retinal model

INTRODUCTION. Ocular toxoplasmosis (OT) is caused by infection of the eye with the parasite *Toxoplasma gondii* and it is the most common cause of eye inflammation in the world. OT has been the subject of different studies and a recent reviews have focused on specific aspects of the disease (Smith et al., 2020 J M Prog Retin Eye Res 81: 100882; Rodriguez Fernandez et al., 2021 Parasitologia. 1: 50-60). However, little is known of the reaction to infection of specific cellular types of the retina, or whether different retinal cell types display different sensitivities to infection. Most experimental models used to date consist of in vitro preparations, also including human retinal cell subset. Alternatively, in vivo experimental models involve local or systemic inoculation of parasites to induce acute disease, or sequential systemic and local parasite inoculations to trigger recurrent disease. An almost unexplored possibility is the use of organotypic, ex vivo retinal explants, which offer the advantage of easy manipulation and fast and reliable observation of the effects of exogenous compounds while maintaining the complexity and the extracellular environment typical of the in vivo setting.

MATERIALS AND METHODS. Extracted adult murine retinas were placed with photoreceptor layer facing downward on rafts of nitrocellulose filters. These filters were first soaked in different concentrations of *T. gondii* (2 x 10^3 *t. gondii*/mL, 20 x 10^3 *t. gondii*/mL and 200 x 10^3 *t. gondii*) and were maintained in culture for 24 hours, 3 days, 7 days or 10 days, changing the culture medium every two days. After incubation the retinas werefixed with 4% paraformaldehyde, cut with a cryostat and processed for immunohistochemistry for *T. gondii* detection.

RESULTS AND CONCLUSIONS. Set up experiments showed a successful infection of the retinal explants by *T. gondii*, but the best concentration of the parasite was not established yet, and we are currently running experiments yet, and we are currently running experiments with different parasite concentrations. The ongoing experiments will allow us to determine in which areas of the retina *Toxoplasma* is most present, and which infection time and parasite concentration are optimal for model establishment.

Systemic profile of immune factors in an elderly Italian population affected by chronic strongyloidiasis

N TIBERTI[1], D BUONFRATE[1], C CARBONE[2], G PIRO[2], Z BISOFFI[1,3], C PIUBELLI[1]

[1]Department of Infectious-Tropical Diseases and Microbiology, IRCCS Sacro Cuore Don Calabria Hospital, Negrar, Italy; [2]Department of Medical and Surgical Sciences, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy; [3]Department of Diagnostics and Public Health, University of Verona, Verona, Italy.

Keywords: *Strongyloides*, Immunology, Tolerance

INTRODUCTION. Strongyloidiasis caused by *Strongyloides stercoralis* is a soil-transmitted helminthiasis affecting an estimated 370 million people and considered one of the most neglected tropical diseases. Although mostly distributed in tropical and subtropical areas, autochthonous infections have also been documented in northeastern Italy, even though the transmission presumably stopped decades ago. Because of its peculiar auto-infective cycle, strongyloidiasis can persist lifelong, but the pathophysiological mechanisms associated with the maintenance of such a chronic infection are yet to be fully deciphered.

MATERIALS AND METHODS. Serum levels of 23 immune factors were retrospectively assessed in a subgroup of participants in a randomised clinical trial for the treatment of strongyloidiasis (Strong Treat). Here we included Italian subjects born between 1931 and 1964 and diagnosed with strongyloidiasis between 2013 and 2017 (Ss+, n = 32). Serum samples obtained before (BT) and 6 months (6M AT) after ivermectin treatment, as well as from age- and gender-matched uninfected controls (CTRL, n = 34) were analysed.

RESULTS AND CONCLUSIONS. The assessed immune factors showed a general reduced concertation in Ss+ patients and a lack of association with eosinophilia. In our cohort, we did not observe the classical shift towards a type 2 immune response, since Th1 and Th2 cytokines were mostly unaltered. Instead, we observed chemokines as particularly affected by the presence of the parasite, since IL-8, CCL3, CCL4 and CCL5 were significantly reduced in concentration in Ss+ subjects compared to CTRL, suggesting that immune cell recruitment to the infection site might be dampened in these patients. This observation was further sustained by a significant increase of CCL4, CCL5 and CCL11 concentrations 6M AT. A significant raised systemic concentration of three growth factors, bFGF, PDGF-BB and IL-7 (haematopoietic growth factor) was also observed post-treatment, indicating a potential involvement in restoring tissue integrity and homeostasis following parasite elimination. These preliminary data suggest that, in order to survive for such a long period, *S. stercoralis* might suppress host responses that could otherwise result in its ejection. Our results offer novel insights in the potential mechanisms of disease tolerance that might take place during this chronic infection, including a potential T-cell hypo-responsiveness and a role for chemokines.

POSTER SOIPA

ZOONOSI PARASSITARIE

Giardia duodenalis and other intestinal parasites in kennels of Tuscany (central Italy)

A AGRESTI[1], F BERRILLI[2], M MAESTRINI[1], I GUADANO PROCESI[2], E LORETTI[3], N VONCI[4], S PERRUCCI[1]

[1]Dipartimento di Scienze Veterinarie- Università di Pisa, Viale delle Piagge n. 2, 56124 Pisa, Italy; [2]Dipartimento di Scienze cliniche e Medicina Traslazionale, Università di Roma "Tor Vergata", Via Montpellier 1, 00133 Roma, Italy; [3]Unità Funzionale Aziendale Igiene Urbana, Az. USL Centro Toscana, Viale Corsica 4, 50127 Firenze, Italy; [4]Dipartimento di Medicina Molecolare e dello Sviluppo, Università di Siena, Via Aldo Moro 4, 53100 Siena, Italy.

Keywords: *Giardia duodenalis*, Intestinal parasites, Kennels, Prevalence, Assemblages

INTRODUCTION. In kennel dogs, the prevalence of intestinal parasite infections is frequently high and may include potential zoonotic species/genotypes.

MATERIALS AND METHODS. Prevalence and potential risk factors of*Giardia duodenalis* were assessed in 168 dogs of different sex and age from four Tuscan kennels (Pistoia, Prato, Florence, Valdarno). Other intestinal parasiteswere also evaluated. Individual dog fecal samples collected from each kennel were examined by parasitological techniques (fresh and stained faecal smears, Mini-FLOTAC) and a commercial rapid immunoassay for the detection of *G. duodenalis* and *Cryptosporidium* faecal antigens. On *Giardia*-positive samples, molecular analysis was performed for genotype identification. Data were statistically analysed ($p \leq 0.05$).

RESULTS AND CONCLUSIONS. Out of the 168 examined dogs, 69 dogs scored positive for *G. duodenalis* (41%). Significant differences ($p \leq 0.05$) were found among kennels and age groups regarding *G. duodenalis* prevalence. The highest prevalence was recorded in the age-group ≤6 months and in the kennel in Pistoia. The potential zoonotic assemblages A and B and the dog-specific assemblage C (Pistoia: A-AII, B, C; Prato: A-AII, B; Florence: A-AII; Valdarno: A and C), were identified. *Toxocara canis* (15/168, 8.93%), *Trichuris vulpis* (6/168 dogs, 3.57%), Ancylostomatidae (2/168, 1.19%) and *Cryptosporidium* spp. (1/169, 0.6%), were also identified. The high prevalence of *G. duodenalis* and the identification of potentially zoonotic parasitic species/genotypes in all examined kennels, underline the need to improve routine parasite control measures and to provide insights into the local transmission dynamics and host-specificity of *G. duodenalis*.

How can vultures contribute to Cystic Echinococcosis control?

F AHMED, A VARCASIA, M SANNA, L CAVALLO, MF SINI, G DESSÌ, F NONNIS, S KNOLL, C TAMPONI, C PASINI, F PORCU, L MELONI, A SCALA, MG CAPPAI, S CARTA, F BERLINGUER

Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Italy.

Keywords: Cystic echinococcosis, Griffon vultures, Control, Livestock

INTRODUCTION. Cystic Echinococcosis (CE) is a parasitic zoonosis endemic in Sardinia with a prevalence of 65.3% in sheep (Varcasia et al., 2020 Parasitol Res. 119: 2207-15). A wildlife conservation program in Sardinia, envision to expand griffon population (*Gyps fulvus*) by utilizing dead carcasses of animals in vultures feeding stations. The study foresees to ascertain, natural foraging habits of vultures in controlled disposal of hazardous biomass, thus may provide intervention for breaking the lifecycle of *Echinococcus granulosus sensu lato* in Sardinia.

MATERIALS AND METHODS. The study was carried out in Sardinia, within the LIFE project for conservation of vultures. A total of 37 artificial feeding stations were established in Natura 2000 network protected sites. A feedingstation is an enclosed space, surrounded by electric fence with no chance of access to canids. Vultures were provided with food consist of bovine, sheep, goat and equine carcasses associated with livestockfarms which were inspected for sanitation and drug presence before feeding the vultures. The concomitant ecosystem services such as incineration and CO_2 cost saved by vultures were also estimated along with.

RESULTS AND CONCLUSIONS. In total, 81,361 kg of biomass was scavenged by vultures saving € 90,041 incineration cost and € 1,054 CO_2 emission cost during the course of project. Biomass removed correspond to 255 individuals from sheep-goat (9,618 kg), 171 individuals from cattle (71,093 kg) and 2 individuals from equids (650 kg). We extrapolate these results to quantify the amount of carrion perhaps be infected with CE, removed by griffon which relate to 5,304 kg from sheep carcass (65.3%), thereby reducing considerable risk and burden of CE in Sardinia. The study, also quantify the amount of biomass that would be removed by griffon in succeeding conservation LIFE project which implies that, 162,722 kg of biomass would be consumed by vultures and 10,608 kg of infected biomass would be decomposed from sheep only, further lowering the disease burden of CE. The results manifest vulture's differential peculiarity of removing infected biomass, for biological control of CE simultaneously saving hefty amount for incineration and carbon emission.

Occurrence and identification of *Ixodes ricinus* - borne pathogens in north-eastern Italy

M BERTOLA, G DA ROLD, S CARLIN, F OBBER, E PORCELLATO, C FALCARO, S ORMELLI, F MONTARSI, S RAVAGNAN

Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy.

Keywords: Tick-borne pathogens, *Ixodes ricinus*, Italy

INTRODUCTION. In Europe, the main vector for tick-borne pathogens (TBP) is *Ixodes ricinus*, the most common tick species in Italy, reaching the highest density in hilly and pre-alpine northern areas. Here, the majority of human cases of Lyme borreliosis (LB) and tick-borne encephalitis (TBE) is notified while other infections are only rarely reported. The aim of this study was to add new knowledge on the presence and occurrence of endemic and emergent pathogens in ticks in north-eastern Italy.

MATERIALS AND METHODS. From 2011 to 2017 ticks were collected by dragging in 20 municipalities (43 sites) in Belluno province. Ticks were morphologically identified, pooled (adults individually, nymphs and larvae in pool) and molecularly processed (rt-PCR and sequencing) for the pathogen identification.

RESULTS AND CONCLUSION. Nine different pathogens were found in *I. ricinus* (n= 2668, all stages) to be circulating in the Belluno province. Overall, 38.4% of screened pools were positive at least for one pathogen belonging to *Rickettsia* (*Rickettsia helvetica* 21.5% and *Rickettsia monacensis* 3.0%) and *Borrelia burgdorferi s.l.* (*Borrelia afzelii* 10.1%, *Borrelia burgdoferi s.s.* 7.1%, *Borrelia valaisiana* 1.5% and *Borrelia garinii* 1.3%). Other pathogen species were *Anaplasma phagocytophilum* (18.9%), *Candidatus* Neoehrlichia mikurensis (cNm) (10.1%) and *Babesia venatorum* (0.3%). TBE virus was not found. Co-infections by two or three pathogens were diagnosed in 18.4% of positive tick pools. Positivity to TBP was found in 85% of monitored municipalities (i.e. *R. helvetica* and *B. afzelii* occurred in 60% and 50% of monitored municipalities respectively). In each municipality, the presence of TBPs varied from one to seven different pathogens in the same year. The present study indicates that the molecular screening of TBPs in questing ticks remains an efficient system for monitoring the circulation of tick-borne diseases (TBD) in a specific area. Our results also suggest to considering *Rickettsia* and *Anaplasma phagocytophilum* in human cases that are not classified as LB or TBE, due to the high prevalence of these pathogens in ticks.

Update of sand fly species distribution in six Italian Regions as a preliminary information for risk-map construction of phlebotomine-borne diseases

G BONGIORNO[1], R BIANCHI[1], T DI MUCCIO[1], C DE LIBERATO[2], M CALZOLARI[3], A MICHELUTTI[4], F MONTARSI[4], F CIPRIANO[5], M DOTTORI[3], G SATTA[5], L GRADONI[1], P ANGELINI[6]

[1]Istituto Superiore di Sanità, DMI, Unit of Vector-borne Diseases, Rome; [2]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Via Appia Nuova 1411, Rome; [3]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "B. Ubertini", Via Bianchi 9, 25124 Brescia; [4]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (Padua); [5]Istituto Zooprofilattico Sperimentale della Sardegna, Sassari; [6]Servizio Prevenzione collettiva e Sanità pubblica - Regione Emilia-Romagna, Italy.

Keywords: Sand fly, Species, Identification, Prevalence

INTRODUCTION. Diseases transmitted by sand flies, in particular those caused by *Leishmania* and *Phlebovirus*, are currently considered to be expanding both in terms of incidence and territory. The factors involved are many, starting with the abandonment of agricultural use of hilly areas and the impact of climate change. The assessment of the historical and geographical dynamics is made complex due to the lack of systematicity and standardization in the reporting of the sand fly data (Busani et al., 2012 Rapporti ISTISAN 12/22). Under the patronage of the italian Ministry of Health, in the frame of West Nile viruses surveillance entomological surveys have been conducted in six Italian regions. Phlebotomine sand flies have been collected along with others hematophagous insects. In this study, we report the results of sand fly identification and their distribution in the investigated regions.

MATERIALS AND METHODS. Attractive traps were set during summer every two weeks, from May till October 2020 near animal shelters. Sand flies collection have been performed also in the previous years (2017-18-19). Morphological identification using only the last segments of the insect's abdomen (3 for female and 4 for male) were performed keeping the specimen refrigerated. This procedure should avoid deterioration of the pathogens that may be contained in the remaining part of the insect body. Hence this part can be usefull for subsequent molecular analysis, which is in progress, to detect natural (parasitological or viral) sand fly infections.

RESULTS AND CONCLUSIONS. The prevalence of sand fly species for the six Region are reported in Table 1

REGION	No. of sites	No. of sand flies	% *Ph. perfiliewi*	% *Ph. perniciosus*
Friuli Venezia Giulia	7	39	0	100
Veneto	9	20	26.3	73.7
Emilia-Romagna	6	1174	98.6	1.2
Tuscany	9	661	96.2	3.8
Latium	1	60	0	100
Sardinia	16	364	11.7	88.3

Tab. 1 Prevalent sand fly species identified by Region being examined for pathogens

EchinoCamp: an integrated approach to control of *Echinococcus granulosus* infection in a hyperendemic area of southern Italy

A BOSCO[1], P PEPE[1], MP MAURELLI[1], P QUARANTA[1], L BALDI[2], P CIARAMELLA[1], F CAPUANO[2], L MORENA[3], R PINTO[3,4], P SARNELLI[3,4], L RINALDI[1,4], G CRINGOLI[1,4]

[1]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, CREMOPAR, Naples, Italy; [2]Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Naples, Italy; [3]Centro di Riferimento Regionale per le Malattie degli Animali Domestici (CReSan), Regione Campania, Naples, Italy; [4]UOD Prevenzione e Sanità Pubblica Veterinaria Regione Campania, Naples, Italy.

Keywords: *Echinococcus granulosus*, Control programme, One health

INTRODUCTION. Cystic echinococcosis (CE) is a severe zoonosis, caused by the larval stage of the tapeworm *Echinococcus granulosus*. This helminth infection is of increasing public health and socio-economic concern due to the considerable morbidity rates that give rise to high economic losses both in the public health sector and in the livestock industry (Torgerson and Macpherson, 2011 Vet Parasitol. 182: 79-95). Control programmes against *E. granulosus* are considered long-term actions which require an integrated approach and high expenditure of time and financial resources (Craig et al., 2017 Adv Parasitol. 96: 55-158). The aim of this study was to develop an integrated approach based on innovative procedures and tools to control CE in a hyperendemic area of southern Italy (Campania region).

MATERIALS AND METHODS. A ten-year regional initiative addressed the control of CE from a holistic perspective based on: i) active and passive surveillance in livestock (using geospatial tools for georeferencing), ii) diagnosis in dogs (using the FLOTAC techniques and molecular analysis), iii) targeted treatment of farm dogs (using purpose-built confinement cages), iv) early diagnosis in livestock (by ultrasonography), v) surveillance in humans (through hospital discharge records analysis), vi) monitoring the food chain (analysing raw vegetables), vii) outreach activities to the general public (through dissemination material, e.g. brochures, gadgets, videos, virtual reality).

RESULTS AND CONCLUSIONS. The integrated programme resulted in a significant reduction of the infection rates of dogs, sheep and other livestock in Campania. Therefore, the procedures and tools developed during the ten-year control programme could be used by regional and national health authorities to target interventions aimed at eliminating CE in animals and humans in other endemic areas.

Taking aim at *Borrelia burgdorferi* s.l. complex: a new molecular tool for *B. garini*, *B. afzelii* and *B. burgdorferi* s.s. detection in *Ixodes ricinus*

G CHIAPPA[1], A CAFISO[1], M PERINI[2], F COMANDATORE[2], M MASSIMINO[1], G GRANDI[3,4], C BAZZOCCHI[1]

[1]Department of Veterinary Medicine, University of Milan, Lodi, Italy; [2]Department of Biomedical and Clinical Sciences, University of Milan, Milan, Italy; [3]Department of Microbiology, National Veterinary Institute (SVA), Uppsala, Sweden; [4]Department of Biomedical Sciences and Veterinary Public Health (BVF), Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden.

Keywords: *Ixodes ricinus*, *Borrelia burgdorferi* s.l., Molecular analyses, Lyme disease

INTRODUCTION. Lyme disease (LD), which affects both humans and animals worldwide, is caused by bacteria of the *Borrelia burgdorferi* s.l. complex mainly associated to the hard tick *Ixodes ricinus* in Europe (Mysterud et al., 2019 Proc Biol ScI. 286: 20190759). Despite the recent improvements in molecular techniques, the detection of the LD causative agents in the hosts is still challenging and imprecise, with the possibility of false negative or false positive results (Parola & Raoul, 2001 Clin Infect Dis. 32: 897-928). Additionally, the identification of the spirochetes to the species level is pivotal both for a correct diagnosis in patients and accurate epidemiological studies in the tick vector. The molecular identification of the bacteria included in the *B. burgdorferi* s.l. complex is extremely complex due to the highly dynamic plasmid composition and the absence of a 'core genome' (Qiu & Martin, 2014 Infect Genet Evol. 27: 576-593). The aim of this work is to develop reliable species-specific targets for the univocal identification of *Borrelia burgdorferi* s.s., *Borrelia garinii* and *Borrelia afzelii*, the main LD agents in Italy.

MATERIALS AND METHODS. The available genomes of *B. burgdorferi* s.l. complex were compared through bioinformatics analyses, and unique fragments specific for each species of interest (*B. burgdorferi* s.s., *B. garini* and *B. afzelii*) were identified. Specific primers and PCR protocols were developed for each species. The newly designed molecular tools were validated on 50 DNA samples extracted from *I. ricinus* and previously tested positive to *B. burgdorferi* s.l. complex.

RESULTS AND CONCLUSIONS. The proposed tools resulted highly sensitive and specific in both qualitative and Real Time PCRs. Indeed, the species-specific primers were effective only for the respective species, thus avoiding cross-reactions with other members of the complex. In conclusion, the present work proposes an innovative approach paving the way to epidemiological studies aimed at evaluating the distribution of LD agents in areas of interest.

A study on the possible use of *Anisakis* as biological indicators of heavy metals contamination in marine environments

A COSTA[1], G CAMMILLERI[1], ML RIZZUTO[1], L PANTANO[1], FG GALLUZZO[1], A BRUNONE[2], E GIANGROSSO[1], MD BUSCEMI[1], V FERRANTELLI[1]

[1]Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy; [2]Biologist Freelance, Palermo, Italy.

Keywords: *Anisakis*, Biological indicators, Ecotoxicology

INTRODUCTION. A bioindicator is an organism that provides information on the environmental conditions of its habitat based on its presence or absence or for its behavior, which vary according to the exposure or toxic effect of environmental chemicals (Peakall, 1994 Ecotoxicology. 3: 157-160). Marine fish are subject to infestation with various species of parasites (Sures et al., 2001 Aquat Ecol. 35: 245-55). This aspect, together with their ability to accumulate heavy metals, suggests that parasites could be used as biological indicators of heavy metals contamination in marine environment (Sures et al., 2001 Aquat Ecol. 35: 245-55). The aim of this work was to determine the ecological parameters useful for the recognition of *Anisakis* spp. as environmental indicators of As, Cd Hg and Pb pollution in the Mediterranean.

MATERIALS AND METHODS. A total of 87 fish samples from Mediterranean (FAO 37.1.3) and 6 specimens of stranded dolphins were examined for the detection of anisakid parasites and the detection of Cd, As, Pb and Hg in the host muscle and in the larvae themselves. The adult forms of anisakids and muscle portions of the host cetaceans were both analysed for the presence of heavy metals listed above.

RESULTS AND CONCLUSIONS. The larvae and adults collected during the inspection belonged to the morphotype I of the genus *Anisakis*.The comparison between the levels of As in *Anisakis* and their hosts showed significantly higher concentrations in the muscle of each host sample examined. Regarding the levels of Cd, species-specific differences were noted, with significantly higher average values of the *Anisakis* larvae with respect to the squid muscle, on the contrary a inverse trend was verified between mackerel muscle and its parasites. The Pb analysis showed significantly higher concentrations of *Anisakis* larvae compared to the muscle portions of their hosts. The comparison of the dolphin samples with the *Anisakis* adults did not reveal any significant differences ($p > 0.05$) in of Cd, Pb, As and Hg values. The bioconcentration factor further confirmed the clear divergence in the accumulation of lead between *Anisakis* and its hosts. *Anisakis* could be therefore confirmed as sensitive indicators of Pb in aquatic ecosystems, showing an evident signal of the load of environmental pollution at sea.

A systematic review on *Eustrongylides* spp. in fish species: preliminary results

L GUARDONE[1], **E POLSINELLI**[1], **F SUSINI**[2], **F MANCIANTI**[1], **A ARMANI**[1]

[1]Department of Veterinary Sciences, University of Pisa, Via delle Piagge 2, 56124 Pisa, Italy; [2]Istituto Zooprofilattico Sperimentale Del Lazio e Della Toscana "M. Aleandri", S.S. Dell'Abetone e Del Brennero 4, 56123, Pisa, Italy.

Keywords: Fish-borne parasites, Fish-borne nematodes, Food safety, Food quality

INTRODUCTION. Consumption of raw fish dishes may favour the transmission of fish-borne zoonoses. *Eustrongylides* spp. (Dioctophymatidae) nematodes have an indirect life cycle in freshwater environments, with several fish species acting as second intermediate or paratenic hosts, and a wide geographical distribution (Xiong et al., 2009 J Parasitol. 99: 137-44). A few human cases have been described, in the USA and South Sudan (Heberard and Ruiz-Tiben, 2014 Am J Trop Med Hyg. 90: 315-17). The preliminary results of a systematic review on the epidemiology of *Eustrongylides* spp. in freshwater fish species globally are presented.

MATERIALS AND METHODS. A bibliographic search was carried out on 3 scientific databases with the keywords "Eustrongylides AND fish" and 2015-2020 as timeframe. The following data was set as inclusion criteria and registered in an Excel file: investigated fish species/genus; sampling site (at least country); number of examined specimens; number of positive specimens/prevalence (P). Additional data were recorded, if present: parasite detection and identification method; mean intensity; larval location (viscera or muscle).

RESULTS AND CONCLUSIONS. Initially 686 scientific papers (SPs) were retrieved. After deduplication and assessment of the inclusion criteria, 61 SPs were reviewed. Most of the studies were conducted in Brazil (n=14 SPs), followed by Iran and USA (7), Italy and Turkey (5), Nigeria (4) and Ethiopia (3). Eastern EU countries were also addressed (5 SPs). Overall, 101 fish species and 6 genera were investigated, with 67 different species and one genus found positive for *Eustrongylides* sp. larvae in at least one SP. P values ranged from 0.13% to 100%. The visceral location was predominant, but larvae in the muscle were reported by ~30% of the SPs. Only 4 SPs (6.6%) used molecular identification. The ten most investigated species (each targeted in ≥3 SPs) were: *Atherina boyeri, Cyprinus carpio, Esox lucius, Hoplias malabaricus, Micropterus salmoides, Oreochromis niloticus, Perca fluviatilis, Rutilus rutilus, Sander lucioperca* and *Silurus glanis*. Predator species showed very high P (85-100%), while lower P rates were reported for non-predators, and *C. carpio* was always negative. Reports also occurred in several commercial freshwater species in Central and Northern Italy. The rising prevalence rates in some areas (Danube basin and the Caspian Sea), possibly due to climate and anthropogenic changes (Urdes et al., 2015Agric Agri Sci Procedia. 6: 277-80; Fallah et al., 2015 J Parasitic Dis. 39: 245-48), as well as the current poor molecular characterization of the genus, suggest the need for further surveys to better understand consumers' health risks and impact on seafood quality.

Cystic echinococcosis of livestock and humans in Lebanon

G JOANNY[1], G DESSÌ[1], C TAMPONI[1], F NONNIS[1], F AHMED[1], C HOSRI[2], A SCALA[1], A VARCASIA[1]

[1]Department of Veterinary Medicine, University of Sassari, Italy; [2]Lebanese University, Faculty of Agronomy and Veterinary Medicine, Dekwaneh, Lebanon.

Keywords: Cystic echinococcosis, Zoonosis, *Echinococcus granulosus s.s.*, *Echinococcus canadensis*

INTRODUCTION. Cystic echinococcosis (CE) is a zoonotic disease caused by the species complex of *Echinococcus granulosus sensu lato* (s.l), endemic in Middle East and the Mediterranean basin, where pastoral activity is widespread (Borhani et al., 2020 PLOS Negl Trop Dis. 14: e0008114). Despite the chronic endemicity of the disease in Lebanon and neighboring, recent data are scant. The objectives of this survey were to evaluate the current epidemiology of CE in sheep and goats of Lebanon, assess the demographic and epidemiologic features of human CE surgical cases over a 14-year period in the five main regions of Lebanon and determine the infecting genotypes.

MATERIALS AND METHODS. A multidimensional approach combining *post mortem* inspection of slaughtered animals, data collection of surgically confirmed cases of human CE from five anatomo- pathology laboratories, and molecular diagnosis of the parasite was conducted.

RESULTS AND CONCLUSIONS. From 2018 to 2020, on 369 slaughtered sheep and 335 goats examined, 62.9% of sheep and 20.9% of goats were found positive for CE. The presence of hydatids varied between organs, showing higher prevalence in liver of sheep and in lungs of goats, however, higher fertility rate of hydatid cyst was observed in lungs for both animals. In humans, from 2005 to 2018, a total of 894 surgically confirmed cases of CE were recorded, with an increasing incidence from 0.53 to 1.94 cases/100,000 inhabitants in 2005 and 2018, respectively. The mean annual surgical incidence was 1.23/100,000 inhabitants. CE is present in Lebanon with an uneven distribution with higher prevalence in Bekaa (29.0%), a rural region where sheep raising is widespread. CE cases were more common in females than in males and a high burden of infection was reported for the age group of 30-39 years. Besides, 66.7% of the cases expressed only liver complications, 20.5% were related to lungs, 7.8% presented cysts in other organs, and 1.3% showed multiple localizations. Molecular diagnosis showed that the majority of isolates were identified as *E. granulosus sensu stricto* and only one isolate from goat was identified as *E. canadensis*. The current study is a step forward to fill the gap of knowledge for the CE in Lebanon and revealed for the first time the presence of three different genotypes G1, G3 and G7.

Tapeworm infection in a Mediterranean Monk Seal (*Monachus monachus*) pup

E MARCHIORI[1], G DOTTO[1], G FRANZO[1], DA RAELE[2], G PENNUZZI[2], R KUCHTA[3], A GUSTINELLI[4], L DI RENZO[5], F MARCER[1]

[1]University of Padova, Dept. Animal Medicine, Production and Health, Legnaro (PD), Italy; [2]Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy; [3]Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic; [4]University of Bologna, Dept. Veterinary Medical Sciences, Ozzano dell’Emilia (BO), Italy; Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise“ G. Caporale”, Laboratorio Ecosistemi Acquatici e Terrestri, Teramo, Italy.

Keywords: *Monachus monachus*, Diphyllobothriidae, Molecular phylogenetic analyses

INTRODUCTION The Mediterranean monk seal (*Monachus monachus*) is the rarest species of pinniped in the world, ranking among the most endangered marine mammal species. Twenty species of Diphyllobothriidae have been reported in pinnipeds, although many of them are still considered *incertae sedis* (Waeschenbach et al., 2017 IJP: Parasites and Wildlife. 47: 831-843). Definitely, one species (*Diphyllobothrium hians*) has been described in Mediterranean monk seal so far and uncertain reports exist upon another species (*D. elegans*) (Scholz et al. 2019 IJP: Parasites and Wildlife. 9: 359-369).

MATERIALS AND METHODS. In January 2020, a female monk seal pup got stranded along Apulian coast and was submitted to necropsy after failed attempt of rescue. Gastrointestinal content was washed with tap water through a series of sieves and the parasites were collected, counted, stored in 70% ethanol and 10% formalin and subsequently morphologically and molecularly studied. The complete sequence of *cox1* gene was amplified (Wicht et al. 2010 J. Clin. Microbiol. 48(9): 3111–3116), sequencedand compared to freely available *Diphyllobothrium* spp. sequences through phylogenetic analysis, performed using the IQ-Tree software. A faecal sample, collected from rectum, was subjected to copromicroscopic analyses (sedimentation and flotation method and McMaster technique; 1.450 high density solution).

RESULTS AND CONCLUSIONS. Thirty-three adult cestodes, morphologically identified as *Diphyllobothrium* sp., were isolated from the lumen of jejunum and diphyllobothriid eggs (2,522 e.p.g.) were found at copromicroscopic analysis. Phylogenetic analysis confirmed such classification. However, no closely related sequences could be identified in the public database, and the collected parasites were part of an independent cluster in the phylogenetic tree.

Further investigations will be useful to understand the taxonomic position of these specimens. Few data are available on the parassitofauna of this host species in particular on potentially zoonotic parasites, such as the Diphyllobothriidae.

Raillietiella hemidactyli (Pentastomida) in *Tarentola mauritanica* geckoes from Linosa island, Italy: a new zoonotic parasite for Europe?

JA MENDOZA-ROLDAN, RP LIA, G ANNOSCIA, R MANOJ, D OTRANTO

Dipartimento di Medicina Veterinaria, Università Degli Studi di Bari Aldo Moro, Valenzano, Italia.

Keywords: Pentastomida, *Raillietiella*, Geckoes, Zoonoses

INTRODUCTION. Pentastomida is a group of parasitic arthropods, related to crustaceans, which develop in the respiratory tract of reptiles (i.e., snakes and lizards) and mammals, such as dogs. Differently from other members of the subclass Pentastomida, the intermediate hosts of *Raillietiella* spp. are insects (e.g., cockroaches), whereas adults develop in the lung of lizards. *Raillietiella hemidactyli* has been reported as zoonotic agent causing the “creeping disease” in Asia. This infection is usually associated to consumption of raw meat of lizards. However, other potential transmission routes to humans are through the accidental ingestion of lizard secretions and feces. Herein, we report the infection of *R. hemidactyli* in invasive Moorish geckoes from Linosa, island and its possible zoonotic implications.

MATERIALS AND METHODS. Lizards from Linosa island (Pelagic archipelago, Sicily) were captured under the frame of a study on reptile zoonotic parasites. Animals were collected by hand, lassoing or collected already dead. Species of reptiles were identified using reference keys, and captured animals were then physically examined, to assess their health status. A small amount of blood was obtained via lizard tail fracture or, when animals were adults and non-gravid females, by cardiocentesis. Fecal samples were also collected from each animal. Captured lizards were humanely euthanized and necropsied. Pentastomids were morphologically identified, and their identity further confirmed through 18S rDNA and cytochrome *c* oxydase subunit 1 amplification and sequencing.

RESULTS AND CONCLUSIONS. Overall, 71 lizards were captured, of which 23 were *Chalcides occelatus*, 24 *Podarcis filfolensis* and 11 *Tarentola mauritanica.* Of the latter species, 3 (4.22%) had adult forms of *R. hemidactyli* pentastomids. Moreover, fecal samples of infected individuals were positive for embryonated eggs of this zoonotic parasite. Results of host and distribution of *Raillietiella* herein presented are new to science. Although, larval stages of *Raillietiella* were already recorded in snakes (dead-end hosts) form Italy, this finding represents the first report of zoonotic infective stages in synanthropic reptiles. Further studies should focus on the prevalence of pentastomids on synanthropic reptiles in other Italian regions to assess the zoonotic risk of infection.

Red foxes as a source of zoonotic scabies in a rural area of Piedmont, Italy

B MORONI[1], M DUTTO[2], M BARRASETAS[1], A MOLINAR MIN[1], L ROSSI[1]

[1]Department of Veterinary Science, University of Turin, Largo Braccini 2, 10095 Grugliasco, Italy; [2]former Medical Entomology Consultant, A.O. S. Croce e Carle, Cuneo.

Keywords: *Sarcoptes scabiei*, Scabies, Zoonosis, Wildlife

INTRODUCTION. *Sarcoptes scabiei* is an obligate parasitic mite affecting more than 100 mammal species worldwide. Traditionally, several host specific varieties of sarcoptic mange are known in different animal species, including humans. Whether host specificity, genetic strain or geographical occurrence of *Sarcoptes scabiei* might influence the transmission of this parasitic mite between animals and human beings is still an ongoing and open debate (Moroni et al., 2021 Parasit Vectors. 14: 1-10). The aim of this study was to describe a severe episode of human scabies in two patients with no travel history tracing the source of the infection using microsatellites as molecular markers.

MATERIALS AND METHODS. In November 2019 a woman and her daughter were referred to a local health unit in a rural area of Piedmont (Italy) for severe pruritus appeared overnight after rescuing a red fox (*Vulpes vulpes*) that showed crusty lesions all over the body. After the PCR protocol, ten *Sarcoptes* microsatellite markers were used to characterize the genetic structure of mites obtained by dermoscopy of the two human patients and skin scrapings of the fox (four and two mites, respectively). All mites were identified as *Sarcoptes scabiei* following morphological criteria. The genetic profiles of mites isolated in the present study were compared to those of mites previously collected from sympatric foxes and Spanish ibexes as outgroup. Population genetics analyses were carried out using Bayesian clustering approach (software STRUCTURE 2.3.4).

RESULTS AND CONCLUSIONS. Dermoscopy from two human patients revealed the presence of *Sarcoptes* eggs, mites of all stages and skin burrows, which imply that the mites transmitted from foxes to humans were able to actively replicate. The Bayesian assignment test revealed the presence of two different *Sarcoptes* -derived clusters (Figure 1), consisting of mites from two human patients, the rescued fox and sympatric foxes, and mites from Spanish ibex. The results demonstrate a clear origin of the human outbreak identifiable in the red fox rescued by the women. Foxes are well-known wildlife hosts for sarcoptic mange in Europe, and previous genetic studies identified them as one of the most common carnivores spreading mange among different wildlife species, and humans as well (Pisano et al., 2019 Emerg Infect Dis. 25: 1235–38). To the best of our knowledge, this is the first genetic report of fox-derived transmission to humans.

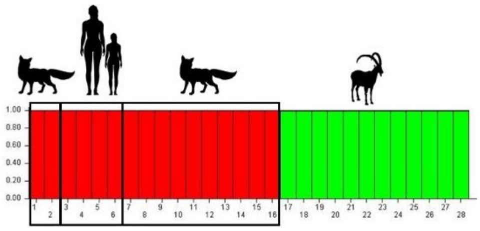

Figure 1. Bar plot of *Sarcoptes*-derived genetic cluster generated with the software Structure 2.3.4 with maximum likelihood $K = 2$. Each mite is represented by a single bar. Red cluster represents mites from the fox-human outbreak in Piedmont (on the left) and mites from sympatric red foxes (on the right). Green cluster represents *Sarcoptes* mites from the Spanish ibex (as outgroup).

Mosquito Alert: a Citizen Science project to monitor mosquitoes in Italy

MOSQUITO ALERT ITALIA TASK FORCE (B CAPUTO)

Dipartimento di Sanità Pubblica e Malattie Infettive, Università di Roma SAPIENZA; Istituto Superiore di Sanità, Roma; Istituto Zooprofilattico delle Venezie, Padova; MUSE-Museo delle Scienze, Trento; Università Alma Mater di Bologna.

Keywords: Mosquitoes, Citizen-science, Smartphone app.

INTRODUCTION. The national plan of the Italian Ministry of Health for the prevention, surveillance and control of arboviruses (PNA, 2020-2025) prescribes entomological surveillance at region level to prevent risk of pathogen transmission by autochthonous and invasive mosquito species. However, the high costs of entomological surveillance restrict the implementation of these activities to few areas/regions where the risk is higher or the public health system is more efficient. Under this scenario, Citizen Science offers the opportunity to complement current entomological approaches and to provide data from the whole national territory.

MATERIALS AND METHODS. In October 2020, the European-funded Aedes Invasive Mosquito (AIM) COST Action (https://www.aedescost.eu/), coordinated by University of Rome SAPIENZA, made available all over Europe MOSQUITO ALERT application for smartphones, with the objective to involve citizen in mosquito surveillance, while educating them on mosquito biology, public health risks and preventive interventions. Citizen can send 3 types of data through MOSQUITO ALERT app: 1) photos of live or dead mosquitoes, which will be identified by 3 expert entomologists and can be exploited for updating the distribution of autochthonous and invasive mosquito species and for developing maps of probability of their presence; 2) photos of larval sites; 3) records of bites, which can be exploited to map and model human-mosquito contact and arbovirus transmission risk. The task Force of MOSQUITO ALERT ITALIA is promoting the app use among citizen in 2021 mosquito-breeding season and will analyse data, produce maps of species distribution and of probability of bites and make them public available.

RESULTS AND CONCLUSIONS. On the short term, we expect that the large-scale use of MOSQUITO ALERT by citizen will provide relevant data for research and surveillance of mosquitoes in Italy, while on the medium/large term this tool could be exploited by public administrations to guide and optimize interventions aimed at reducing mosquito nuisance and mosquito-related public health risks. Whether or not these objectives are achieved depends on number of records sent by users. We expect that SOIPA community is the ideal one for understanding the relevance of the project and contribute mosquito photos and bite records.

Seroprevalence of *Toxoplasma gondii* among sylvatic rodents in Poland

J NOWICKA[1], M GRZYBEK[1], B BIERNAT[1], D ANTONOVÁ[2], K TOŁKACZ[3], A BAJER[4], M ALSARRAF[4], J BEHNKE-BOROWCZYK[5], J PALEOLOG[6], JM BEHNKE[7]

[1]Medical University of Gdańsk, Poland; [2]Slovak Academy of Sciences, Košice, Slovakia; [3]Polish Academy of Sciences, Warsaw, Poland; [4]University of Warsaw, Poland; [5]Poznań University of Life Sciences, Poland; [6]University of Life Sciences in Lublin, Poland; [7]University of Nottingham, United Kingdom.

Keywords: *Toxoplasma gondii*, Rodents, Seromonitoring, Rodent-borne diseases

INTRODUCTION. There is currently considerable interest in understanding the transmission of pathogens and the range of different variables that influence infection dynamics. Wild rodents pose a particular threat to human communities because they constitute the most abundant and diversified group of all living mammals. *Toxoplasma gondii* is an intracellular Apicomplexan parasite with a broad range of intermediate hosts, including humans and rodents. Rodents are considered to be reservoirs of infection for their predators that include cats, pigs and dogs.

MATERIALS AND METHODS. We conducted a multi-site, long-term study on *T. gondii* in northeastern Poland. Our objectives were to monitor the seroprevalence of *T. gondii* in the four abundant vole species found in the region (*Myodes glareolus, Microtus arvalis, Microtus agrestis, Alexandromys oeconomus*) and to assess variation in seroprevalence attributable to both intrinsic and extrinsic factors that were quantified. A bespoke enzyme-linked immunosorbent assay was used to detect antibodies against *T. gondii*.

RESULTS AND CONCLUSIONS. We detected *T. gondii* antibodies in the sera of all four rodent species with an overall seroprevalence of 5.5% (3.6% for *M. glareolus* and 20% for other vole species). Seroprevalence in bank voles varied significantly between host age and sex). These results contribute to our understanding of the distribution and abundance of *T. gondii* in voles in Poland and confirm that *T. gondii* circulates also in *M. glareolus* and *Microtus/Alexandromys spp*. Therefore, they may potentially play a role as reservoirs of this parasite in the sylvatic environment.

This research was funded through the 2018-2019 BiodivERsA joint call for research proposals under the BiodivERsA3 ERA-Net COFOUND programme. JN and MG were supported by the National Science Centre, Poland, under the BiodivERsA3 programme (2019/31/Z/NZ8/04028).

Clustered cases of human ophtalmomyiasis in tourists caused by *Oestrus ovis*

C PASINI, C TAMPONI, S MIGNACCA, E CONTU, G DESSÌ, A VARCASIA, L MELONI, S KNOLL, L CAVALLO, F PORCU, MF SINI, F NONNIS, A SCALA

Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Italy.

Keywords: Ophthalmomyiasis, Sheep bot fly, *Oestrus ovis*

INTRODUCTION. *Oestrus ovis* is the most common cause of human ophthalmomyiasis (D'Assumpcao et al., 2019 J Investig Med High Impact Case Rep. 7: 2324709619835852; Pupić-Bakrač et al., 2020 Parasitol Res. 119: 783-93). Herein we present, two clinical cases of ophthalmomyiasisrecorded in in two young and healthy women in North Sardinia in 2018.

MATERIALS AND METHODS. The anamnesis of both women reported any contact with wild or farm animals. The patients suddenly felt a foreign body inside their eyes. The clinical examination showed blepharospasm, conjunctival hyperaemia and tiny mobile and translucent larvae, 1 mm in size. Larvae were collected and sent to the Parasitology Laboratory of the Veterinary Department of Sassari. The larvae were identified using the morphological keys described by Zumpt (1965 Butterworths, London). Genomic DNA of the larva was extracted using a commercial kit and a fragment of the *Cox1* gene was amplified by a PCR (Ward et al., 2005 Philos Trans R Soc B. 360: 1847-57). PCR products were sent to an external sequencing service (Eurofins Genomics, Ebersberg, Germany) and the sequences obtained were compared with those available on the NCBI database.

RESULTS AND CONCLUSIONS. The larvae were morphological identified as first instar (L1) of *O. ovis*. The genetic analysis confirmed the morphological identification. Myiasis caused by *O. ovis* should be considered in people returning from abroad presenting foreign body sensation in the eyes, nose or throat (Brini et al., 2019Parasitol Res. 118: 3217-21; Dìez-Gonzalez et al., 2020Med Clin. 155: 566-67). Although ophtalmomyiasis is rare, the number of reported cases is probably underestimated (Pubic'-Bakrac et al., 2020Parasitol Res. 119: 783-93). Global warming predisposes future increase of *O. ovis* prevalence in humans which emphasizes the need for mandatory reporting and surveillance of disease (Pubic'-Bakrac et al., 2020Parasitol Res. 119: 783-93) and greater awareness among patients and medical professionals.

Towards an integrated approach for monitoring toxoplasmosis in southern Italy

P PEPE[1], A BOSCO[1], G MANGIERI[1], F CAPUANO[2], L BALDI[2], A GIORDANO[2], A MANCUSI[2], M BUONANNO[2], L MORENA[3], R PINTO[4], P SARNELLI[4], G CRINGOLI[1,3], L RINALDI[1,3]

[1]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, CREMOPAR, Naples, Italy; [2]Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Naples, Italy; [3]Centro di Riferimento Regionale Sanità Animale (CReSan), Regione Campania, Naples, Italy; [4]UOD Prevenzione e Sanità Pubblica Veterinaria Regione Campania, Naples, Italy.

Keywords: *Toxoplasma gondii*, Monitoring, Risk factors, One Health

INTRODUCTION. Toxoplasmosis is a widespread worldwide zoonotic infection caused by the intracellular protozoan *Toxoplasma gondii* (Dubey et al., 2020 Vet Parasitol. 288: 109185). This protozoan infection is considered one of the most important food-borne parasitic zoonoses globally (Opsteegh et al., 2016 Final Report EFSA). Beyond its impact on public health, toxoplasmosis has also important veterinary implications, because it causes in livestock miscarriage or congenital malformations with negative economic impacts (Hill et al., 2015 Anim Health Res Rev. 6: 41-61). In order to reduce the spread of this infection, an integrated monitoring programme aimed to deepen the epidemiological data on toxoplasmosis and to identify the risk factors which may favour *T. gondii* infections in animals and humans was conducted in an endemic area of southern Italy (Campania region).

MATERIALS AND METHODS. The monitoring activities started in 2019 and were based on the following strategies: (i) parasitological analysis and risk factors for *T. gondii* in livestock (sheep, goats, cattle and water buffalo) farms; (ii) serological and molecular monitoring in meat-producing livestock during the slaughter's activities; (iii) hospital discharge records (HDRs); (iv) outreach activities (information, dissemination and health education) to farmers, vet practitioners, school-age children and pregnant women.

RESULTS AND CONCLUSIONS. The present study shed light on the current epidemiological situation of *T. gondii* in the Campania region of southern Italy, confirming a very high seroprevalence of this infection in livestock (up to 93.1 % in sheep farms) in southern Italy and highlighting the potentially significant public health risk in this area.

Based on these findings, reducing *T. gondii* infection in animals is of fundamental importance to prevent foodborne transmission of *T. gondii* to humans according to the One Health perspective. Furthermore, due to the impact of toxoplasmosis on public and veterinary health, a greater institutional awareness of the pathways of infection and comprehensive and transdisciplinary actions to control transmission are needed.

Small bowel volvulus by *Ascaris suum* in a 75-year-old man: a case report

G ROMANO[1], P PEPE[2], S CAVALLERO[3], P COCIANCIC[2,4], L DI LIBERO[1], G GRANDE[1], G CRINGOLI[2], S D'AMELIO[3], L RINALDI[2]

[1]Department of General Surgery, Frieri-Criscuoli Hospital, Sant'Angelo dei Lombardi, Avellino, Italy; [2]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, CREMOPAR, WHO Collaborating Centre for diagnosis of intestinal helminths and protozoa (ITA-116), Naples, Italy; [3]Department of Public Health and Infectious Diseases, Section of Parasitology, Sapienza University of Rome, Rome, Italy; [4]Centro de EstudiosParasitológicos y de Vectores (CEPAVE-CONICET-UNLP-asociado a CICPBA), La Plata, Buenos Aires, Argentina.

Keywords: *Ascaris suum*, Zoonosis, Pigs, Southern Italy

INTRODUCTION. *Ascaris lumbricoides* and *Ascaris suum* are the most common soil-transmitted helminths of humans and domestic pigs, respectively. Both species produce cross-infections between pigs and humans being potentially zoonotic (Betson et al., 2014 J Infect Dis. 210: 932-41). However, the zoonotic potential is often underestimated especially in the developed countries (Cavallero et al., 2013 PLoSNegl Trop Dis. 7: e2170.). This study was aimed to present a case of human ascariasis caused by *A. suum* in southern Italy.

MATERIALS AND METHODS. A 75-year-old man presented to the department of surgery in southern Italy (Avellino province, Campania region) complaining of abdominal pain and vomiting. The patient lived in a rural area of the province and had raised chickens and pigs for his family's consumption. Physical examination revealed bloating and abdominal tenderness. Computed tomography (CT) scan exposed multiple air-fluid levels in the epigastrium and left hypochondrium with some fluid in mesenteric recesses and signs of bowel obstruction. The day after the hospitalization, the symptoms persisted so the patient was referred to surgery. During exploratory laparotomy a small bowel volvulus with mesenteritis was evident and surprisingly an intraluminal worm was detected. The palpation of the entire bowel did not show the presence of other helminths. A stool sample was collected and analysed with the FLOTAC dual technique (Cringoli et al., 2010 Nat Protoc. 5: 503-15) whereas the recovered worm was subjected to morphological and molecular analysis (Zhu et al., 1999 Int J Parasitol. 29: 469-78).

RESULTS AND CONCLUSIONS. Faecal examination revealed the presence of unfertilized *Ascaris* eggs with an intensity of 16 eggs per gram of faeces. The worm was identified as an adult female of *A. suum* on morphological and molecular analysis. The patient was treated with mebendazole 100 mg twice a day for three days. The post-operative course was regular with re-alimentation after 3 days and discharge after 12 days. This report highlights *A. suum* as a relevant agent of human zoonosis in rural areas non-endemic for *A. lumbricoides*.

Prevalence of intestinal parasites infecting free-ranging non-human primates in Colombia

S RONDÓN[1], S CAVALLERO[1], A LINK[2], C GONZÁLEZ[3], S D'AMELIO[1]

[1]Department of Public Health and Infectious Diseases, Parasitology Section, Sapienza University of Rome; [2]Departamento de Ciencias Biológicas, Laboratorio de Ecología de Bosques Tropicales y Primatología, Universidad de Los Andes; [3]Departamento de Ciencias Biológicas, Centro de Investigaciones en Microbiología y Parasitología Tropical, Universidad de Los Andes.

Keywords: Non-human primates, Intestinal parasites, Fragmented forest, Colombia

INTRODUCTION. Neotropical non-human primates (NHP) have been found infected with a diversity of intestinal zoonotic protozoan and metazoan parasites of public health concern (Solórzano-García and Pérez-Ponce de León, 2018 Int J Primatol. 39: 155-82). Ecosystem transformation increases contact between humans and NHP (Trejo-Macías et al., 2007 Int J Primatol. 28: 931-45), leading to zoonotic parasite transmission. This study aimed to assess the prevalence of intestinal parasites in free-ranging NHP, living in five forest fragments in Colombia and to perform parasite identification to the lowest possible taxonomic level.

MATERIALS AND METHODS. In 2019 and 2020, fecal samples were collected from NHP immediately after defecation, and stored in 10% formalin solution and 96% ethanol. Faecal smears with 1% iodine solution and 0.85% saline solution were performed (Botero and Restrepo, 2012 Parasitosis humanas. Quinta edición). In this study, samples microscopically classified as positive for Ascarididae and *Blastocystis* sp. were processed for molecular characterization (Cavallero et al., 2013 PLosNegl Trop Dis. 7: e2170; Mattiucci et al., 2016 Epidemiol Infect. 144: 635-46).

RESULTS AND CONCLUSIONS. Overall, 160 fecal samples were collected from individuals from six platyrrhine genera: *Alouatta seniculus* (n=46)*, Ateles hybridus* (n=13)*, Aotus griseimembra* (n=5)*, Cebus versicolor* (n=20)*, Saimiri cassiquiarensis* (n=73), and *Sapajus* sp. (n=3). Around 90% of the samples were positive for intestinal parasites. Protozoans (*Blastocystis* sp., Balantiididae, *Dientamoeba* sp., Entamoebidae, *Giardia* sp.), cestodes (*Hymenolepis* sp.), trematodes (*Controrchis* sp.), nematodes (Ascarididae, Trichuridae, *Strongyloides* sp., *Trypanoxyuris* sp., Ancylostomatidae), and acanthocephalans were observed. *Ascaris lumbricoides* and *Blastocystis hominis* (ST8) were identified using molecular biology techniques. The finding of parasites with zoonotic potential suggests epidemiological implications in NHP conservation and human health, particularly in highly transformed ecosystems.

Occurrence of *Eustrongylides excisus* (Nematoda: Dioctophymatidae) in European perch (*Perca fluviatilis*) and Great cormorant (*Phalacrocorax carbo*) in Northern Italy, Lake Annone

A RUSCONI[1], P PRATI[2], R BRAGONI[2], M CASTELLI[1], U POSTIGLIONE[1], D SASSERA[1], E OLIVIERI[1]

[1]Dipartimento di Biologia e Biotecnologie, Università di Pavia, Italy; [2]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Pavia Department, Italy.

Keywords: *Eustrongylides excisus, Perca fluviatilis*, *Phalacrocorax carbo sinensis*, Waterborne zoonoses

INTRODUCTION. The genus *Eustrongylides* includes zoonotic nematodes that infect fish species and fish-eating birds of freshwater ecosystems (Measures, 1988 Can J Zool. 66: 885-95). The number of new reports has recently increased worldwide, suggesting that the parasite is widely present and/or spreading to novel areas. Lake Annone is a pre-mountainous lake located in Alps area in Northwest Italy. This is a very peculiar area, in which the status of the aquatic environment together with the presence of both wintering and resident cormorants (the main definitive host of *Eustrongylides*) provide exceptional suitable conditions for the nematode life cycle. The aim of this study was to evaluate the occurrence of *Eustrongylides* in the paratenic host *Perca fluviatilis* (European perch) and in the definitive host, *Phalacrocorax carbo sinensis* (great cormorant) foraging in the basin.

MATERIALS AND METHODS. A total of 114 European perch and 48 cormorants were examined for the occurrence of *Eustrongylides*. All parasites collected were identified by microscopic examination and molecular analysis.

RESULTS AND CONCLUSIONS. Overall, 11 specimens of European perch (9.6%) and 13 individuals of cormorants (27%) harboured nematodes identified as fourth-stage larvae and adults of *Eustrongylides excisus*, respectively. The herein observed prevalence of *Eustrongylides* appears to be intermediate between the higher values in cormorant breeding areas in northern Europe and the lower prevalence observed in their wintering sites in southernmost Europe (Branciari et al., 2016 J Food Saf. 5: 3-6; Goncharov et al., 2018 Vestn Zool. 52: 137-44). Considering that in lake Annone, wintering cormorants coexist with newly formed breeding colonies, our results suggest a North-South gradient of the prevalence levels of *Eustrongylides*. Therefore, it could be interesting to investigate on the historical and recent diffusion dynamics and paths of *Eustrongylides* and its bird hosts from different areas.

Identification of cestodes in veterinary diagnostics: evaluation of two PCR methods

S SGUBIN[1], V CAGNIN[1], G DA ROLD[1], E QUARANTA[1], G SIMONATO[2], S PASQUALOTTO[1], A ZOROASTER[1], E PORCELLATO[1], S RAVAGNAN[1]

[1]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padua, Italy; [2]Department of Animal Medicine, Production and Health, University of Padova, Italy.

Keywords: Cestode, Molecular identification, Veterinary diagnostics

INTRODUCTION. The molecular identification can support the morphological identification of cestodes (eggs, larvae and adults) that can be difficult. The aim of the study was to evaluate the ability of two PCR methods to identify cestodes at the species level.

MATERIALS AND METHODS. The COI-PCR assay, targeting the cytochrome oxidase gene (COI) (Bart et al., 2006 Parasitol Res.98: 130-37) and the Multiplex PCR amplifying the ND1 gene for *Echinococcus multilocularis* (394bp) and the 12SrRNA for *Echinococcus granulosus* (117bp) and *Taenia* spp. (271bp) (Trachsel et al.,2007 Parasitology.134: 911-20) were performed on 162 samples of feces (foxes n=138, dogs n=9, badger n=1) and 14 of cysts collected from ruminants. Amplicons of COI and 12SrRNA *Taenia* PCR were sequenced for species identification, whereas *E. granulosus* vs *E. multilocularis* can be identified based on different Multiplex PCR amplicons length. All feces were positive for cestode eggs at copromicroscopy.

RESULTS AND CONCLUSIONS. *E. granulosus* was identified in 7 out of 14 cysts with both PCR methods. Results of fecal samples are shown in Fig. 1. Multiplex PCR confirmed all COI-PCR positive samples, except for 5 *Taenia* spp., and it was able to identify 7 more cestodes that were hidden by coinfection: 2 *E. multilocularis* and 5 *Taenia* spp. Two COI-PCR negative samples resulted positive (*Taenia crassiceps* and 1 co-infection *E. multilocularis* and *T. crassiceps*). In conclusion, the two methods gave the same results for cysts. For faecal samples, often coinfected, Multiplex PCR is more suitable, asCOI-PCR detects only the prevalent cestode. Multiplex PCR seems to be more sensitive in the detection of *E. multilocularis* (3 more were found) and it is less expensive as it does not require always sequencing. For phylogenetic analysis, COI-PCR is the method recommended.

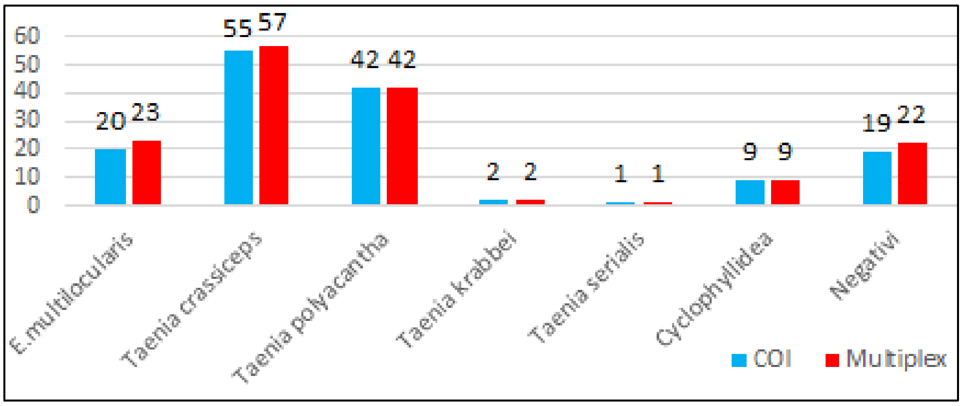

Fig.1 Results of two PCR methods (COI PCR and Multiplex PCR) applied to faecal samples.

Bayesian statistical models to evaluate the efficacy of traditional and innovative mosquito control interventions

C VIRGILLITO[1,2], M MANICA[2], G MARINI[2], B CAPUTO[1], R ROSÀ[2,3], A DELLA TORRE[1]

[1]Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome; [2] Department of Biodiversity and Molecular Ecology, Research and Innovation Centre Edmund Mach Foundation, San Michele all' Adige, Trento; [3]Center Agriculture Food Environment, University of Trento, San Michele all' Adige, Trento.

Keywords: Statistical models, Bayesian regression, Mosquito control

INTRODUCTION. We tested the flexibility of Bayesian approaches to analyze results of two field experiments conducted in Italy: Exp1) a conventional larval control intervention using *Bacillus thuringiensis* subsp. *israelensis* and *Bacillus sphaericus* in rural ditches, the main larval habitat of West Nile Virus vector*, Culex pipiens*; Exp2) an innovative approach to reduce abundance of adult *Aedes albopictus* based on the release of incompatible males transinfected with a foreign *Wolbachia* (ARwP) endosymbiont.

MATERIALS AND METHODS. Generalized Additive Mixed Models (GAMM) were developed to model either larvae/pupae (Exp1) or viable egg (Exp2) abundance overtime in treated vs untreated sites, accounting for multiple locations and repeated samplings. A two-stage Generalized Linear Model (GLM) was developed for Exp2 to infer the exposure of field-collected females to mating with released ARwP-males.

RESULTS AND CONCLUSION. GAMM approaches predict: in Exp1, full effectiveness of the treatment up to 21 days (100% larval and pupal mortality) and a gradual increase in survival up to 4 weeks post-treatment; In Exp2, a lower percentage (up to 35%) of viable eggs in treated than in control site. The GLM model predicts the proportion of females classified as mated with ARwP males is 41.78%(95% CI 33.18–50.80). Overall, the application of Bayesian regression approaches has an added value compared to conventional statistical methods in the analysis of data on mosquito control experiments as it allows to predict mosquito temporal dynamics and other relevant parameters (e.g. mating probability), as well as effect of the treatments in different ecological-climatic settings.

POSTER SOIPA

PARASSITOLOGIA VETERINARIA E UMANA

Blastocystis in the marine environment: new insights into the genetic diversity and distribution in cetaceans and marine turtles from the Mediterranean Sea

R ACO-ALBURQUEQUE[1], S GABRIELLI[1], M PALOMBA[1,2], F MARCER[3], E MARCHIORI[3], M SANTORO[2], S MATTIUCCI[1]

[1]Department of Public Health and Infectious Diseases, Sapienza-University of Rome; [2]Department of Integrative Marine Ecology, Anton Dohrn; [3]Department of Animal Medicine, Production and Health, University of Padua.

Keywords: *Blastocystis*, SSU rDNA, Subtypes, Marine mammals, Italy

INTRODUCTION. *Blastocystis* is widespread in several terrestrial animal hosts, including humans; a large genetic heterogeneity has been so far demonstrated with the characterization of distinct 22 subtypes (STs). However, its investigation in aquatic organisms remains still limited (Gantois et al., 2020, Microorganisms, 8(3), 460). In the present study, a large survey for the presence of *Blastocystis* was carried out by screening frozen stools collected directly from rectum comprising 25 samples from 5 cetacean species (*Balaenoptera physalus, Physeter macrocephalus, Grampus griseus, Stenella coeruleoalba, Tursiops truncatus*) and 12 from the loggerhead seaturtle (*Caretta caretta),* stranded along the Italian Mediterranean Sea coast during the years 2011-2021.

MATERIALS AND METHODS. Direct sequences analysis of the total SSU rDNA gene was carried out, as previously described. The prevalence of the parasite in the examined samples was about 2.7%. The phylogenetic analysis shows that the isolates from *B. physalus* and *C. caretta* correspond to potential new STs: they are forming distinct phylogenetic lineages with respect to those STs so far known and genetically characterized. The findings seem to support the hypothesis that distinct STs of *Blastocystis* are adapted to marine mammals and turtles.

RESULTS AND CONCLUSIONS. The examined marine samples were not so far found colonized by other STs identified in humans and other terrestrial animals, as the possible result of bioaccumulation in those top-predator animals through a marine trophic-web and/or from coastal contaminated seawater by animal waste. However, a limit in the detection of other STs in those samples would be also related to the long-time storing of the examined faecal isolates. The present survey represents the first data regarding ST distribution in several Mediterranean cetaceans and marine turtles and provides new insights into its genetic diversity and host range. Despite the fact that the detected STs have been not so far identified in humans, the potential risk of the zoonotic transmission of those new STs to humans, is discussed.

This study was supported by: Sapienza University of Rome, Grandi Progetti di Ricerca Ateneo Sapienza (2020) and IILA, International Italian-Latin American Organization [Fellowship IILA-MAECI/DGCS assigned to R. Aco Alburqueque]

Unravelling the development of the female mosquito proboscis using X-ray Tomography

I ARNOLDI[1,3], A NEGRI[2,3], B BISAGLIA[2,3], I VAROTTO-BOCCAZZI[2,3], R NODARI[2,3], S EPIS[2,3], P GABRIELI[2,3]

[1]IUSS, Pavia, 27100, Italy; [2]Department of Biosciences and Pediatric Clinical Research Center "Romeo ed Enrica Invernizzi", University of Milan, Milan, 20133, Italy; [3]Italian Malaria Network, University of Milan, Milan, 20133, Italy.

Keywords: Mosquitoes, X-ray Tomography, Development

INTRODUCTION. The proboscis of adult female mosquitoes is formed by the labium, an external sheath retracted during feeding, and by a fascicle of six stylets, which are inserted in the vertebrate host skin. Among them, the labrum mostly forms the food channel, while the hypopharynx carries the salivary duct. The other two paired appendices, the maxillae, which carry a row of proximally directed teeth, and the mandibles help the penetration of the dermis during the feeding process. Thus far, very little is known about the formation of this complex, mainly because the use of classical approaches, such as Transmission Electron Microscopy, is heavily hampered by the presence of multiple cuticle layers, preventing proper fixation, embedding, and cutting of the samples. For this reason, we investigated the morphological features of the head and the buccal apparatus of larvae, pupae and adult mosquitoes of the species *Anopheles stephensi*, *Aedes albopictus* and *Aedes aegypti* using X-ray Tomography.

MATERIALS AND METHODS. We collected 25 samples for *Ae. albopictus* (4th instar larva, 15 female pupae at different time points after pupation, 7 male pupae, female and male adults), 11 samples for *Ae. aegypti* (4th instar larva, 9 female pupae, female adult) and 12 samples for *An. stephensi* species (4th instar larva, 10 female pupae, female adult). The X-ray diffraction datasets were collected at the synchrotron facility (Villigen, CH) and analyzed in both the absorption based and edge-enhanced radiography, using Gridrec and Paganini reconstruction guidelines. Segmentations and 3D reconstructions were performed using Slicer 3D Software.

RESULTS AND CONCLUSIONS. By analyzing a total of 48 datasets, we compared the morphology of the buccal apparatus of close-related species, such as *Ae. albopictus* and *Ae. aegypti*, as well as of species belonging to different genera (*Anopheles* vs *Aedes*), unravelling the gradual modifications of the mouth occurring during the pupal development. Our study paves the way for a deeper understanding of the events taking place during mosquito metamorphosis and for the investigation of other organs and organisms using X-ray Tomography.

Identification of mosquitoes: new simplified keys for morphological analysis

I BERNARDINI[1,2], M DI LUCA[1], F SEVERINI[1]

[1]Istituto Superiore di Sanità, Dipartimento di Malattie Infettive, Roma, Italy; [2]Sapienza Università di Roma, Dipartimento di Sanità Pubblica e Malattie Infettive, Roma, Italy.

Keywords: Mosquitoes, Simplified keys, Morphological identification, Vectors

INTRODUCTION. In medical and veterinary entomology, the morphological identification is an important tool to recognize vectors of pathogens as mosquitoes, sand flies and ticks. Analysis is based on dichotomous keys (on paper or digital support), that follow a single pathway of character choices up to an end point, i.e. the identification of taxon. When personnel with expertise in entomology are not available, it is necessary to implement simplified tools suitable for achieving targeted objectives. Aim of this study is to create a simplified identification keys of mosquitoes at genus level, for personnel working in areas with potential circulation of vector-borne diseases. This work is a part of the ISS project "Capture, Characterization, and Control of vector arthropods and related pathogens – C3-Lab" carried out in collaboration with Ministry of Defence and University of Rome "Sapienza".

MATERIALS AND METHODS. To create this new taxonomic support, all the morphological keys available for Italian and African species were analyzed. Simplified identification technology was developed in digital form using PowerPoint software – Microsoft® in a step-by-step dichotomic procedure. Practically, the operator chooses the characteristics relating to the genera analysed in a simple scheduling system, including figures and description of morphological details with taxonomic value. Additionally, the main information on the vector and invasive species is reported in the summary sheets. All the selected characters will easily and uniquely guide operator towards a mosquito genus.

RESULTS AND CONCLUSIONS. To have a prior knowledge of mosquitoes in an area chosen as a theatre of operations, and consequently of the potential diseases circulating, non-specialized personnel can use these digital keys that allow to quickly identify mosquito genera, using any technological support (smartphone, tablet, or computer). Moreover, during sampling procedures, specimens can be screened in real time, and all characteristics are preserved for identification and eventual molecular analysis of pathogens. This new practical support allows the identification of different subfamilies and genera using only a few diagnostic characters, currently only for Italian and African (Djibouti and Niger) mosquitoes. Future studies could be aimed at simplifying morphological keys for genera and species of any endemic area.

Questionnaire survey of *Thelazia callipaeda* prevalence in Italy and vector samplings indicate Lazio region as a hot-spot of thelaziosis

I BERNARDINI[1,2], RP LIA[3], S MANZI[2], C POGGI[2], V PETRARCA[2], F BEUGNET[4], J MACA[5], MS LATROFA[3], D OTRANTO[3], M POMBI[2]

[1]Istituto Superiore di Sanità, Dipartimento di Malattive Infettive, Rome, Italy; [2]Sapienza Università di Roma, Dipartimento di Sanità Pubblica e Malattie Infettive, Roma, Italy; [3]Università degli Studi di Bari, Dipartimento di Medicina Veterinaria, Valenzano, Italy; [4]Boehringer-Ingelheim, Lyon, France; [5]Czech Entomological Society, Praha, Czech Republic.

Keywords: Eyeworm, Zoonosis, Case reports, Epidemiology

INTRODUCTION. Thelaziosis is a zoonotic disease widespread in Asia and an emerging threat in Europe caused by the oriental eyeworm *Thelazia callipaeda*, transmitted through lachryphagous activity of *Phortica variegata* (Otranto et al., 2005 Trends Parasitol. 21: 1-4). In European countries, increasing cases of thelaziosis have been registered during last 20 years in domestic and wild animals, respectively (do Vale et al., 2020 Parasite. 27). In addition, several reports of this disease are described in humans, with 11 cases diagnosed in Europe until now. In order to evaluate the burden of this disease in Italy, an on-line survey has been administered on veterinarians. This information has also been associated to *T. callipaeda* prevalence in vector populations focusing on Lazio region.

MATERIALS AND METHODS. A questionnaire was distributed through the Federazione Nazionale dei Medici Veterinari Italiani requesting information about clinical records of thelaziosis cases. Sampling of *lachryphagous drosophilids* was conducted in 4 areas of Lazio region: Manziana (RM), Gattaceca (RM), Nomentum Park (RM), Foglino (RM) and Turona park (VT). Collected specimens were morphologically identified and molecularly tested by PCR (*cox1*) to detect *T. callipaeda* (Otranto et al., 2005 Mol Cell Probes. 19: 306-13).

RESULTS AND CONCLUSIONS. A total of 408 records were obtained from questionnaire administration, reporting an overall presence of *T. callipaeda* in 15 out of 17 regions, with higher participation in Lazio (66), Emilia-Romagna (65) and Veneto (57). Most thelaziosis cases have been observed in dogs (82%), followed by cats (10%) and a few other animal cases. The majority of detections were in Lazio (27%) and Piemonte (25%). The first clinical detection dates 1980 in Frosinone (Lazio), with an increasing trend through the years, particularly after 2015. A total of 1316 lachrypagous *P. variegata* were collected, of which 23 (1.7%; 95% CI: 0.6 – 2.8%) tested positive for *T. callipaeda*, particularly in Manziana, were the eyeworm was stably diagnosed from 2017 to 2020. These results indicate that *T. callipaeda* is widespread among mammal hosts throughout Italy, particularly in Lazio region, which seems to be a hot-spot of transmission for this disease.

Natural infections of *Sergentomyia minuta* with kinetoplastid flagellates detected by gold standard methods in Rome province

G BONGIORNO[1], **T DI MUCCIO**[1], **L GRADONI**[1], **A GIACOMI**[2], **M POMBI**[2], **S GABRIELLI**[2], **M GRAMICCIA**[1]

[1]Istituto Superiore di Sanità, Department Infectious Diseases, Unit of Vector-borne Diseases, Rome, Italy; [2]Department of Public Health and Infectious Diseases, La Sapienza University of Rome, Italy.

Keywords: *Sergentomyia minuta*, *Leishmania* (*Sauroleishmania*) *tarentolae*, *Trypanosoma platydactyli*

INTRODUCTION. *Sergentomyia* genus includes a widespread group of phlebotomine sand fly species, *S. minuta* being the only member recorded in Italy. When Italian specimens of this species were examined for kinetoplastid flagellates by gold standard methods (*sensu* Seblova et al., 2014 Parasit Vectors. 7: 22) (gut dissection, microscopy evaluation of developmental stage and load of detected morphotypes, culture isolation and biochemical/molecular typing), they had always been found infected by *Leishmania* (*Sauroleishmania*) *tarentolae* and/or *Trypanosoma platydactyli* (Pozio et al., 1986 in: *Leishmania*. Taxonomie et phylogenése, IMEEE, Montpellier 149-55; Maroli et al., 1988 Trans R Soc Trop Med Hyg. 82: 227-28; Gramiccia et al., 1989 Ann Parasitol Hum Comp. 64: 154-56; Gramiccia, 2013 EDENext final report, data on file). Since *Leishmania* (*Leishmania*) *infantum* DNA has recently been identified in Italian *S. minuta* specimens (Latrofa et al., 2018 Vet Parasitol. 253: 39-42; Pombi et al., 2020 Med Vet Entomol. 34: 470-75), we aimed to check whether a gold-standard approach could confirm natural *L. infantum* infections in females of this species from an area where *S. minuta* has been found positive by *Leishmania* PCR.

MATERIALS AND METHODS. A survey was performed in Monteporzio Catone, Rome, from July till September 2017. CDC light traps were placed along unpaved roads bordered by dry stone walls, far away from animal shelters to minimize collection of species different from *S. minuta*. Live females were identified upon dissection and examined for flagellates as described above. Typing of cultured organisms was carried out by ribosomal ITS1-PCR RFLP and sequencing is in progress (Di Muccio et al., 2015 PLoS One. 10: e0129418).

RESULTS AND CONCLUSIONS. Out of 129 *S. minuta* specimens dissected, 6 (4.7%) harboured massive infections by mobile and culturable flagellates: 3 by epi- and trypomastigotes morphologically identical to *T. platydactyli* ISS reference strains; 1 by promastigotes typed molecularly as *L. tarentolae*; and 2 by a mixed infection (pro- and trypomastigotes) in which promastigotes were also identified as *L. tarentolae*; hence, no *L. infantum* viable infection has been found. Whereas these data confirm *S. minuta* as a vector of two distinct Trypanosomatidae species, further studies, including experimental infections, are required to clarify if this sand fly species may permit full development and transmission of *L. infantum*.

Morphological identification and molecular characterization of *Anopheles algeriensis*Theobald, 1903 (Diptera: Culicidae) from Apulia region

M MENEGON[1], F SEVERINI[1], D BOCCOLINI[1], L TOMA[1], I VASCO[2], MA CAFIERO[2], M DI LUCA[1], DA RAELE[2]

[1]Istituto Superiore di Sanità, Dipartimento di Malattie Infettive, Reparto di Malattie Trasmesse da vettori, Roma; [2]Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata.

Keywords: COI, ITS2, *Anopheles algeriensis*, Apulia region

INTRODUCTION. *Anopheles algeriensis*, formerly considered competent for *Plasmodium* parasites, is a species distributed in the Mediterranean area and more rarely found in Central and Northern Europe. In Italy, the disappearance of suitable breeding sites has been affecting the occurrence of this species, once common along the Southern coasts and islands. In 2020, in the framework of monitoring activities of the IZSPB 1/18 RC Project on residual anophelism activities, adultsof *An. algeriensis* were collected in sites close to two different brackish lake basins in Foggia province, Apulia region. Given the noteworthy record of such a rare *Anopheles* species, an in-depth molecular investigation was performed.

MATERIALS AND METHODS. Mosquitoes were collected in two farms located near Lesina and Salso lakes, to the north and south of GarganoPromontory respectively, using CDC and BG sentinel® traps. Specimens were morphologically identified and a sub-sample of *An. algeriensis* was amplified and sequenced for COI (Folmer et al., 1994 Mol Mar Biol Biotechnol 3:294-9) and ITS2 markers (Marinucci et al., 1999 Insect Mol Biol 8:469-80). GenBank datasets of *An. algeriensis* COI sequences from Spain, Germany and Sweden and ITS2 sequences available only from Spain were used for sequence alignments and to generate the respective phylogenetic trees (NJ method-Tajima-Nei distance).

RESULTS AND CONCLUSIONS. In total, 494 specimens were identified, showing the typical morphological characteristic of the species**.** In addition, a sub-sample (15% of the total) was molecularly characterized. Phylogenetic tree generated for COI sequences showed that Apulian populations form a cluster with German and Swedish specimens, whereas specimens from Spain form a distinct cluster. Alike, ITS2 phylogenetic tree showed two-separated clusters, one including Apulian *An. algeriensis* sequences and another one formed by Spanish *An. algeriensis* sequences. As already observed in previous studies (Delgado-Serra et al. 2021 J Med Entomol 58:608-15), both COI and ITS2 sequence analyses confirm the presence of two separate subgroups in the *An. algeriensis* species. Further investigation should be carried out to better investigate molecular diversity of *An. algeriensis* populations from different countries.

Cheese infesting mites: experience in a small dairy in Umbria (Italy)

I MORETTA[1], E MEARINI[2], MA PRINCIPATO[1,3]

[1] Dip. di Medicina Veterinaria, Università degli Studi di Perugia; [2]Dottore in Produzioni Animali, Perugia; [3]Centro di Ricerca Urania, Perugia.

Keywords**:** *Tyrophagus putrescentiae, Glycyphagus domesticus,* Cheese

INTRODUCTION. The presence of infesting arthropods in any agri-food manufacturing factory is unacceptable, as the risks associated with parasites include: spread of pathogens, contamination of work surfaces and products, direct damage to foodstuffs, occupational diseases and image damage. In particular, during the seasoning phase, hams and cheeses are frequently subjected to mite infestations, which erode the external surface, creating a dustiness that ends up devaluing the products themselves and also their quality, altering organoleptic characteristics. We present the results of a study carried out in an Umbrian dairy, in order to verify the presence of mites infesting aged cheeses.

MATERIALS AND METHODS. This parasitological survey took place in a small dairy in Assisi (Umbria, Italy), in May-December 2019, sampling the cheeses present in the seasoning cell by scotch test. The samples were taken monthly on cow, sheep, goat or mixed cheeses. In total, n°36 forms of cheese were examined; n°3 scotch tests were carried out on each form on different areas, observed through the stereomicroscope and present mites were identified by optical microscope. The day and time of the withdrawals have not been agreed in advance with the operators of the dairy, in order to avoid manipulations on the cheeses.

RESULTS AND CONCLUSIONS. The results showed the presence of two mite species: *Tyrophagus putrescentiae*, a very important pest from an economic point of view, and *Glycyphagus domesticus*, important as a pathogenic species for humans, with the consequent possibility of developing diseases both in the sector operators and in the final consumers. *T. putrescentiae* were found on n°25 samples (70%); in n°15 (42%) this mite represents the only infesting species, while in n°10 (28%) it is present in mixed infestations with *G. domesticus*. N°11 cheeses (30%) were negative. Although very limited, data show that the infestation by *T. putrescentiae* begins already after about a month of maturing, reaching high levels in the 5°-6° month of maturing. At this point begin to appear *G. domesticus*, testifying to the fact that this mite is not a primary pest of animal products, but generally complicates infestations caused by other species. The use of a simple monitoring method, such as scotch test, allows to constantly check the status of the products; it also identifies not only the typically pest species, but also those potentially pathogenic to humans.

The Kubic FLOTAC Microscope: a new tool for helminth eggs diagnosis in veterinary and human field

MP MAURELLI[1], A AMADESI[1], C SANSONE[2], S MARRONE[2], M GRAVINA[2], A BOSCO[1], B CELANO[3], L RINALDI[1], G CRINGOLI[1]

[1]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, CREMOPAR, Naples, Italy; [2]Department of Electrical Engineering and Information Technology (DIETI), University of Naples Federico II, Naples, Italy; [3]OfficinaElettronicaSPA S.r.l., Naples, Italy.

Keywords: Mini-FLOTAC, Helminth eggs, Veterinary parasitology, Human parasitology

INTRODUCTION. In the last decades several studies have been focused on the development of semi-automated and automated systems for assessing Faecal Egg Count (FEC) in the veterinary and human fields (Vercruysse et al., 2018 Parasitology 145: 1655-64). The use of these new diagnostic technologies is beginning to offer potential solutions to overcome gaps and limitations of FEC techniques (i.e. human errors and time for analysis).In this study a new automated system for diagnosis of helminth eggs in veterinary and human fields is presented, the Kubic FLOTAC Microscope (KFM) (Cringoli et al., 2021 Parasitology 148: 427-34).

MATERIALS AND METHODS. The KFM is a compact and portable digital microscope designed to analyse faecal specimens prepared with the Mini-FLOTAC/FLOTAC in both field and laboratory settings. This system can be remotely controlled via software by an external device, i.e. smartphone, tablet or PC, or via internet it is possible to transfer the captured pictures to other laboratories, that could be very useful to create a network or to support operators directly in the field. Recently, an Artificial Intelligence (AI) based predictive model was developed to perform a rapid, easy and precise automated recognizing and counting of parasitic elements. For this purpose, a dataset with 18,944 objects (parasitic eggs) was used including samples of gastrointestinal nematodes from large and small ruminants, as well as *Trichuris vulpis* and *Toxocara canis* from dogs and *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Trichuris trichiura* from humans (confirmed by experts).

RESULTS AND CONCLUSIONS. The KFM combines the high sensitivity, accuracy and precision of the Mini-FLOTAC/FLOTAC techniques with a reliable AI system that was able to recognize 99.0% of the eggs analysed. Therefore, the KFM is a promising automated system for a rapid and accurate assessment of FEC to improve the diagnosis of veterinary and human parasitic infections.

Aedes koreicus as an emerging invader in the Lombardy region

A NEGRI[1,3], I ARNOLDI[1,2], R NODARI[1,3], I VAROTTO-BOCCAZZI[1,3], B BISAGLIA[1], G CATTANEO[1], P GABRIELI[1,3], S EPIS[1,3]

[1]Department of Biosciences and Pediatric Clinical Research Center "Romeo ed Enrica Invernizzi", University of Milan, Milan, Italy; [2]Department of Biology and Biotechnology, University of Pavia, Pavia, Italy; [3]Inter University Center for Malaria Research, Italian Malaria Network, University of Milan, Milan, 20133, Italy.

Keywords: Invasive species, Monitoring, Control strategies

INTRODUCTION. The monitoring of mosquito invasive species is a crucial action to prevent new invasions or limit their spread where they are already established. Several mosquitoes demonstrated significant invasive capability, causing ecological, economic and health problems in different parts of the world. Among them, *Aedes koreicus* is a new alien species in Europe, vector of nematodes and viruses, that was firstly described in northern Italy in 2011.

MATERIALS AND METHODS. After a monitoring activity of invasive mosquito species (IMS) between July and October 2020, hundreds of mosquitoes at immature stages and eggs were sampled in the Bergamo district. To identify mosquitoes, we performed morphological analyses and molecular screenings on several loci. The ND4, COI, D2 and ITS2 genes were amplified, and the obtained sequences used for phylogenetic analysis.

RESULTS AND CONCLUSIONS. Morphological observations and molecular analysis were coherent in supporting the identification of part of the collected mosquitoes as *Ae. koreicus*. We identified 50 individuals of *Ae. koreicus*, all belonging to the insular variant Jeju-do island, already described in Italy, Germany, Belgium, Russia and Hungary. The finding of *Ae. koreicus* in the pre-alpine area in the Bergamo district, particularly at the end of the summer season, is a further confirmation of the adaptation of these mosquitoes to specific climate and environmental conditions; our novel georeferentiated record will thus contribute to the prediction of future expansion patterns for this species.

Novel nuclear markers for the identification of the sibling species of the *Anisakis simplex* (s.l.) complex in a multilocus genotyping approach

M PALOMBA[1,2], E BELLO[1,3], R ACO-ALBURQUEQUE[1], P CIPRIANI[4], G NASCETTI[3], S MATTIUCCI[1]

[1]Dpt. of Public Health and Infectious Diseases, Lab. affiliated Ist. Pasteur Italy-Fond. Cenci-Bolognetti, Sapienza-University, Rome; [2]Dpt of Integrative Marine Ecology, Anton Dohrn; [3]Dpt. Ecological and Biological Sciences, Tuscia University, VT;[4]Institute of Marine Research (IMR), Bergen, Norway.

Keywords: *Anisakis simplex* (s. l.) complex*, Nuclear markers,* Multilocus genotyping

INTRODUCTION. Three closely related taxa are recognized in the *Anisakis simplex* (s.l.) complex: *A. pegreffii*, *A. simplex* (s.s.) and *A. berlandi* (Mattiucci et al., 1997 J Parasitol. 83: 401–16; 2014 J Parasitol. 100: 199–214), distributed in several intermediate/paratenic (fish and squids) and definitive hosts (cetaceans) species (Mattiucci et al., 2018 Adv Parasitol. 99: 93-263). Recently, a partial genome library of *A. pegreffii* allowed to develop diagnostic markers in the three species inferred from DNA microsatellite loci (SSRs) (Mattiucci et al., 2019 Parasitology 146:1-51; Bello et al., 2020 Parasite 27:9; 2021 Infect Genet Evol. 92: 104887), and at the locus *nas10*nDNA (Palomba et al., 2020 Parasite 27 39). Aim of the present study was to investigate and validate novel nuclear gene loci on those species, to be used in a multilocus genotyping approach for the identification of the parental taxa and their mixed ancestry in allopatric and sympatric areas included in their range of distribution.

MATERIALS AND METHODS. Based on the partial genome deposited of *A. simplex* (s.l.) (Łopienska-Biernat et al., 2019, Int J Parasitol. 49: 933–94), oligonucleotideprimers were designed for the sequencing of the superoxidedismutase (*Sod*) and adenylate-kinase (*Adk*) gene loci of *Anisakis*, by using PRIMER3 plus. PCR conditions were then adjusted. A total of 189 specimens of the three *Anisakis* spp., collected in hosts from several areas included in their geographic range, were sequenced at the both newly and previously scored loci. Clustal X 2.0 software (Thompson et al., 1997 Nucleic Acids Res. 25: 4876-82) was used for sequence alignment. Bayesian clustering algorithm by STRUCTURE 2.3.3 (Pritchard et al., 2000 Genetics 155: 945-59) of the datasets from new (*Sod, Adk*) and previous (*nas*10*, SSRs*) diagnostic loci, was used.

RESULTS AND CONCLUSIONS. The novel nuclear markers have shown several fixed alternativenucleotide positions - i.e., diagnostic at 100%- between the three species. STRUCTURE analysis allowed the worms assignment (100% of probability) to the three "pure parental" taxa; mixed ancestry between *A. pegreffii* and *A. berlandi* was also discovered. The novel nuclear diagnostic marker will be useful in future studies on a multi-locus genotypingapproach to study hybridization and/or introgression events occurring between the three species in sympatric areas.

Granted by: Grandi Progetti di Ricerca Ateneo Sapienza (2020).

Novel PCR-based genotyping approach allowed to detect spreading of pyrethroid resistance in *Aedes albopictus* across Italy

V PICHLER[1], E MANCINI[2], M MICOCCI[1], M CALZETTA[1], D ARNOLDI[3], A RIZZOLI[3], V LENCIONI[4], F PAOLI[4], R BELLINI[5], R VERONESI[5], S MARTINI[6], A DRAGO[6], C DE LIBERATO[7], A ERMENEGILDI[7], RP LIA[8], D OTRANTO[8], J PINTO[9], A DELLA TORRE[1], B CAPUTO[1]

[1]Dipartimento di Sanità Pubblica e Malattie Infettive, Università Sapienza, Rome, Italy; [2]Dipartimento di Biologia e Biotecnologie 'C. Darwin', Università Sapienza, Rome, Italy; [3]Department of Biodiversity and MolecularEcology, Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Trento, Italy; [4]Section of Invertebrate Zoology and Hydrobiology, MUSE-Science Museum, Trento, Italy; [5]Centro Agricoltura Ambiente "G. Nicoli", Crevalcore, Italy; [6]Entostudio snc, Padua, Italy; [7]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Rome, Italy; [8]Dipartimento di Medicina Veterinaria, Università di Bari, Valenzano, Italia; [9]Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisboa, Portugal.

Keywords: *Aedes albopictus*, Insecticide-resistance, Vector control, *Kdr* genotyping

INTRODUCTION. The highly invasive mosquito species *Aedes albopictus* has become a major health concern in Italy and other temperate regions due to its capacity to transmit arboviruses such as Dengue and Chikungunya. Pyrethroid insecticides are fundamental tools to counteract autochthonous arbovirus transmission, but extensive use of these compounds to control mosquito nuisance is selecting insecticide resistance in mosquito populations worldwide. Phenotypic signature of resistance to pyrethroids (PyR) has been already highlighted in *Ae. albopictus* populations from the native range and in invasive populations from temperate regions (Italy, Spain and US). Among pyrethroid target-site resistance mechanisms, a mutation in position 1016 (V1016G) of the voltage-sensitive sodium channel has been shown to confer the highest levels of PyR in *Ae. albopictus* populations from Vietnam and Italy.

MATERIALS AND METHODS. We genotyped 516 *Ae. albopictus* specimens collected from 2017 to 2019 in 37 sites in Trentino, Veneto, Emilia Romagna, Toscana, Lazio and Puglia regions by an AS-PCR assay we developed to genotype the V1016G allele and to allow large scale monitoring of the occurrence and spread of this mutation in wild populations.

RESULTS AND CONCLUSIONS. The mutated 1016G allele was detected in almost all examined sites (i.e. 31/37) and in all regions, except Trentino. Highest frequencies (between20–45%) of 1016G allele were observed in coastal touristic sites of Emilia Romagna and Lazio regions, where pyrethroid treatments are extensively implemented, mostly for mosquito nuisance reduction. The widespread presence of this mutation in Italian *Ae. albopictus* populations should serve as a warning bell, advocating for increased monitoring and management of a phenomenon which risks neutralizing the only weapon today available to counteract (risks of) arbovirus outbreaks.

Monitoring tick exposure and Rickettsiales in hunters and hunting dogs: a citizen science approach

G SGROI[1], R IATTA[1], RP LIA[1], V VENEZIANO[2,3], G ANNOSCIA[1], D OTRANTO[1]

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari Aldo Moro, Italia; [2]Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli Federico II, Napoli, Italia; [3]Osservatorio Faunistico Venatorio, Regione Campania, Napoli, Italia.

Keywords: Citizen-science, Italy, Rickettsiae, Zoonosis

INTRODUCTION. Following the evidence of high prevalence of *Rickettsia* spp. in *Dermacentor marginatus* from wild boars in hunting areas of southern Italy, this study aimed to assess the occurrence of spotted fever group (SFG) rickettsiae in ticks collected from hunters and hunting dogs sharing the same environments.

MATERIALS AND METHODS. From October 2020 to May 2021, wild boar hunters (n=316) were involved in tick collection from themselves and their dogs (n=315) in different regions of southern Italy (i.e., Basilicata, Calabria and Campania). All ticks collected were morphologically identified and molecularly screened through the amplification of a fragment of *glt*A gene for *Rickettsia* species and the positive samples were further tested for *omp*A gene of the SFG rickettsiae. Finally, positive samples to *omp*A gene were sequenced and compared to those available in GenBank.

RESULTS AND CONCLUSIONS. Twenty-nine hunters (i.e., 9.2%; 95%CI: 6.5-12.9) and 134 dogs (i.e., 42.5%; 95%CI: 37.2-48.1) were infested by at least one tick. A total of 281 ticks was collected, 48 (17.1%) from hunters and 233 (82.9%) from dogs, being *D. marginatus* (n=252; 89.7%) the most prevalent, followed by *Rhipicephalus sanguineus* sensu lato (n=21; 7.5%), *Rhipicephalus turanicus* (n=5; 1.8%) and *Ixodes ricinus* (n=3; 1.1%). Overall, 44 ticks scored positive to *Rickettsia* spp. (i.e., 15.6%; 95%CI: 11.9-20.4) by using the combined *glt*A/*omp*A approach, being 12 sequenced as *Rickettsia slovaca*. These findings revealed a high occurrence of *D. marginatus* and related *R. slovaca* in rural areas of southern Italy, suggesting a relevant risk of exposure to ticks and zoonotic *Rickettsia* species for hunters and their dogs during hunting activities. The role of hunters to monitor the circulation of ticks in rural areas may be considered an effective example of the citizen-science approach, supporting the cooperation towards public health stakeholders.

INDICE DEGLI AUTORI