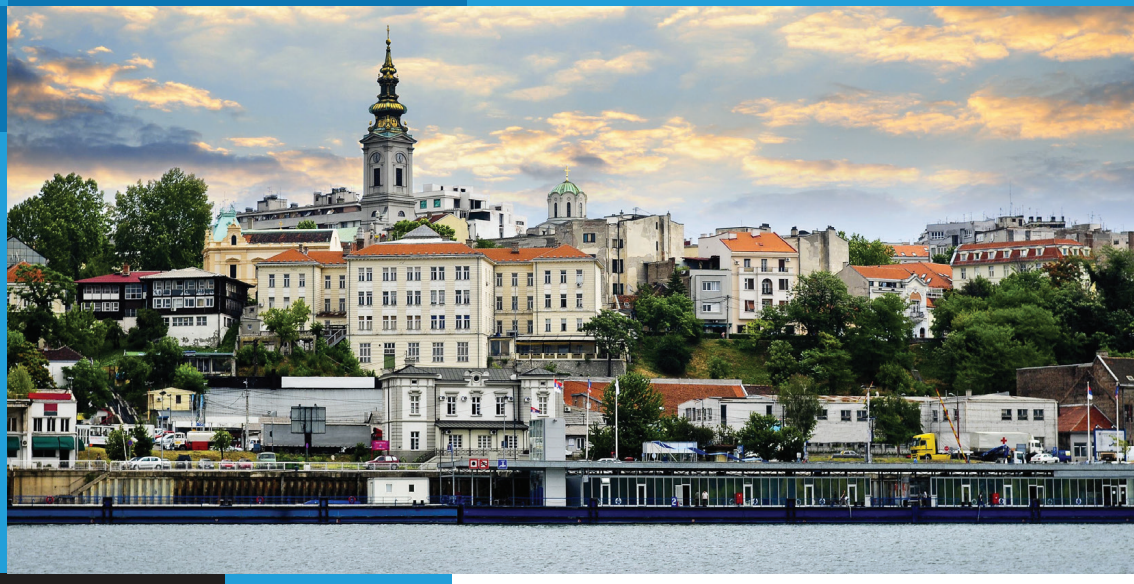
13th EUROPEAN MULTICOLLOQUIUM OF PARASITOLOGY

emop 2021 XIII

changing climate
changing parasites

Programme
& Abstract
Book



Belgrade, Serbia
October
12-16, 2021

13th European Multicolloquium of Parasitology
Belgrade, Serbia
October 12-16, 2021

PROGRAMME
&
ABSTRACT BOOK

IMPORTANT NOTICE:
The abstracts included in this book are the proceedings of the 13th European Multicolloquium of Parasitology, as provided by the authors. The Organizers of the EMOP2021 are not responsible for the scientific content of the abstracts.

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EDITORIAL

Thomas Romig

President of the EMOP 2021 Scientific Committee

European Federation of Parasitologists

Olgica Djurkovic-Djakovic

President of the EMOP2021 Organizing Committee

Serbian Society for Parasitology

Dear colleagues,

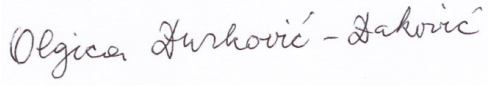
On behalf of the Organizing Committee, the Serbian Society for Parasitology and the European Federation of Parasitologists (EFP), it is our great pleasure to welcome you to the 13th European Multicolloquium of Parasitology (EMOP XIII, Belgrade, Serbia, 12-16 October 2021). Here, you will find the programme and the abstracts of all communications to be presented.

At the heart of this edition of the EMOP is CHANGE. Changes that the world is currently going through, including climate change, migrations of both people and animals, and changes in food habits, favour the persistence and contribute to the re-emergence of parasitic infections at the global level. We tried to capture this in the motto of EMOP 2021, back when it was supposed to be EMOP 2020. The mere fact that this is the first time in its 50-year long tradition that an EMOP has had to be postponed (for more than a year after the originally set dates), speaks even louder about the changes that we are living through. In this case, of course, changes caused by the covid-19 pandemic that has claimed more lives and disrupted life like no other peacetime event in a hundred years.

So, we should all be proud that there will be an EMOP at this time, and that we are meeting, whether on-site or online, to exchange knowledge and ideas, and even share some hugs, or smiles at least. And there is an exciting programme to benefit from, on the latest discoveries and technological developments, tackling major current global issues such as Climate change and parasite re-emergence, Migrations and parasites, Food and Water-borne parasitology, the One Health approach to combatting parasitic diseases, to mention just a few. In addition, because of the geographical position of the host country, developments in the field in the region of South East Europe are under the spotlight.

The number of papers submitted to EMOP 2021 that you can find in this volume may not be as large as would have been expected before the “new normal”. But it has been an endeavour to reach this point, both from us as organizers and from you as participants. Moreover, whatever the programme has lost in quantity may have been made up in quality, since the structure of the conference consists largely of symposia on particular topics organized by leaders in the field, with invited talks by top experts. This means our programme represents not only a rich learning experience, but also an excellent cross-section of current developments and perspectives in the broad field of parasitology in Europe and beyond.

We wish you all a stimulating and fulfilling congress.



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PARASITE

CONTENTS

PROGRAMME AT A GLANCE

Tuesday, October 12, 2021

	Room Pacific
14:00-19:00	REGISTRATION
17:30-18:15	Opening Ceremony
18:15-19:00	**Opening Talk** **Plenary Lecture**
19:00-20:30	Welcome Reception

Wenesday, October 13, 2021

	Room Pacific	Room Atlantic	Room Mediterranean	Room Baltic
07:30	REGISTRATION			
9:00-9:45	**Plenary Lecture**			
10:00-11:30	Cryptosporidium	Diversity of Echinococcus and other taeniids	Parasite taxonomy, systematics and phylogeny in the molecular - Part I	6th KE Mott Symposium I
11:30-12:00	Coffee Break			
12:00-13:30	Trichinella	Giardia	Parasite taxonomy, systematics and phylogeny in the molecular - Part II	6th KE Mott Symposium II
13:30-14:30	Lunch Break & POSTER VIEWING			
14:30-16:00	Protozoan infections in livestock and their control – zoonotic and animal health aspects	Clinical and Tropical Parasitology	**bioMérieux** Symposium	Host-parasite interactions
16:00-16:30	Coffee Break			
16:30-18:00	Workshop: Publishing in parasitology	Protozoa in food & environment – methods used in different environmental matrices	Combatting anthelmintic resistance in ruminants (COMBAR) – COST Action CA16230	

Thursday, October 14, 2021

	Room Pacific	Room Atlantic	Room Mediterranean	Room Baltic
07:30	REGISTRATION			
9:00-9:45	**Plenary Lecture**			
10:00-11:30	Malaria	The microbiome of parasites and role in diseases	6th International Workshop on Arctic Parasitology – IWAP 6.0e	6th KE Mott Symposium III
11:30-12:00	Coffee Break			
12:00-13:30	Wildlife parasitology	Migrants and migrating parasites	EVPC – European Veterinary Parasitology College Symposium	Novel perspectives for diagnosis and treatment
13:30-14:30	Lunch Break & POSTER VIEWING			
14:30-16:00	Malaria: Selected abstracts	Toxoplasma genetic diversity	International projects in parasitology	Paleoparasitology
16:00-16:30	Coffee Break			
16:30-18:00	**EFP General Assembly**	Helminth immunomodulation and interactions with the microbiome	OIE Collaborative Centre on Foodborne Zoonotic Parasites Symposium	

Friday, October 15, 2021

	Room Pacific	Room Atlantic	Room Mediterranean	Room Baltic
07:30	REGISTRATION			
9:00-9:45	**Plenary Lecture**			
10:00-11:30	Foodborne and waterborne parasites: changing climate, changing trends, changing parasites - Part I	Dirofilariosis in Europe today	SEE Toxoplasmosis	Fish parasitology: Anisakis & anisakiasis
11:30-12:00	Coffee Break			
12:00-13:30	Foodborne and waterborne parasites: changing climate, changing trends, changing parasites - Part II	Other ectoparasites: from biology to control	Trichinellosis and Trichinella infection in SEE: current status	Fish parasitology: Ecology and adaptation of fish parasites
13:30-14:30	Lunch Break & POSTER VIEWING			
14:30-16:00	Foodborne and waterborne parasites: Panel Discussion & Selected abstracts	Diagnosis and epidemiology of visceral leishmaniasis	SEE Dirofilariosis & other emerging vector-borne zoonoses	**Young Scientist Award Session**
16:00-16:30	Coffee Break			
16:30-18:00	Hot clinical topics in toxoplasmosis	Leishmaniasis: Selected abstracts	COMBAR Management Committee Meeting (by invitation only)	
20:30	Farewell Party			

Saturday, October 16, 2021

	Room Pacific	Room Atlantic	Room Mediterranean
08:00	REGISTRATION		
9:00-10:30	A One Health approach to manage parasitic infections	14th International Symposium of Geospatial Health – GnosisGIS - Part I	SEE Vectors and vector-borne pathogens
10:30-11:00	Coffee Break		
11:00-12:30	Wildlife parasitology: Selected abstracts	14th International Symposium of Geospatial Health – GnosisGIS - Part II	SEE One Health
12:30-13:15		**Closing Talk** **Plenary Lecture**	
13:15-14:00		Farewell ceremony	

DETAILED PROGRAMME

	DAY 1, Tuesday, October 12, 2021
	Room Pacific
14:00-19:00	REGISTRATION
17:30-18:15	Opening Ceremony
18:15-19:00	**Opening Talk - Plenary Lecture** **Bernadette Abela-Ridder**
19:00-20:30	**Welcome Reception**

	DAY 2, Wednesday, October 13, 2021
	Room Pacific
07:30	REGISTRATION
9:00-9:45	**Plenary Lecture** **Bruno Gottstein:** *Echinococcus multilocularis* / alveolar echinococcosis: Basics for potential immunotherapy and vaccination
10:00-11:30	**Cryptosporidium** **Organizer / Moderator: Simone Mario Cacciò**
10:00-10:13	**Simone Mario Cacciò**: Comparative genomics of *Cryptosporidium parvum*
10:13-10:26	**Frits Franssen**: Finding *Cryptosporidium* in the (metagenomics) haystack
10:26-10:39	**Gabriela Certad**: Pathogenic mechanisms of cryptosporidiosis
10:39-10:47	**Ruchika Shakya**: Interactions between *Cryptosporidium parvum* and bovine coronavirus during sequential and simultaneous co-infection of HCT-8 cells
10:47-10:55	**Ambre Baillou**: Characterization of intestinal macrophages and dendritic cell subsets in neonatal lambs and calves at homeostasis and following *Cryptosporidium parvum* infection
10:55-11:08	**Fabrice Laurent**: Current knowledge and toward new promising therapies to control cryptosporidiosis
11:08-11:16	**Ayman El-Badry**: Therapeutic and Nitazoxanide-enhancer effect of a novel curcumin nanocomposite in *Cryptosporidium* infected immunocompromised mice
11:16-11:30	Discussion
11:30-12:00	**Coffee Break**
12:00-13:30	**Trichinella** **Organizer / Moderator: Ljiljana Sofronić-Milosavljević**
12:00-12:25	**Dante Zarlenga**: Horizontal gene transfer provides insights into the evolutionary history and biology of *Trichinella*
12:25-12:45	**Natasa Ilić**: How *Trichinella spiralis* -derived extracellular vesicles affect dendritic cells
12:45-13:05	**Maria Angeles Gomez-Morales**: What does the humoral immune response in *Trichinella* infections tell us?
13:05-13:12	**Sylwia Grzelak**: *Trichinella britovi* recombinant excretory-secretory 21 kDa protein and chymotrypsin-like protein for IgG antibodies level detection in trichinellosis in mice and pigs
13:12-13:19	**Anqi Wang**: A *Trichinella spiralis* new born larvae specific protein, Ts-NBL1, interacts with host's cell vimentin
13:19-13:30	Discussion
13:30-14:30	**Lunch Break & Poster Viewing**
14:30-16:00	**Protozoan infections in livestock and their control – zoonotic and animal health aspects** **Organizers / Moderators: Gereon Schares & Walter Basso**
14:30-14:43	**Gema Alvarez Garcia**: Besnoitiosis in Europe: impact of the disease and possibilities to control
14:43-14:56	**Luis Miguel Ortega-Mora**: Neosporosis in sheep: more important than previously thought?
14:56-15:09	**Walter Basso, Gereon Schares**: *Toxoplasma gondii* and *Neospora caninum* infections in South American camelids
15:09-15:16	**Radu Blaga, Myriam Thomas**: Anatomical distribution of *Toxoplasma gondii* in naturally and experimentally infected lambs
15:16-15:23	**Karin Troell**: *Cryptosporidium* species and subtypes in Swedish rodent populations - concern for zoonotic transmission
15:23-15:36	**Caroline Frey**: *Tritrichomonas foetus* or what else? Insights from epidemiology and genetic comparisons
15:36-15:43	**Dorien Mabille**: Assessing the role of neutrophils in vector-transmitted *Trypanosoma brucei* infections
15:43-16:00	Discussion
16:00-16:30	**Coffee Break**

13.10.2021	Room Pacific
16:30-18:00	**Workshop: Publishing in parasitology** **Organizer / Moderator: Julia Walochnik**
	Andy Thompson: International Journal for Parasitology: Parasites and Wildlife **Pikka Jokelainen:** Parasite Epidemiology & Control **Eskild Petersen:** International Journal of Infectious Diseases **Julia Walochnik:** Parasitology Research

13.10.2021	Room Atlantic
10:00-11:30	**Diversity of Echinococcus and other taeniids** **Organizer / Moderator: Thomas Romig**
10:00-10:15	**Thomas Romig, Marion Wassermann:** *Echinococcus* phylogeny and nomenclature: an update
10:15-10:30	**Alessandro Massolo**: The transmission of *Echinococcus* species: how ecology explains most of it
10:30-10:45	**Adriano Casulli**: Do we know the real burden of human cystic and alveolar echinococcosis in Europe?
10:45-10:55	**Azzurra Santoro**: Molecular epidemiology of *Echinococcus multilocularis* in Europe: a preliminary picture
10:55-11:05	**Abdou Malik Da Silva**: Individual patterns of *Echinococcus multilocularis* infection in foxes in an endemic area of alveolar echinococcosis
11:05-11:30	Discussion
11:30-12:00	**Coffee Break**
12:00-13:30	**Giardia** **Organizers / Moderators: Marco Lalle & Christian Klotz**
12:00-12:20	**Kurt Hanevik**: From infection to clinical giardiasis and beyond
12:20-12:40	**Christian Klotz**: In vivo and in vitro models for giardiasis: what's new
12:40-13:00	**Scott Dawson**: Advances in *Giardia* genomics and genome manipulation
13:00-13:20	**Marco Lalle**: Drugs and drug resistance in *Giardia*: what we don't know yet
13:20-13:30	Discussion
13:30-14:30	**Lunch Break & Poster Viewing**
14:30-16:00	**Clinical and tropical parasitology** **Organizers / Moderators: Maria Antoniou & Miloš Korać**
14:30-14:50	**Laurence Millon**: Surveillance of alveolar echinococcosis in France: report of the National Reference Centre for Echinococcosis
14:50-15:05	**Nadia El Dib**: Disseminated strongyloidiasis in cases with different presentations
15:05-15:20	**Dušan Lalošević**: Pseudoparasitism – an issue for patients and parasitologists alike
15:20-15:30	**Anja Šterbenc**: Fatal *Strongyloides stercoralis* hyperinfection syndrome following heart transplantation
15:30-15:40	**Eya Ben Salah**: First report of qualitative and quantitative differences of *Echinococcus granulosus* immunoreactive proteins between "relapsed" and "non-relapsed" CE-paediatric patients and investigation of the most promising early prognostic candidates
15:40-16:00	Discussion
16:00-16:30	**Coffee Break**
16:30-18:00	**Protozoa in food & environment – methods used in different environmental matrices** **Organizer / Moderator: Isabelle Villena**
16:30-16:50	**Karen Shapiro**: Detection of *Toxoplasma gondii* in environmental matrices: Current methods and future directions for mitigation of toxoplasmosis
16:50-17:05	**Isabelle Villena**: Detection of *Toxoplasma gondii* in food and environmental matrices: Update on methods and future directions for food safety.
17:05-17:12	**Nadia María López Ureña**: Environmental contamination with *Toxoplasma gondii* oocysts: a systematic review
17:12-17:19	**Gianluca Marucci**: Molecular method for detection of *Toxoplasma gondii* oocysts in leafy-green vegetables: method selection, validation and SOP development
17:19-17:26	**Marco Lalle**: How to improve risk assessment capacity for foodborne protozoan parasites in the EU: evaluation, validation, and standardization of a molecular method for detection of *Cryptosporidium* spp. oocysts in ready-to-eat salad.

13.10.2021	Room Atlantic
17:26-17:33	**Romy Razakandrainibe**: Beware, *Cryptosporidium parvum* oocysts remain infective after homemade cottage cheese production.
17:33-17:40	**Damien Costa**: Focusing on waterborne and foodborne cryptosporidiosis outbreaks that have occurred in France in recent years (2017-2020)
17:40-17:47	**Lilian Bahia-Oliveira**: *Toxoplasma* -free food production in Brazil, current situation and future perspectives
17:47-18:00	Discussion

13.10.2021	Room Mediterranean
10:00-11:30	**Parasite taxonomy, systematics and phylogeny in the molecular era - Part I** **Organizer / Moderator: Aneta Kostadinova** **Co-moderator: Roman Kuchta**
10:00-10:20	**Simonetta Mattiucci**: Insights into the systematics, evolution and ecology of anisakid nematodes in the genomic era
10:20-10:40	**Isabel Blasco-Costa**: Exploring parasitic flatworm phylogeography with ddRAD data
10:40-10:50	**Sonja Dumendiak**: Cestodes in African wildlife: Dark taxa and cryptic species
10:50-11:00	**Darya Krupenko**: Digenea life cycles in the subtidal communities of the White Sea
11:00-11:30	Discussion
11:30-12:00	**Coffee Break**
12:00-13:30	**Parasite taxonomy, systematics and phylogeny in the molecular era - Part II** **Organizer / Moderator: Aneta Kostadinova** **Co-moderator: Isabel Blasco-Costa**
12:00-12:20	**Roman Kuchta**: *Spirometra* tapeworms in Europe in the molecular era: a neglected human parasite or a matter of concern?
12:20-12:40	**Omar Amin**: Variability in the Acanthocephala
12:40-12:50	**Iva Prikrylova**: Exploring parasite diversity across the African continent: A new genus of Gyrodactylidae (Monogenea) from Lake Kariba, Zimbabwe
12:50-13:00	**Boris Efeykin**: Mitochondrial genomes of neglected groups of parasitic nematodes: evolutionary analyses and comparative informativity
13:00-13:30	Discussion
13:30-14:30	**Lunch Break & Poster Viewing**
15:00-16:00	**bioMerieux Symposium** **Valeria Meroni:** Diagnosis of TORCH infections in pregnancy; Toxoplasmosis last updates 2020
16:00-16:30	**Coffee Break**
16:30-18:00	**Combatting anthelmintic resistance in ruminants (COMBAR) – COST Action CA16230** **Organizers / Moderators: Laura Rinaldi & Smaragda Sotiraki**
16:30-16:40	**Johannes Charlier**: COST Action COMBAR: combatting anthelmintic resistance in ruminants in Europe
16:40-17:00	**Jozef Vercruysse**: Roadmaps for the coordination of global research into helminth infection control in farmed ruminants
17:00-17:20	**Stanislav Simin**: Combating anthelmintic resistance in ruminants: a Serbian perspective
17:20-17:30	**Panagiota Ligda**: Presence of *Ostertagia ostertagi* and *Fasciola hepatica* antibodies in bulk tank milk from cattle herds in Greece
17:30-17:40	**Herve Hoste**: Potential use of agroindustrial by-products containing tannins for the integrated control of gastrointestinal nematodes in ruminants
17:40-17:50	**Nikol Reslová**: Multiplex real-time PCR assays for the identification and semi-quantitative assessment of strongyle nematodes in faeces
17:50-18:00	**Laura Rinaldi**: Monitoring the efficacy of anthelmintics and early detection of drug resistance in sheep from southern Italy
18:00-18:15	Discussion

13.10.2021	**Room Baltic**
10:00-11:30	**6th KE Mott Symposium I** **Organizers / Moderators: Santiago Mas-Coma & Bonnie Webster**
10:00-10:25	**Bonnie Webster**: Genetic diversity of *Schistosoma bovis* , *S. haematobium* and their hybrid forms
10:25-10:50	**Poppy Lamberton**: Using choice modelling to identify popular and affordable alternative interventions for schistosomiasis in Uganda
10:50-11:00	**Eglantine Mathieu-Bégné**: No pre-zygotic isolation mechanisms between *Schistosoma haematobium* and *Schistosoma bovis* parasites: from mating interactions to differential gene expression
11:00-11:10	**Jessica Clark**: Translating from egg- to antigen-based indicators for *Schistosoma mansoni* elimination targets: A Bayesian latent class analysis study
11:10-11:30	Discussion
11:30-12:00	**Coffee Break**
12:00-13:30	**6th KE Mott Symposium II** **Organizers / Moderators: Santiago Mas-Coma & Bonnie Webster**
12:00-12:25	**M. Adela Valero**: Impact of fascioliasis reinfection on Fasciola hepatica egg shedding: relationship with the immune-regulatory response
12:25-12:35	**Jana Ilgová**: Changes of the peptidase profile during *Fasciola hepatica* embryonation evidenced by omics data.
12:35-12:45	**Alejandra De Elias-Escribano**: DNA multimarker characterization of *Fasciola gigantica* from Algeria
12:45-12:55	**Raquel Sánchez-Marqués**: Long-term effects of temperature on embryonic development and hatching success of *Fasciola hepatica* miracidia
12:55-13:05	**Santiago Mas-Coma**: Fascioliasis in preschool age children: Unexpectedly inversed gender ratio regarding that in school children and adults
13:05-13:30	Discussion
13:30-14:30	**Lunch Break & Poster Viewing**
14:30-16:00	**Host-parasite interactions** **Moderator: Alisa Gruden-Movsesijan**
14:30-14:40	**Mathieu Claes:** A nanobody-based in situ knockdown approach for functional proteomics in *Trypanosoma brucei*
14:40-14:50	**Judit Serrat:** *Fasciola hepatica* juveniles: exploiting the fibrinolytic system to migrate through host tissues
14:50-15:00	**Manfred Schreiber:** Effect of *Mesocestoides corti* and *Taenia crassiceps* larvae on melanoma tumors in mice
15:00-15:10	**Alicia Diosdado:** The interaction with the haemostatic system of the host as a possible survival mechanism for migrating parasites: the third larval stage of *Ascaris suum* as a model
15:10-15:20	**Judit Serrat:** The tegument of *Fasciola hepatica* juveniles contains proteins that interact with laminin, a major component of the intestinal basal lamina
15:20-15:30	**Robert Stryiński:** Exploration of the proteome of human intestinal epithelial cell line (CACO-2) exposed to extracellular vesicles of *Anisakis simplex* L3 larvae – parasite-host interactions overview
15:30-15:40	Discussion
16:00-16:30	**Coffee Break**

	DAY 3, Thursday, October 14, 2021
	Room Pacific
07:30	REGISTRATION
9:00-9:45	**Plenary Lecture** **Sanjeev Krishna:** Musings of a malariologist from the lab and the clinic
10:00-11:30	**Malaria** **Organizer / Moderator: Sanjeev Krishna**
10:00-10:20	**Steffen Borrmann**: Chemo-attenuated live *Plasmodium falciparum* immunization
10:20-10:40	**Paul Davis**: When the test-line appears: making lateral flow tests for *Plasmodium vivax* lactate dehydrogenase with increased sensitivity, lower cost and greater availability

14.10.2021	**Room Pacific**
10:40-11:00	**Suzana Blesic**: Understanding climate and environmental drivers of rural hospital admissions for diarrhoeal disease, malaria, pneumonia, and asthma in South Africa
11:00-11:20	**Michael Ramharter**: Drugs for the treatment of malaria: What is in the clinical development pipeline?
11:20-11:30	Discussion
11:30-12:00	**Coffee Break**
12:00-13:30	**Wildlife parasitology** **Organizer / Moderator: Pikka Jokelainen**
12:00-12:20	**Pikka Jokelainen, Karen Shapiro**: Wildlife as sentinels and indicators for transmission of *Toxoplasma gondii*
12:20-12:40	**Jairo Alfonso Mendoza Roldan, Domenico Otranto**: Wildlife and reptiles as sentinels for zoonotic vector-borne diseases
12:40-13:00	**Vaidas Palinauskas, Elena Platonova**: Avian malaria parasites: annual visitors and potential threats to wild birds
13:00-13:10	**Gérald Umhang**: Grey wolves as sentinels for the presence of *Echinococcus* spp. and other gastrointestinal parasites in France
13:10-13:20	**Silvia Rondón**: Molecular characterization and prevalence of intestinal parasites infecting non-human primates in Colombia
13:20-13:30	Discussion
13:30-14:30	**Lunch Break & Poster Viewing**
14:30-16:00	**Malaria: Selected abstracts** **Organizers / Moderators: Sanjeev Krishna**
14:30-14:45	**Ravinder Sehgal**: Avian Malaria: Tropical deforestation and host specificity
14:45-15:00	**Kim van Bergen**: Evaluation of a novel real-time PCR assay for the detection, identification and quantification of *Plasmodium* species causing malaria in humans.
15:00-15:10	**Christelle Pomares**: Travel to Cameroon, fever, thrombocytopenia and malaria positive test: how much would you bet on the diagnosis?
15:10-15:20	**Jelena Srbljanović**: Aminoquinolines afford resistance to cerebral malaria in susceptible mice
15:20-16:00	Discussion
16:00-16:30	**Coffee Break**
16:30-18:00	**EFP General Assembly**

14.10.2021	**Room Atlantic**
10:00-11:30	**The microbiome of parasites and role in diseases** **Organizers / Moderators: Cinzia Cantacessi & Nolwenn Dheilly**
10:00-10:23	**Nolwenn Dheilly**: Diversity and evolution of viruses of parasitic flatworms (Phylum Platyhelminthes, group Neodermata)
10:23-10:41	**Marco Lalle**: Giardiavirus and friends: genomic and functional analysis expand our knowledge on viruses inhabiting the protozoan parasite *Giardia duodenalis*
10:41-10:59	**Jaelle Brealey**: Microbiome inception: an intestinal cestode shapes a hierarchical landscape of distinct microbial communities nested within the host
10:59-11:09	**Maria Pakharukova**: Comparative analysis of host and parasite microbial communities in experimental models of three liver fluke infections
11:09-11:19	**Serge Ankri**: Epitranscriptomics regulation of stress survival in the parasite *Entamoeba histolytica* by queuine from the gut microbiota
11:19-11:30	Discussion
11:30-12:00	**Coffee Break**
12:00-13:30	**Migrants and migrating parasites** **Organizer / Moderator: Zeno Bisoffi**
12:00-12:20	**Anna Färnert**: Screening of malaria in migrants
12:20-12:40	**Ana Requena-Méndez**: Strongyloidiasis in migrant populations: Is systematic screening the best strategy?
12:40-13:00	**Francesca Tamarozzi**: Imaging in parasitic diseases: when, how and why
13:00-13:20	**Ivana Čolović Čalovski**: Intestinal parasitic infections in migrants passing through Serbia
13:20-13:30	Discussion
13:30-14:30	**Lunch Break & Poster Viewing**

14.10.2021	**Room Atlantic**
14:30-16:00	**Toxoplasma genetic diversity** **Organizer / Moderator: Marie-Laure Dardé**
14:30-14:50	**Marie-Laure Dardé**: Analysing the genetic diversity of *Toxoplasma gondii* in an European country via human samples
14:50-15:10	**Lokman Galal**: Massive introgressions of *Toxoplasma gondii* domestic alleles in the Americas coincide with the recent introduction of the domestic cat.
15:10-15:30	**Pavlo Maksimov**: Genome-wide single nucleotide variation in *Toxoplasma gondii* type II isolates from Europe
15:30-15:40	**Aleksandra Uzelac**: Virulence and underlying mechanisms of four distinct lineage III variant genotypes of *Toxoplasma gondii*
15:40-15:50	**Azra Hamidović**: How rodent invasion and *Toxoplasma gondii* genetic diversity in West Africa are linked: a comparison between two african countries, Senegal and Benin
15:50-16:00	Discussion
16:00-16:30	**Coffee Break**
16:30-18:00	**Helminth immunomodulation and interactions with the microbiome** **Organizers / Moderators: William Harnett & Ljiljana Sofronić-Milosavljević**
16:30-16:50	**William Harnett**: The anti-inflammatory parasitic worm product ES-62 modulates the gut microbiome
16:50-17:10	**Susanne Hartmann**: Intestinal nematodes release antimicrobials and benefit from microbiota-driven host immune regulation
17:10-17:30	**Alisa Gruden-Movsesijan**: New delivery system for *Trichinella spiralis* antigens - accelerated approach to autoimmunity treatment
17:30-17:50	**Padraic Fallon**: How do *Schistosoma* eggs break the gut?
17:50-18:00	Discussion

14.10.2021	**Room Mediterranean**
10:00-11:30	**6th International Workshop on Arctic Parasitology – IWAP 6.0e** **Organizer / Moderator: Antti Oksanen**
10:00-10:20	**Antti Oksanen**: Changing climate, changing parasites in the Arctic
10:20-10:30	**Tetiana Kuzmina**: Helminth communities of teleost fishes as indicators of ecological changes in the Antarctic marine ecosystems
10:30-10:40	**Antti Oksanen**: Changing morphology of *Moniezia* spp. eggs
10:40-10:50	**Christen Rune Stensvold**: Host specificity and genetic diversity of *Blastocystis* and *Entamoeba* in muskoxen in Greenland as determined by metabarcoding
10:50-11:00	**Rebeca Berg**: Endoparasites detected in faecal samples from muskoxen (*Ovibos moschatus*) in Greenland
11:00-11:10	**Kirill Galaktionov**: Which parasites will be able to cross the Arctic under conditions of climate changes? An example of marine and coastal bird digeneans
11:10-11:20	**Katarzyna Tołkacz**: Vertical transmission of *Babesia microti* and the effect of concurrent *Bartonella* spp. infection on its success
11:20-11:30	Discussion
11:30-12:00	**Coffee Break**
12:00-13:30	**EVPC – European Veterinary Parasitology College Symposium** **Organizers / Moderators: Smaragda Sotiraki & Edwin Claerebout**
12:00-12:15	**Aránzazu Meana**: EVPC quod est et quo vadit (past, present and future of European Veterinary Parasitology College)
12:15-12:45	**Luís Cardoso**: Animal leishmaniosis in Europe: vertebrate hosts other than dogs
12:45-13:00	**Hubertus Hertzberg**: Re-orientation of parasite-management in adult horses in Switzerland
13:00-13:15	**Georgina Deak**: The current situation of *Angiostrongylus vasorum* in Romania
13:15-13:30	Discussion
13:30-14:30	**Lunch Break & Poster Viewing**

14.10.2021	**Room Mediterranean**
14:30-16:00	**International projects in parasitology** **Organizer / Moderator: Adriano Casulli**
14:30-14:45	**Pikka Jokelainen**: TOXOSOURCES: *Toxoplasma gondii* sources quantified
14:45-15:00	**Adriano Casulli**: Multi-centre study on *Echinococcus multilocularis* and *Echinococcus granulosus* s.l. in Europe: development and harmonization of diagnostic methods in the food chain (MEME project)
15:00-15:15	**Simone Mario Cacciò**: A brief excursion into PARADISE
15:15-15:30	**Adriano Casulli**: Molecular-epidemiological studies on pathways of transmission and long lasting capacity building to prevent cystic echinococcosis infection (PERITAS project)
15:30-15:40	**Francesca Tamarozzi**: The "European Register of Cystic Echinococcosis" (ERCE) becoming "International" (IRCE)
15:40-16:00	Discussion
16:00-16:30	**Coffee Break**
16:30-18:00	**OIE Collaborative Centre on foodborne zoonotic parasites Symposium** **Organizer / Moderator: Isabelle Vallee**
16:30-16:50	**W. Brad Scandrett**: OIE Collaborating Centre activities at the Canadian Food Inspection Agency's Centre for Food-borne and Animal Parasitology
16:50-17:10	**Xuelin Wang**: OIE Collaborating Center for Foodborne Parasites in Asian-Pacific Region
17:10-17:30	**Isabelle Vallee**: OIE Collaborating centre for Foodborne Zoonotic parasites (European region): overview of activities
17:30-17:50	**Bin Tang**: Prevalence of *Clonorchis sinensis* infection in residents and fish in China
17:50-18:00	Discussion

14.10.2021	**Room Baltic**
10:00-11:30	**6th KE Mott Symposium III** **Organizers / Moderators: Santiago Mas Coma & Bonnie Webster**
10:00-10:25	**Maria Dolores Bargues**: Genetics and geography of lymnaeid vectors in the highest human fascioliasis hyperendemic: Key points within a One Health control initiative
10:25-10:50	**Santiago Mas-Coma**: Ordering reservoir species priorities in a One Health control action for human fascioliasis: A large complexity of experimental and field studies
10:50-11:00	**Bonnie Webster**: The importance of snail molecular xenomonitoring for accurately identifying schistosomiasis transmission
11:00-11:10	**Barbora Šmídová**: Nitric oxide hinders the infection with the neuropathogenic schistosome *Trichobilharzia regenti* in mice, partly by inhibiting its vital peptidases
11:10-11:30	Discussion
11:30-12:00	Coffee Break
12:00-13:30	**Novel perspectives for diagnosis and treatment** **Moderator: Dušan Lalošević**
12:00-12:10	**David Becerro-Recio**: Differential protein expression in the early host-parasite interaction in fasciolosis: towards new vaccine candidates
12:10-12:20	**Marc Kaethner**: The Achilles' heel of the fox tapeworm? - Investigation of the threonine metabolism of *Echinococcus multilocularis*
12:20-12:30	**Sheena Chaudhry**: Dual inhibition of the *Echinococcus multilocularis* energy metabolism
12:30-12:40	**Roman Memedovski**: Activity and mechanism of action of mefloquine derivatives against *Echinococcus multilocularis*
12:40-12:50	**Christina Saghaug**: Genetic diversity in the metronidazole metabolism genes nitroreductase 1 and 2 in susceptible and refractory clinical samples of *Giardia lamblia*
12:50-13:00	**Neda Bauman**: Computational image analysis reveals the structural complexity of *Toxoplasma gondii* tissue cysts
13:00-13:10	**Darko Mihaljica**: In silico characterization of the *Ixodes ricinus* AV422 salivary protein immunogenicity
13:10-13:30	Discussion
13:30-14:30	**Lunch Break & Poster Viewing**

14.10.2021	**Room Baltic**
14:30-16:00	**Paleoparasitology** **Organizers / Moderators: Matthieu Le Bailly & Gholamreza Mowlavi**
14:30-14:50	**Matthieu Le Bailly**: New approaches and data synthesis in paleoparasitology. 15 years of studies in the Neolithic period
14:50-15:10	**Piers Mitchell**: Parasite infection in the Roman Period: Change over time from Pre-Roman to the Medieval Periods
15:10-15:20	**Kévin Roche**: A combined approach to study parasites in the past: microscopy and paleogenetics
15:20-15:40	**Gholamreza Mowlavi**: Paleoparasitology and new practical perspectives
15:40-15:50	**Gholamreza Mowlavi:** The status of parasitic infection in Chehrabad salt mine
15:50-16:00	Discussion
16:00-16:30	**Coffee Break**

	DAY 4, Friday, October 15, 2021
	Room Pacific
07:30	REGISTRATION
9:00-9:45	**Plenary Lecture** **James Cotton:** Genomic insights into the unusual population genetics of *Leishmania*
10:00-11:30	**Foodborne and waterborne parasites: changing climate, changing trends, changing parasites - Part I** **Organizers / Moderators: Lucy Robertson & Joke van der Giessen**
10:00-10:30	**Nynke Hofstra**: Effect of climate and environmental changes on the distribution of WBP/FBP
10:30-11:00	**Lapo Mughini-Gras**: Are source attribution and transmission of FBP influenced by climate change?
11:00-11:20	**Joke van der Giessen**: Risk ranking and evaluation of surveillance systems for foodborne parasites in Europe
11:20-11:30	Discussion
11:30-12:00	**Coffee Break**
12:00-13:30	**Foodborne and waterborne parasites: changing climate, changing trends, changing parasites - Part II** **Organizers / Moderators: Lucy Robertson & Joke van der Giessen**
12:00-12:15	**Olgica Djurković-Djaković**: Case study: Unusual toxoplasmosis cases in Serbia potentially associated with imported meat
12:15-12:30	**Simone Mario Cacciò**: Influence of climate change on the occurrence of cryptosporidiosis outbreaks in Europe
12:30-12:45	**Jerome Boissier**: Emergence of bilharziasis in Corsica: where are we now?
12:45-13:00	**Thomas Romig**: Transmission of *Echinococcus multilocularis* – new ideas emerging?
13:00-13:15	**Leah Lourenco**: Transmission of *Fasciola hepatica* from the intermediate host *Galba truncatula* to the definitive host *Ovis aries* under drought conditions in a Danish nature area
13:15-13:30	Discussion
13:30-14:30	**Lunch Break & Poster Viewing**
14:30-16:00	**Foodborne and waterborne parasites: Panel discussion (Session I and II) followed by selections of submitted abstracts** **Moderator: Lucy Robertson**
14:30-15:15	**Panel discussion**: "How do we see climate change affecting transmission of WBP and FBP in Europe?" (Previous presenters in symposium (sessions I and II) along with **Angelo Maggiore** from EFSA and **Edoardo Pozio**, former director of EURL-Parasitology)
15:15-15:25	**Gérald Umhang**: From feed to fork: contamination of lettuces by eggs of *Echinococcus multilocularis* and others *Taenidae* species
15:25-15:35	**Maria Pakharukova**: Foodborne trematode *Opisthorchis felineus* infection: mechanistic insights into biliary neoplasia formation
15:35-15:45	**Filip Dámek**: Tropism of *Toxoplasma gondii* in the tissues of experimentally infected pigs
15:45-16:00	Discussion
16:00-16:30	**Coffee Break**

15.10.2021	Room Pacific
16:30-18:00	**Hot clinical topics in toxoplasmosis** **Organizer / Moderator: Florence Robert-Gangneux**
16:30-16:50	**Jose Gilberto Montoya**: Prophylactic and treatment approaches for toxoplasmosis in immunocompromised patients (ICP): Challenges and gaps
16:50-17:10	**Florence Robert-Gangneux**: Toxoplasmosis in transplant recipients: new epidemiological trends, diagnosis and prevention
17:10-17:20	**Tijana Štajner**: A prospective study of the incidence of *Toxoplasma infection* after HSCT and heart transplantation
17:20-17:25	Discussion
17:25-17:40	**Fabrizio Bruschi**: If toxoplasmosis is forever could prevention and treatment reduce the risk of psychiatric disorders?
17:40-17:55	**Isabelle Villena**: Toxoplasmosis and behavioural disorders: controversial association
17:55-18:00	Discussion
20:30	**Farewell Party**

15.10.2021	Room Atlantic
10:00-11:30	**Dirofilariosis in Europe today** **Organizer / Moderator: Fernando Simón Martín**
10:00-10:15	**Luigi Venco**: Main aspects to be solved in the management of canine and feline dirofilariosis
10:15-10:30	**Fernando Simón Martín**: Current status of human dirofilariosis. A scoping review
10:30-10:45	**Hans-Peter Fuehrer:** *Dirofilaria immitis* and *D. repens* : current risk of spreading in Central and Northern Europe
10:45-11:00	**Javier González-Miguel**: Molecular relationships between *Dirofilaria* and hosts. From survival to pathology
11:00-11:15	**Domenico Otranto**: Other filariae of dogs in Europe
11:15-11:23	**Ljubica Spasojević Kosić**: Tumor necrosis factor alpha in dogs with heartworm disease
11:23-11:30	Discussion
11:30-12:00	**Coffee Break**
12:00-13:30	**Other ectoparasites: from biology to control** **Organizer / Moderator: Domenico Otranto**
12:00-12:20	**Bruno Chomel**: *Bartonella* spp. and their vectors: coevolution and zoonotic aspects
12:20-12:40	**Fred Beugnet**: Veterinary parasitology discovery in animal health – overview and ISOXAZOLINE example
12:40-13:00	**Emanuele Brianti**: *Acanthocheilonema reconditum* and its vectors. A look into the biology of an unusual filarioid
13:00-13:10	**Jacques Sevestre**: Detection of emerging tick-borne disease agents on the French Riviera
13:10-13:30	Discussion
13:30-14:30	**Lunch Break & Poster Viewing**
14:30-16:00	**Diagnosis and epidemiology of visceral leishmaniasis** **Organizer / Moderator: Jean-Pierre Gangneux**
14:30-14:50	**Jean-Pierre Gangneux**: Visceral leishmaniasis: what's new in 2021
14:50-15:10	**Maria Antoniou**: Visceral leishmaniasis: Diagnosis and epidemiology
15:10-15:30	**Jerome Depaquit**: *Leishmania martiniquensis* and *Mundinia* transmission: what's new?
15:30-15:40	**Laura Dirkx**: Long-term hematopoietic stem cells as sanctuary niche during treatment failure in visceral leishmaniasis.
15:40-15:50	**Christelle Pomares**: Identification of adipocytes as target cells for *Leishmania infantum* parasites
15:50-16:00	Discussion
16:00-16:30	**Coffee Break**
16:30-17:30	**Leishmaniasis: Selected abstracts** **Organizer / Moderator: Jean-Pierre Gangneux** **Co-moderator: Guy Caljon**
16:30-16:40	**Guy Caljon**: Drug resistance and treatment failure in visceral leishmaniasis: what do sand fly and rodent infections teach us?

15.10.2021	Room Atlantic
16:40-16:50	**Luca Galluzzi**: Development and application of a MLST panel for the identification of informative polymorphisms in *Leishmania infantum* strains in the Mediterranean region
16:50-17:00	**Dimitri Bulté**: Miltefosine enhances infectivity of a miltefosine-resistant *Leishmania infantum* strain by attenuating the antileishmanial immune response
17:00-17:10	**Sarah Hendrickx**: The impact of drug resistance of visceral *Leishmania* species on the parasite-vector-host interaction
17:10-17:20	**Jovana Stefanovska**: Seroprevalence of leishmaniosis in stray dogs in North Macedonia
17:20-17:30	Discussion

15.10.2021	Room Mediterranean
10:00-11:30	**SEE Toxoplasmosis** **Organizers / Moderators: Branko Bobić & Barbara Šoba**
10:00-10:15	**Branko Bobić**: *Toxoplasma* infection in Southeast Europe
10:15-10:30	**Tudor Rares Olariu**: Human toxoplasmosis in Western Romania
10:30-10:45	**Barbara Šoba**: Slovenian national screening programme for prevention of congenital toxoplasmosis
10:45-11:00	**Aleksandra Uzelac**: Genotypes of *Toxoplasma gondii* circulating in South-Eastern Europe, a region of intercontinental strain exchange
11:00-11:10	**Olivera Lijeskić**: Postnatal ocular toxoplasmosis in immunocompetent patients in SEE: a case series and review of the literature
11:10-11:30	Discussion
11:30-12:00	**Coffee Break**
12:00-13:30	**Trichinellosis and Trichinella infection in SEE: current status** **Organizer / Moderator: Vasile Cozma**
12:00-12:20	**Davor Balić:** Trichinellosis and *Trichinella* infection in Croatia: current status
12:20-12:40	**Zsolt Boros:** Trichinosis in Romania: updates regarding *Trichinella* spp. infections in wild animal species
12:40-13:00	**Saša Vasilev**: Serbian *Trichinella* story
13:00-13:30	Discussion
13:30-14:30	**Lunch Break & Poster Viewing**
14:30-16:00	**SEE Dirofilariosis & other emerging vector-borne zoonoses** **Organizer / Moderator: Suzana Otašević**
14:30-14:50	**Suzana Otašević**: Dirofilariosis & thelaziosis: emerging vector-borne zoonoses on the territory of the Central Balkans
14:50-15:10	**Anastasia Diakou**: *Dirofilaria* infections: The One Health approach
15:10:15:30	**Ilona Dóczi**: Epidemiology of dirofilariosis in Hungary – past and present
15:30-16:00	Discussion
16:00-16:30	**Coffee Break**
16:30-19:00	**COMBAR Management Committee Meeting** (by invitation only)

15.10.2021	Room Baltic
10:00-11:30	**Fish parasitology: Anisakis & anisakiasis** **Organizer / Moderator: Ivona Mladineo**
10:00-10:20	**Alfonso Navas**: Anisakis´s "omics": Transcriptomics, Proteomics and Metagenomics revelations
10:20-10:40	**Serena Cavallero**: A miRNA catalogue from third-stage larvae and exosomes of *Anisakis pegreffii*
10:40-11:00	**Jerko Hrabar**: Unwanted guest – a rat model for studying early immune response to an unusual human pathogen, *Anisakis pegreffii*
11:00-11:20	**Ivona Mladineo**: *Anisakis* and anisakiasis - an old pathogen and an emerging disease
11:20-11:30	Discussion
11:30-12:00	**Coffee Break**
12:00-13:30	**Fish parasitology: Ecology and adaptation of fish parasites** **Organizer: Ivona Mladineo** **Co-moderators: Serena Cavallero & Jerko Hrabar**
12:00-12:20	**Andrea Gustinelli**: Negligible risk of zoonotic anisakid nematodes in farmed fish from European mariculture

15.10.2021	Room Baltic
12:20-12:30	**Mélanie Gay**: Length and depth are major drivers of *Anisakis* levels in a zooplankton-feeding fish
12:30-12:40	**Eglantine Mathieu-Bégné:** A fine-scale analysis reveals microgeographic hotspots maximizing infection rate between a parasite and its fish host
12:40-12:50	**Eglantine Mathieu-Bégné**: Investigating the role of parasite plasticity in parasite host shift: evidences from a transcriptomic approach
12:50-13:00	**Maureen Duflot**: *Cryptocotyle* (metacercariae) parasitic communities from seven commercial fish species sampled in the English Channel and the North Sea
13:00-13:10	**Chahinez Bouguerche**: Tell me what you eat, I'll tell you what you are! A study of a hyperparasite *Cyclocotyla bellones* (Monogenea, Platyhelminthes) using integrative taxonomy
13:10-13:30	Discussion
13:30-14:30	**Lunch Break & Poster Viewing**
14:30-16:00	**Young Scientist Award Session**
14:30-15:00	**Simone Morelli**: Efficacy of a spot-on combination containing 10% w/v imidacloprid and 1% w/v moxidectin in the treatment of *Troglostrongylus brevior* infection in cats
15:00-15:30	**Robert Stryinski**: Exploration of the proteome of human intestinal epithelial cell line (CACO-2) exposed to extracellular vesicles of *Anisakis simplex* L3 larvae – parasite-host interactions overview
15:30-16:00	Discussion
16:00-16:30	**Coffee Break**

	DAY 5, Saturday, October 16, 2021
	Room Pacific
07:30	REGISTRATION
9:00-10:30	**A One Health approach to manage parasitic infections** **Organizers / Moderators: Frank Katzer & Rachel Chalmers**
9:00-9:18	**Martha Betson**: Zoonotic transmission of intestinal helminths in the Philippines
9:18-9:36	**Rachel Chalmers**: New approaches to investigating zoonotic *Cryptosporidium* outbreaks
9:36-9:54	**Joke van der Giessen**: Public Health impact of foodborne parasite in a One Health approach
9:54-10:12	**Lucy Robertson**: Squeezing *Giardia* under the One Health umbrella
10:12-10:20	**Francesca Tamarozzi**: Pilot survey of cystic echinococcosis in Masaai livestock-keeping communities of Northern Tanzania
10:20-10:30	Discussion
10:30-11:00	**Coffee Break**
11:00-12:30	**Wildlife parasitology: Selected abstracts** **Organizer / Moderator: Pikka Jokelainen**
11:00-11:10	**Martin Heinrich Richter**: Insights into monitoring and prevalence studies of circulating zoonotic pathogens in German wildlife
11:10-11:20	**Filip Dámek**: *Toxoplasma gondii* seroprevalence in European wildlife: a systematic review
11:20-11:30	**Kaya Stollberg**: Moderate to substantial agreement between direct and indirect detection of *Toxoplasma gondii* in game
11:30-11:40	**Carolyn Kästner**: Identification of *Alaria alata* mesocercariae by MALDI-TOF mass spectrometry
11:40-11:50	**Olivera Bjelić Čabrilo**: Parasite load of nematode species in *Apodemus flavicolis*: Effects of host spleen size, body mass, body condition and sex
11:50-12:00	**Branka Pejić**: Ectoparasite bat flies (Diptera: Nycteribiidae) of Schreiber's bent-winged bat and their fungus parasite
12:00-12:30	Discussion

16.10.2021	Room Atlantic
9:00-10:30	**14th International Symposium of Geospatial Health – GnosisGIS - Part I** **Organizers / Moderators: Laura Rinaldi & Robert Bergquist** **Co-moderator: Sherif Amer**
9:00-9:10	**Sherif Amer**: Spatial and temporal analysis of SARS-CoV-2 concentration in wastewater in The Netherlands
9:10-9:20	**Samuel Manda**: A Spatial Analysis of COVID-19 in African countries: Evaluating the Effects of COVID-19 Vulnerability Risk Factors

16.10.2021	Room Atlantic
9:20-9:40	**Robert Bergquist**: Comparing spatio-temporal distribution of the most common human parasitic infections in Iran: a systemic quantitative literature review
9:40-9:50	**Roula Zougheibe**: COVID-19 pandemic related worries compared to everyday life: Evidence from a cross-sectional national survey of Australian families
9:50-10:00	**Justine Blanford**: Cycling to get my vaccination: how accessible are the COVID-19 vaccination centers really in the Netherlands by bicycle?
10:00-10:10	**Fedor Korennoy:** COVID-19 in the Russian Federation: Regional Differences and Public Health Response
10:10-10:20	**Matteo Mazzucato**: E.V.E.: An integrated system for the management of environmental data, to support veterinary epidemiology
10:20-10:30	Discussion
10:30-11:00	**Coffee Break**
11:00-12:30	**14th International Symposium of Geospatial Health – GnosisGIS - Part II** **Organizers / Moderators: Laura Rinaldi & Robert Bergquist** **Co-moderator: Sherif Amer**
11:00-11:10	**Nils Tjaden**: Chikungunya beyond the tropics: A threat for Europe?
11:10-11:20	**Natalia Shartova**: Spatial patterns of West Nile virus distribution in the endemic area with focus on the urban environment
11:20-11:40	**Laura Rinaldi**: Predictive tools for assessing the infection risk by rumen flukes in small ruminants of southern Italy
11:40-12:00	**Anna-Sofie Stensgaard**: Changing patterns of snail-borne diseases
12:00-12:10	**Fedor Korennoy:** Evaluating spatial risks of emerging animal diseases introduction in the Republic of Kazakhstan: the case of African swine fever and Peste des petits ruminants
12:10-12:30	Discussion
12:30-13:15	**Closing Talk - Plenary Lecture** **Eskild Petersen**: Parasitology in the 21st century
13:15-14:00	**Farewell ceremony (incl. Awards; announcement of EMOP XIV host)**

16.10.2021	Room Mediterranean
9:00-10:30	**SEE Vectors and vector-borne pathogens** **Organizer / Moderator: Snežana Tomanović**
9:00-9:18	**Dimosthlakis Chochlakis**: Global warming and infectious diseases: the need to catch them before they catch us
9:18-9:36	**Dušan Petrić**: Impacts of climate change on West Nile disease and vectors in Serbia (1985 – 2100)
9:36-9:54	**Snežana Tomanović**: Should we be scared of a tick bite (in Southeastern Europe)?
9:54-10:02	**Alexander Lindau**: The occurrence of *Hyalomma* spp. in Germany – results of a citizen science study
10:02-10:10	**Gorana Veinović**: Small rodents as hosts of tick-borne pathogens in Serbia
10:10-10:18	**Ratko Sukara**: Knowledge, attitude and practices of general population, professionally tick-exposed persons and health care workers of tick-borne encephalitis and tick-borne diseases in Serbia
10:18-10:30	Discussion
10:30-11:00	**Coffee Break**
11:00-12:30	**SEE One Health** **Organizer / Moderator: Ivana Klun**
11:00-11:20	**Judit Plutzer**: C*ryptosporidium* and *Giardia* in the eastern part of Europe: the One Health perspective
11:20-11:40	**Ivana Ćirković**: Is Serbia ready for the One Health approach in the field of AMR control?
11:40-12:00	**Sara Savić**: Leishmaniasis and a One Health approach
12:00-12:20	**Ivana Klun**: *Toxoplasma gondii* infection and the One Health approach in Serbia
12:20-12:30	Discussion

PLENARY LECTURES

Bernadette Abela-Ridder

Department for the Control of Neglected Tropical Diseases
World Health Organization

Dr Bernadette Abela-Ridder works at World Health Organization (WHO) leading the work on diseases associated with a human animal interface at the Department for the Control of Neglected Tropical Diseases (NTDs). Previously she worked WHO Department of Food Safety and Zoonoses, US Food and Drug Administration on antimicrobial resistance, for l'Institut de recherche pour le développement (IRD) in Cameroon on emergence of simian immunodeficiency viruses from non-human primates including bushmeat, the Food and Agriculture Organization of the U.N. on veterinary public health, and in clinical veterinary practice.

Bruno Gottstein
Institute of Infectious Diseases
Faculty of Medicine
University of Bern

After obtaining his PhD in 1982, Bruno Gottstein became a research assistant at the Institute of Parasitology at the University of Zürich (with Prof. John Eckert), where he habilitated in 1992. He spent two research sabbaticals in the US, one at the CDC in Atlanta, and one at the NIH in Bethesda. In 1992, he became full professor and director at the newly founded Institute of Parasitology at the University in Bern. He left this position as emeritus in 2019, and subsequently got a research professorship in parasitology at the Institute of Infectious Diseases at the Medical Faculty and at the University Hospital of the University of Bern.

Prof. Gottstein's main research topic is the development of immunotherapy for human alveolar echinococcosis and vaccine development against alveolar echinococcosis in various primates.

Echinococcus multilocularis / ALVEOLAR ECHINOCOCCOSIS: BASICS FOR POTENTIAL IMMUNOTHERAPY AND VACCINATION

Bruno GOTTSTEIN
Institute for Infectious Diseases, Faculty of Medicine, University of Bern, Switzerland

Infection with the larval (metacestode) stage of *Echinococcus multilocularis* causes alveolar echinococcosis (AE), a serious hepatic disorder. Upon infection, we know that the metacestode of E. multilocularis, once established in the liver, outwardly protects with a tight layer represented by a carbohydrate-rich extracellular matrix, termed the laminated layer (LL). This LL is crucial for parasite survival and proliferation as it is able to protect the parasite from host's innate or subsequent specific immune reactions. The nature and orientation of this immune reaction will trigger resistance or susceptibility to disease development. In general, there is accumulating evidence that the immune fitness and respective response upon E. multilocularis infection is crucially involved in the host-parasite interplay. A weak or impaired immune responsiveness definitively promotes metacestode proliferation and metastatic progression, such as following immunosuppressive therapy for malignant and inflammatory diseases, liver transplantation, or rarely upon immune deficiency by AIDS.
From the point of view of parasite survival strategy, there evidenced that a periparasitically induced immune anergy is one of the key mechanisms of the metacestode to yield immune evasion, strong interconnected with upregulating regulatory T-cell mechanisms in the host. A deeper understanding of these immunomodulating mechanisms will support the development of immunotherapeutics to treat AE and/or to curatively support conventional parasitostatic medication with benzimidazoles.
As with other taeniid cestodes, immunization of intermediate hosts with *E. multilocularis* recombinant oncospheral antigens have demonstrated efficacy by preventing disease development in experimentally infected susceptible rodents, and first vaccination trials have now been carried out in naturally exposed primates.

Sanjeev Krishna

Professor of Molecular Parasitology and Medicine
St George's University of London

Sanjeev Krishna is Professor of Molecular Parasitology and Medicine at St George's University of London. He completed degrees at Cambridge and Oxford, and studied malaria in Thailand. A former Wellcome Trust Senior Research Fellow in Clinical Science, he joined St George's in 2000. He was elected a Fellow of the Academy of Medical Sciences in 2004 and awarded an ScD by the University of Cambridge in 2007. Professor Krishna maintains a wide-ranging programme of research spanning basic research into the function of *Plasmodium* transporters and their value as drug targets. He has identified mechanisms of drug resistance and novel diagnostic approaches for parasitic and other infections. Clinical studies have improved treatments for malaria by simplifying regimens, and validating different routes for administration. He has studied the emergent infection caused by *P. knowlesi* in Malaysian Borneo.

MUSINGS OF A MALARIOLOGIST FROM THE LAB AND THE CLINIC

Sanjeev KRISHNA
s.krishna@sgul.ac.uk, St George's University of London, London, UK; Institute of Infection and Immunity, St George's University of London, London, UK

Highly collaborative studies on malaria in the past 40 years have linked clinical aspects of disease to laboratory-based advances. Clinical trials have sought to improve treatment regimens and our understanding of the pathophysiology of disease. Laboratory studies have aimed to discover new ways to kill parasites, how some existing antimalarials work and how they may fail. Case studies on the pathophysiology of lactic acidosis and hypoglycaemia, improvements in treatments with quinine and artemisinins, the discovery of parasite encoded transporter proteins as drug targets, and transporters as drug resistance mediators will be described to illustrate different facets of studies on malaria. A general lesson that has emerged is the length of time between first making observations and findings eventually yielding tangible benefits. Some recent history will be revisited to demonstrate.

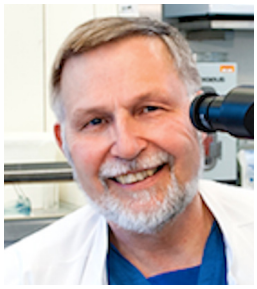
James Cotton
Parasite Genomics Group
Wellcome Sanger Institute

James Cotton is a senior staff scientist in the parasite genomics group at the Wellcome Sanger Institute. He works on the population genomics of neglected tropical disease parasites including Leishmania and Guinea worm. A particular focus has been combining population genomics and other genetic approaches to understanding anthelminthic resistance in livestock GI nematodes and in Schistosoma mansoni. He also has an ongoing interest in comparative genomics of nematodes, and has played a major role in a number of de novo genome sequencing projects for parasitic nematodes.

GENOMIC INSIGHTS INTO THE UNUSUAL POPULATION GENETICS OF *Leishmania*

James A. COTTON
Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridge CB10 1SA, United Kingdom

We have now generated whole-genome sequence data for over 500 Leishmania isolates, and these data are starting to provide unprecedented resolution of the genetics of Leishmania populations. In particular, we now have sufficient data from different populations of Leishmania to compare their population genetics directly, without the complication of different molecular markers being used. Natural populations of Leishmania are having sex, but the importance – and perhaps even the mechanism – of genetic exchange seems to vary between different populations. This is compounded by the presence of extensive aneuploidy, where chromosome copy number varies rapidly between isolates and even within isolates. The interplay of sexual reproduction and aneuploidy variation makes the genetics of Leishmania populations rather complex, and impacts how we interpret genetic data in Leishmania: for example, we show that the observed deficit of heterozygosity can be explained by aneuploidy rather than frequent inbreeding. Some recent data that suggests that at least some of this complexity is present even within a single patient infection.



Eskild Petersen

Professor Emeritus of Infectious Diseases
Faculty of Health Science, Aarhus University, Denmark

Eskild Petersen is Professor Emeritus of Infectious Diseases, Institute for Clinical Medicine, Faculty of Health Science, Aarhus University, Denmark. He chairs the ESCMID Emerging Infections Task Force, Basel, Switzerland. Professor Petersen graduated from the Medical School, University of Aarhus in 1978; received his Diploma in Tropical Medicine and Hygiene from the University of Liverpool in 1980; a specialist degree in infectious diseases in 1985; and a specialist degree in tropical medicine in 1988.
He spent two year doing field work on malaria in Liberia supported by a WHO TDR grant. From 1993 to 2007 he was principal investigator or co-investigator on a series of EU grants studying congenital toxoplasmosis with special focus on screening programs.
Professor Petersen has vast experience in journal editorship. Currently, he is Editor-in-Chief of the International Journal of Infectious Diseases.
Professor Petersen is internationally renowned for his extensive contributions to the fields of global health, travel medicine and emerging infections, with a publication record of over 330 original papers in peer reviewed journals an an H-index of 42. He has edited several textbooks, including “Infectious Disease: a Geographic Guide”.

PARASITOLOGY IN THE 21st CENTURY

Eskild PETERSEN
Professor Emeritus, Institute for Clinical Medicine, Faculty of Health Sciences, University of Aarhus, Denmark;
Chair, ESCMID Emerging Infections Task Force

Parasitology is a medical and scientific discipline studying helmints and protozoans. Infections are often zoonotic and there is a huge overlap with veterinarians on epidemiology, diagnostic methods and prevention. Clinical parasitology in humans focus on diagnostics, pathology and treatment and is an integrated part of diagnostic microbiology, infectious diseases, tropical medicine and public health.
I will focus on clinical parasitology in humans, where to me there are exciting opportunities in the 21st century.
First. Diagnostic parasitology has very much focused on microscopy, be it feces or blood. We need to embrace the molecular revolution and introduce PCR methods where we can. The PCR methods have a far better sensitivity and the challenge is to determine if a low number of parasites are causing illness or not.
Second. The molecular revolution opens up for studies of the genetic bases for drug resistance. This has so far been used in studies of falciparum malaria resistant to different drugs, lately the artemisinins, but need to be expanded to Giardia, Entamoeba and Leishmania.
The molecular revolution also opens up for studies using molecular epidemiology to trace transmission routes for instance through food transmitted infections like Toxoplasma.
Third. Vaccines. We have the first approved vaccine against malaria but so far it is not known if it reduced childhood mortality due to falciparum malaria. Leishmania and Toxoplasma vaccines remains in the experimental stage with no known human studies.
The success of the RNA and Adenovirus vaccines against COVID-19 open up for new technologies which must be tried in parasitic infections, both protozoan and helminths.

INVITED LECTURES & ORAL PRESENTATIONS

CRYPTOSPORIDIUM
Organizer / Moderator: Simone Mario Cacciò

INVITED LECTURES

COMPARATIVE GENOMICS OF *Cryptosporidium parvum*

Giulia CORSI[1], Swapnil TICHKULE[2], Anna Rosa SANNELLA[3], Paolo VATTA[3], Francesco ASNICAR[4], Nicola SEGATA[4], Aaron JEX[2], Cock VAN OOSTERHOUT[5], Simone M. CACCIO'[3,6]

[1]University of Copenhagen, Copenhagen, Denmark; [2]University of Melbourne, Melbourne, Australia; [3]Istituto Superiore di Sanità, Rome, Italy; [4]University of Trento, Trento, Italy; [5]University of East Anglia, Norwich, United Kingdom; [6]simone.caccio@iss.it, Istituto Superiore di Sanità, Rome, Italy

Background. The zoonotic parasite *Cryptosporidium parvum* is a major and global cause of diarrheal disease in humans and animals. The parasite shows extensive genetic variability, with many zoonotic variants (subtypes) identified. During modern husbandry practices, livestock can become infected with different subtypes. Furthermore, gene flow between previously isolated parasite populations is facilitated by increased globalization, and the close human-animal contact elevates the risks of spill over and spillback events to humans. Altogether, these factors increase the opportunity for genetic exchanges during the obligatory sexual phase of the parasite.

Objectives. To examine the role of gene flow and recombination in the population structure and evolution of *C. parvum* by comparing whole genome sequences from human and animal isolates collected across Europe, USA, Egypt and China.

Material and Methods. Whole genome sequences were generated by NGS (Illumina) at high coverage. A bioinformatics workflow was used to process raw sequence data and perform phylogenetic, clustering, population genetics and recombination analyses.

Results. Phylogenetic analyses identified three strongly supported clusters, yet the isolates within each cluster were from different host species, geographic origins and subtypes (IIa and IId). We detected many recombination events involving human and animal isolates from the three clusters, and show that the genomic regions affected by genetic exchanges are elevated in nucleotide diversity. Furthermore, these regions are enriched for genes encoding for signal peptides, transmembrane domains and single amino acid repeats.

Conclusion. Our results suggest that genetic exchanges provide novel substrate for natural selection at genes involved in virulence evolution, and that elevated levels of gene flow and recombination play an important role in the evolution of *C. parvum*.

Funding source: This work was funded by the European Union's Horizon 2020 Research and Innovation Programme, under grant agreement № 643476 (project COMPARE).

FINDING *Cryptosporidium* IN THE (METAGENOMICS) HAYSTACK

Frits F.J. FRANSSEN[1], Ingmar JANSE[1], Dennis JANSSEN[1], Simone M. CACCIO[2], Paolo VATTA[2], Joke W.B. van der GIESSEN[1], Mark W. J. van PASSEL[1,3]

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Background. Parasites often have complex developmental cycles that account for their presence in a variety of difficult-to-analyse matrices, including faeces, water, soil and food. Detection of parasites in these matrices still involves laborious methods. Untargeted sequencing of nucleic acids extracted from those matrices in metagenomic projects may represent an attractive alternative method for unbiased detection of these pathogens.

Objectives. Here, we show how publicly available metagenomic datasets can be mined to detect parasite specific sequences, and generate data useful for environmental surveillance.

Material and Methods. We used Kraken2 which uses exact k-mer matches and allows for a fast evaluation and taxonomic identification of reads to the lowest common ancestor. Secondly, we used the Burrows-Wheeler Aligner (BWA-MEM), or the k-mer alignment (KMA), to identify taxa of interest based on the alignment of metagenomic reads to selected query reference sequences.
Results. The protozoan parasite Cryptosporidium parvum was used as a test organism, and we show that detection is influenced by choice of the reference sequence. Use of the whole genome yields high sensitivity but low specificity, whereas specificity is improved through the use of signature sequences.
Conclusion. Querying metagenomic datasets for parasites is feasible and relevant, but requires optimization and validation. Nevertheless, this approach provides access to the large, and rapidly increasing, number of datasets from metagenomic and meta-transcriptomic studies, allowing unlocking hitherto idle signals of parasites in our environments.

Funding source: This work was in part financially supported by the European One Health project PARADISE grant agreement № 773830 and by the Netherlands Food and Product Safety Authority (NVWA).

PATHOGENIC MECHANISMS OF CRYPTOSPORIDIOSIS

Gabriela CERTAD
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The pathophysiological mechanisms of *Cryptosporidium* infection are multifactorial and not completely understood. Some advances achieved recently revealed that the infection by *C. parvum* induces cytoskeleton remodeling and actin reorganization through the implication of several intracellular signals involving, for example, PI3K, Src, Cdc42 and GTPase. It was also reported that the infection by *C. parvum* leads to the activation of NF-κB, known to induce anti-apoptotic mechanisms and to transmit oncogenic signals to epithelial cells. Despite the growing evidence about the hijacking of cellular pathways potentially being involved in cancer onset, this information has rarely been linked to the tumorigenic potential of the parasite. However, several evidences support an association between *Cryptosporidium* and the development of digestive neoplasia. To explore the dynamics of *Cryptosporidium* infection, our team implemented an animal model of cryptosporidiosis using corticoid dexamethasone-treated adult SCID (severe combined immunodeficiency) mice, orally infected with *C. parvum* or *C. muris* oocysts. Intriguingly, *C. parvum*-infected animals developed digestive adenocarcinoma even when they were infected with a single oocyst. In parallel, mechanisms involved in this neoplastic process were explored, and the pivotal role of the Wnt pathway together with the alteration of the cytoskeleton was emphasized. Recently, a microarray assay allowed the detection of cancer-promoting genes and pathways highly up regulated in the group of *C. parvum* infected animals when compared to non-infected ones. Moreover, an epidemiological study conducted in Lebanon by our team reported a significant higher prevalence of *Cryptosporidium* among patients with recently diagnosed colon cancer before any treatment when compared to the control group. These results suggest that *Cryptosporidium* is associated with human colon cancer being a potential etiological agent of this disease. More research in the field is required in order to identify mechanisms and molecular factors involved in this process.

CURRENT KNOWLEDGE AND TOWARD NEW PROMISING THERAPIES TO CONTROL CRYPTOSPORIDIOSIS

Fabrice LAURENT[1,2], Gergö MOTAN[4], Lindon MOODIE[4], Caroline THERESINE[1], Tiffany PEZIER[1], Julie TOTTEY[1], Sonia LAMANDE[1], Céline BARC[3], Christian HEDBERG[4]
[1]INRAE-Tours University UMR1282-ISP, team Apicomplexa and Mucosal Immunity, 37380 Nouzilly, France; [2]fabrice.laurent@inrae.fr; [3]INRAE UE1277 Infectiology of farm, model and wild animals facility, 37380 Nouzilly, France;[4]Chemical Biology Center (KBC), Institute of Chemistry, Umeå University, Umeå, Sweden

Background. Cryptosporidiosis consists in acute gastrointestinal infections in young children and is one of the two main leading causes of diarrhea in newborn calves in France and England resulting in substantial financial losses for farmers and a source of contamination for humans. Despite the seriousness of cryptosporidiosis and the recent strong mobilization of the scientific community to find for new therapeutic strategies (eg Swale *et al*. PMID: 31694928), the obtention of new potent drugs targeting these pathogens is still urgently awaited.

Objective Identification of new potent orally active anti-Cryptosporidium compounds able to prevent and control an established infection in order to increment the poor number of therapeutics available.
Material and Methods. A targeted library of approximately 1000 compounds was initially screened for anti-Cryptosporidium activity leading to the identification of 3 compounds with a best EC50 around 700 nM. Medicinal chemistry guided by structure activity relationship (SAR) via *in vitro* assays in HCT-8 cell cultured with transgenic Cryptosporidium was applied to optimise efficiency. Best compounds from *in vitro* selection were evaluated as oral therapeutics in mouse models (neonatal mice, IFNγ-/-) and neonatal lambs.
Results. Successive *in vitro* assays following chemical improvement allowed to identify several drugs active in the nanomolar range, with the best candidates with EC50 in low sub nanomolar range. Both mice models and lamb experiments confirmed the strong efficacy (parasite load; weight gain) of these soluble and orally administrable compounds. No adverse effects were detected in the *in vivo* experiment.
Conclusion. The strong efficacy of the best compounds combined to low toxicity revealed their robust selectivity for Cryptosporidium, thus revealing the strong potential of these new therapeutics which hopefully will be able to participate in the future arsenal of therapeutics required for optimal cryptosporidiosis control.

ORAL PRESENTATIONS

INTERACTIONS BETWEEN *Cryptosporidium parvum* AND BOVINE CORONAVIRUS DURING SEQUENTIAL AND SIMULTANEOUS CO-INFECTION OF HCT-8 CELLS

Ruchika SHAKYA[1,2], Alejandro JIMENEZ-MELENDEZ[1], Lucy J. ROBERTSON[1], Mette MYRMEL[1]
[1] Faculty of Veterinary Medicine, Norwegian University of Life Sciences (NMBU), Ås, Norway; [2]ruchika.shakya@nmbu.no

Background. Neonatal diarrhoea in calves is one of the major health problems in the cattle industry. Bovine coronavirus (BCoV) and *Cryptosporidium parvum* are among the most prevalent enteropathogens in neonatal calves. Although co-infections have been associated with a greater severity of disease, there is limited information on any impact of co-infections on the pathogens themselves.
Objectives. The main objective was to investigate whether there is an influence from one pathogen on the other during sequential and simultaneous co-infections *in vitro.*
Material and Methods. An *in vitro* infection model using HCT-8 cells was employed. HCT-8 cells were cultured with: (i) BCoV for 24h, followed by *C. parvum* and further incubation for up to 48h, (ii) *C. parvum* for 24h, followed by BCoV and further incubation for up to 48h, and (iii) BCoV and *C. parvum* in a mixed inoculum for 24h. Copy numbers for both pathogens were determined by (RT)-qPCR, and immunostaining followed by confocal microscopy or flow cytometry to determine the infection rates.
Results. The results from simultaneous co-inoculation showed that the entry of viral particles was favoured when *C. parvum* sporozoites were present, although elevated virus copy numbers were no longer evident after 24h. The attachment of BCoV to the sporozoites was probably due to specific binding, as investigations with other viruses showed no attachment. Flow cytometry results revealed that *C. parvum* and BCoV infected 1-11% and 10-20% of cells, respectively, with only 0.04% of cells showing double infections, which was corroborated by confocal microscopy.
Conclusion. The present study demonstrated that sequential inoculation did not provide any advantage – or disadvantage – to either of the infectious agent. However, the results from simultaneous infection showed that more virus particles entered the cells when *C. parvum* sporozoites were also present, suggesting an attachment between BCoV and *C. parvum* sporozoites.

Funding source: Internal funding from Virology and Parasitology units (PARAFAG, NMBU)

CHARACTERIZATION OF INTESTINAL MACROPHAGES AND DENDRITIC CELL SUBSETS IN NEONATAL LAMBS AND CALVES AT HOMEOSTASIS AND FOLLOWING *Cryptosporidium parvum* INFECTION

Ambre BAILLOU[1,2,5], Thierry CHAUMEIL[3], Céline BARC[3], Yves LEVERN[4], Alix SAUSSET[4], Julie SCHULTHESS[5], Pauline PELTIER-PAIN[5], Sonia LACROIX-LAMANDE[1], Fabrice LAURENT[1]

[1]UMR1282 Infectiologie et Santé Publique, INRAE Centre Val de Loire, Université François Rabelais de Tours, 37380 Nouzilly, France; [2]ambre.baillou@inrae.fr, UMR1282 Infectiologie et Santé Publique, INRAE Centre Val de Loire, Université François Rabelais de Tours, 37380 Nouzilly, France; [3]Plateforme d'Infectiologie Expérimentale (PFIE), INRAE Centre Val de Loire, 37380 Nouzilly, France; [4]Plateforme de cytométrie, INRAE Centre Val de Loire, 37380 Nouzilly, France; [5]Phileo by Lesaffre, 137 rue Gabriel Péri, 59700, Marcq-en-Barœul, France

Background: Cryptosporidiosis is a poorly controlled zoonosis caused by an intestinal parasite, *Cryptosporidium parvum* (*Cp*), with a high prevalence in livestock (cattle, sheep, goats). Young animals are particularly susceptible to this infection due to the immaturity of their intestinal immune system. In a neonatal mouse model, we previously demonstrated the importance of the innate immunity and in particular of CD11c+CD103+CD11b- conventional dendritic cells (cDC) among mononuclear phagocytes (MP) in controlling the acute phase of *Cp* infection. During infection, in response to chemokine production by infected epithelial cells, newly recruited cDCs produce IL12 and IFNγ contributing to the elimination of the parasite. According to the well-established mouse cDC classification, this Batf3+DC subpopulation corresponds to the cDC1 subset.
Objectives: The aim of this project was to better characterize intestinal MP subpopulations in neonatal lamb and calf at homeostasis and during *Cp* infection. As in the mouse model, the parasite invades and multiplies mainly in the ileum of animals. However, a peculiarity of young ruminants is the presence of a large ileal Peyer's patch (lymphoid tissue) that extends all along the ileum. MP were therefore analyzed in lymphoid and non-lymphoid intestinal tissues of lambs and claves.
Material and Methods: We performed phenotypic and functional analyses of mononuclear phagocytes by flow cytometry and by transcriptomic methods (FLUIDIGM®) respectively, in the distal jejunum, jejunal and ileal Peyer's patches.
Results & Conclusion: We characterized a population of macrophages and three subpopulations of cDC. We demonstrated that the subset identified as cDC1, according to the current common classification of cDC in different species (human, mouse, pig, sheep and chicken), increases with the age of animal. This might be linked with the decrease in sensitivity to *C. parvum* observed with the age. We are currently investigating the evolution of cDC1 subset during *C. parvum* infection.

THERAPEUTIC AND NITAZOXANIDE-ENHANCER EFFECT OF A NOVEL CURCUMIN NANOCOMPOSITE IN *Cryptosporidium* INFECTED IMMUNOCOMPROMISED MICE

Ayman A. EL-BADRY[1], Reem Y. AL-JINDAN[1], Eman S. El-WAKIL[2], Rabindran JERMY[3]

[1]aelbadry@kasralainy.edu.eg, aaelbadry@iau.edu.sa - Department of Clinical Microbiology, College of Medicine, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia; [2]Parasitology Department, Theodor Bilharz Research Institute, Giza, Egypt; [3]Deanship of Scientific Research & Department of Nano-Medicine Research, Institute for Research and Medical Consultations, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

Background. Hybrid nanocomposites (NCs), based on natural components, offer multiple advantages of drug functionalization and structural stability. *Cryptosporidium* is a zoonotic enteric protozoan parasite of public health and veterinary importance, causing gastroenteritis that can end fatally. Its treatment by nitazoxanide (NTZ) which has side effects and do not result in a full cure.
Objectives. The current study goal is to design novel biocompatible and bioavailable curcumin NC and to assess its therapeutic effect in *Cryptosporidium* infected immunocompromised mice.
Material and Methods. *Cryptosporidium parvum* oocysts were harvested and purified from feces of infected calves. Molecularly characterized and used to infect immunocompromised mice. Mice were divided into groups (controls and therapeutic). Infected mice were sacrificed, with assessment of parasitological, histopathological, and oxidative stress parameters.
Results. The new nano-formulation of curcumin as a hybrid NC improved its bioavailability and biocompatibility. As a therapeutic agent, curcumin NC significantly reduced infection burden and improved infected intestinal mucosa, which was dose dependent added to its antioxidant effect. Impressively, the combination of curcumin

NC with half dose of NTZ gave full (100%) therapeutic effects compared with 59% with full dose of NTZ.
Conclusion. Using cheap, simple, reproducible and scalable techniques, we nano-formulated innovative bioactive curcumin-based NC. This NC is a safe, naturally based, eco-friendly, economical, bioavailable and biocompatible potential anti-*Cryptosporidium* nanotherapeutic with antioxidant and intestinal-protective role. It has a strong synergistic effect when combined with NTZ (etiological treatment) and significantly reduces required therapeutic dose by 50%.

TRICHINELLA
Organizer / Moderator: Ljiljana Sofronić-Milosavljević

INVITED LECTURES

HORIZONTAL GENE TRANSFER PROVIDES INSIGHTS INTO THE EVOLUTIONARY HISTORY AND BIOLOGY OF *Trichinella*

Dante ZARLENGA
USDA, Agricultural Research Service, Animal Parasitic Diseases Laboratory, Beltsville, Maryland 20705 USA
dante.zarlenga@usda.gov

Background. Deciphering processes that drive species diversity is key to understanding parasitism and help advance control strategies. Genomic and transcriptomic data on both parasitic and free living nematodes have been emerging at astonishing rates allowing more holistic investigations of evolution and the host:parasite interface. Sequencing has shed light on horizontal gene transfer (HGT) as a driving force in evolution and parasitism, a paradigm that has occurred independently many times among the Nematoda rather than as a single event.
Results. During the sequencing of the genome of *Trichinella spiralis*, we identified a cyanase gene which is typically found only in plants, bacteria and to a lesser extent fungi. In a database search, we identified a small subset of other Nematoda that also harbor this gene; however, the gene was not found in free-living worms or in organisms of the crown clade. The gene product from *T. spiralis* is biologically functional in recombinant form and resides naturally in the worm hypodermis. Phylogenetic analyses showed that cyanase proteins from the clade I nematodes *Trichinella* spp., *Trichuris* spp., and *Soboliphyme baturini*, (Subclass: Dorylaimia) formed a large, well-supported monophyletic clade with plant cyanases whereas all other cyanases within the Nematoda were monophyletic with those of bacterial origin.
Conclusions These results are consistent with: 1) independent HGT of the cyanase gene within parasitic nematodes but from multiple Kingdoms; 2) functional integration of the gene and encoded protein into the biology of *Trichinella*; 3) acquisition of the gene by members of the Dorylaimia over 400 million years ago prior to the divergence of the Trichinellida and Dioctophymatida, and; 4) early ancestors of the genus *Trichinella* having an association with plants or marine-derived nitrite-oxidizing bacteria given that cyanases from marine cyanobacteria and fungi clustered independent of the plant cyanases and *Trichinella*.

HOW *Trichinella spiralis*-DERIVED EXTRACELLULAR VESICLES AFFECT DENDRITIC CELLS

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Trichinella spiralis is a very promising candidate for modulation of immune response in sense of biasing the inflammatory towards anti-inflammatory type of response. This effect is achieved via its excretory secretory muscle larvae (ES L1) antigens which affect the maturation status and function of dendritic cells (DCs) as the most potent antigen presenting cells. ES L1 induces the tolerogenic status of DCs which leads to the mitigation of Th1 type of response and the activation of regulatory type of immune response both *in vitro* and *in vivo*. ES L1 treated DCs successfully alleviated the severity of experimental autoimmune encephalomyelitis, the animal model of human disease multiple sclerosis. Recent discovery of *T. spiralis* extracellular vesicles (TsEVs) suggested that the induction of a complex regulation of the immune response requires simultaneous delivery of different signals in nano-sized packages. This study aimed to explore whether TsEVs bare the similar potential as ES L1 to influence the status of DCs in initiation, progression and regulation of immune response. TsEVs were enriched from conditioned medium of *T. spiralis* muscle larvae by differential centrifugation and used for treatment of human monocyte derived DCs. TsEVs induced low expression of HLA DR and CD40, moderate CD83 and CD86 and increased expression of ILT3 and CCR7 on treated DCs, i.e. they induced tolerogenic DCs.

DCs generated this way possess the capacity to polarize T cell immune response towards regulatory type, with increased proportion of IL-10 and TGF-β producing cells. These findings indicated that the ability of TsEVs to induce tolerogenic DCs favoring anti-inflammatory responses may be helpful in coping with diseases that involve Th1/Th17-, but also Th2-mediated inflammation, such as different autoimmune and allergic diseases, suggesting that potential TsEVs application could be a new therapeutic approach designed for treatment of inflammatory disorders.

Funding source: Institutional financing agreement №. 451-03-9/2021-14/ 200019

WHAT DOES THE HUMORAL IMMUNE RESPONSE IN *Trichinella* INFECTIONS TELL US?

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The diagnosis of *Trichinella* infections in the human host is based on the humoral immune response, in particular on the determination of the specific IgG. Moreover, the presence of such isotype in animals provides pivotal epidemiological information on parasite-host contacts. After infection, seroconversion most often occurs between the third- and fifth-weeks post infection (p.i.), although it can also take place earlier (12 days) or later (60 days) p.i., and the IgG responses can persist for years. Other isotypes such as IgM, IgE and IgA are known to be present, but their significance remains unclear. On the other hand, *Trichinella* antigens are heterogeneous, some epitopes are shared with other organisms and those highly specific maybe insufficiently sensitive due to their different expression in the developmental stages. The pleotropic immune response that accompanies trichinellosis is complicated and the data is still evolving on how best to diagnose infections. However, the actual tools for diagnosis and surveillance of these infections, as well as the knowledge on the biology of these parasites, might be exploited and combined to gather key information on the epidemiology of theses infections. For example, in epidemiological investigations during a human outbreak, in which the source of infection could not be traced, and muscle biopsies were not available, the specific IgG pattern of recognition of sera from infected people on *Trichinella* proteins by western blot, showed to be an excellent tool for the identification of the aetiological agent. Furthermore, monitoring susceptible animals from different settings may provide evidences on the risk for *Trichinella* transmission or on the circulation of these parasites among those animals.

ORAL PRESENTATIONS

Trichinella britovi RECOMBINANT EXCRETORY-SECRETORY 21 kDa PROTEIN AND CHYMOTRYPSIN-LIKE PROTEIN FOR IgG ANTIBODIES LEVEL DETECTION IN TRICHINELLOSIS IN MICE AND PIGS

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Background. Trichinellosis occurs by consuming raw or inadequately cooked meat containing muscle larvae of the *Trichinella*. This parasite can infect a wide variety of hosts, including humans, causing a food-borne parasitic zoonosis. *Trichinella britovi* is the second most commonly identified species among infected animals and is one of the *Trichinella* spp. which may affect human health.
Objectives. Our study aimed to recognize *T. britovi*-specific proteins, obtain them in a recombinant form in the yeast expression system vector and verify its immunological properties, as a potential molecules for the *Trichinella* infection diagnosis.
Material and Methods. Proteomic analysis performed with *T. britovi* adult worm excretory-secretory antigen and muscle larvae crude extract revealed 21 kDA excretory-secretory (ES21) protein and chymotrypsin-like protein (CTRL). These proteins were chosen for expression in heterologous system. Muscle larvae from previously infected mice with *T. britovi* reference strain (ISS002) were used for total RNA isolation. cDNA

synthetized on RNA template were used for amplification of ES21 and CTRL coding sequences. PCR products were cloned into *Pichia pastoris* expression system vector. The obtained rTb-ES21 and rTbCTRL proteins were used in ELISA to detect the level of IgG antibodies in the sera of mice and pigs experimentally infected with different doses of *T. britovi*.

Results. Both proteins enabled detection of increase level of anti-*Trichinella* IgG antibodies in sera of infected mice, whereas the reactiveness with rTbCTRL was stronger. The rTbCTRL efficacy for IgG antibodies detection in pig sera was determined as 51 days post infection.

Conclusion. The study presents new proteins of *T. britovi* antigen which may serve to identify changes in IgG antibodies profile in sera of animals infected with *Trichinella* spp.

Funding source: Financial support for this study was provided by the National Science Centre Poland (grant UMO-2015/18/E/NZ6/00502).

A *Trichinella spiralis* NEW BORN LARVAE SPECIFIC PROTEIN, *Ts*-NBL1, INTERACTS WITH HOST'S CELL VIMENTIN

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Background. *Trichinella* has a special relation with its host due to a unique intracellular location within the feeder cell, derived from skeletal muscle fibre. *Ts*-NBL1 is considered to be a key protein for early invasion and feeder cell formation in *Trichinella spiralis* infection. This study is the first to report Yeast two-hybrid systems (Y2H) approach for screening protein interactions of *Trichinella*.

Material and Methods. *Ts*-NBL1 interactants were screened by Y2H using human cDNA libraries. The major identified potential interacting protein was further confirmed by GST co-affinity purification and immuno-colocalization by confocal microscopy.

Results. 193 cultured colonies were screened, and corresponded to 20 potential interacting proteins. Among these 193 colonies, vimentin was the most interesting potential interactor of *Ts*-NBL1 which was found in 25 yeast colonies. GST pull-down assay confirmed the interaction between *Ts*-NBL1 and vimentin. Further studies by qPCR evidenced that *Ts*-NBL1 up-regulates vimentin expression on mRNA level in mammalian cells.

Conclusion. This study confirmed vimentin as an important interactor for *Ts*-NBL1. The discovery of host proteins interacting with *Ts*-NBL1 will help to suggest that *Ts*-NBL1 contributes to the capsule formation and help for understanding the mechanisms involved in *Trichinella* survival in the host.

Funding source: China Scholarship Council; DIM1HEALTH Île-de-France grant; LABEX IBEID grant ANR-10-LABX-62-IBEID.

PROTOZOAN INFECTIONS IN LIVESTOCK AND THEIR CONTROL – ZOONOTIC AND ANIMAL HEALTH ASPECTS
Organizers / Moderators: Gereon Schares & Walter Basso

INVITED LECTURES

BESNOITIOSIS IN EUROPE: IMPACT OF THE DISEASE AND POSSIBILITIES TO CONTROL

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Bovine besnoitiosis, caused by *Besnoitia besnoiti,* is a chronic and debilitating disease spread in Southwest Europe and with reported outbreaks in Central and Northern Europe. In Northern mountainous regions of Spain, herd and intra herd prevalence rates may rise to 50%, 87% and higher than 50%, respectively, with 12.5-16.7% clinical incidence rates. Besnoitiosis is one of the most relevant cattle diseases in extensive husbandry systems. Reproductive failure is the major concern as males may develop sterility with azoospermia during both acute and chronic infection. Initially bulls develop fever and orchitis that may end up with atrophy of the testes characterized by marked inflammatory infiltrate, fibrosis, testicular degeneration, and hyperkeratosis. Azoospermia might be a consequence of: i) thermoregulation failure associated to vascular and scrotal skin lesions; and ii) blood-testis barrier damage induced by an intense inflammatory response. There are not effective drugs or licenced vaccines. Thus, control is limited to diagnosis coupled to management measures. The detection of subclinically and acutely infected cattle are two main diagnostic challenges to be tackled. Special attention should be paid to sub-fertile or sterile bulls, open cows, cows that calve late in the calving season and cows with lesions in the udder that may impair appropriate calf lactation. Annual regular monitoring of bull's semen quality and *Besnoitia* infection prior to breeding season are key recommendations. A rigorous control program adapted to the herd health status can control the disease with an improvement of the herd reproductive parameters based on a previous experience. The diagnostic approach and management decisions will be presented.

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NEOSPOROSIS IN SHEEP: MORE IMPORTANT THAN PREVIOUSLY THOUGHT?

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Neospora caninum is an apicomplexan parasite which causes neosporosis, known to be one of the most important infectious causes of abortion in cattle. In recent years, the importance of *N. caninum* as an abortifacient in sheep has increased. However, the economic and epidemiologic importance of *N. caninum* infection in ovine flocks remains unknown. Ovine neosporosis has been described worldwide and the global seroprevalence ranged from 2% to 36%. In flocks suffering the infection, it has been described that 35-40% of *N. caninum* seropositive sheep experience reproductive failure. Moreover, detection rates of *N. caninum* DNA in aborted lambs ranged from 7% to 20%. In contrast to cattle, only three isolates have been obtained so far from sheep. Recently, it has been demonstrated that recrudescence of a chronic infection and the subsequent endogenous transplacental transmission (the most frequent route of transmission in cattle) is also highly efficient in sheep (66-93%), being the presence of stillbirths the main clinical outcome. Ovine experimental models of *N. caninum* infection show some relevant advantages over the bovine model (such as the lower gestational period, easier handling and lower cost of facilities and maintenance) and have being

used as proof-of-concept to test drugs and vaccines. However, the pathogenesis of neosporosis in sheep is poorly understood and, in contrast to the clinical outcome in cattle, infection during mid-pregnancy in sheep results in severe clinical outcome, since most of the dams abort or, less frequently, give birth to weak lambs. To date, the recrudescence of infection after experimental challenge has not been achieved. Similar to cattle, control measures should aim to avoid endogenous and exogenous transplacental transmission. We will present the first and successful attempt to control neosporosis in a sheep flock with high abortion rates through progressive culling of seropositive dams and replacement with seronegative offspring.

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Toxoplasma gondii AND *Neospora caninum* INFECTIONS IN SOUTH AMERICAN CAMELIDS

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Toxoplasma gondii and *Neospora caninum* infections represent major causes of abortion in ruminants. Only few studies worldwide showed that these protozoal infections may also occur in South American camelids (SAC), occasionally associated with abortions and fatal disease. In Europe, the popularity and interest for SAC has shown a notable increase in the last years, but local information on the epidemiology of *T. gondii* and *N. caninum* infections in SAC is scarce. Differences in types of husbandry, contact with definitive hosts, geographical factors and/or co-infections with other agents might influence their occurrence and/or clinical significance. In South America, reported prevalence estimates for *T. gondii* and *N. caninum* antibodies in SAC between 3.0 and 44.0% and 0.3 – 42.0%, respectively. In Europe, antibodies against *T. gondii* and *N. caninum* were detected in a few SAC in Germany (14/32 and 0/32, respectively) and Czech Republic (4/9 and 1/9, respectively). In addition, a recent nationwide serological cross-sectional study in Switzerland, using ELISA and immunoblot, showed prevalences for *T. gondii* antibodies of 82.3% (308/374) in alpacas and 84.8% (167/197) in llamas, with 99.2% (131/132) of the farms presenting seropositive animals. Besides, 3.5% (13/374) of the alpacas and 2.5% (5/197) of the llamas, evidenced antibodies against *N. caninum*, and 9.1% (12/132) of the farms had seropositive animals. In that study, the variables "(older) age" and "female sex" were identified as risk factors for *T. gondii* infection and "absence of cats in the farm during the last two years" as a protective factor. Besides, a further study in Switzerland revealed antibodies against *T. gondii* in one aborted foetus and one stillborn cria, indicating the occurrence of congenital infection. The high *T. gondii* seroprevalence suggests that SAC meat might represent an infection source for humans, as it is often eaten undercooked as "gourmet dish".

Funding sources: Verein zur Förderung der Forschung im Gesundheitssektor von Lamas und Alpakas e.V., Kronberg im Taunus, Germany.

Tritrichomonas foetus OR WHAT ELSE? INSIGHTS FROM EPIDEMIOLOGY AND GENETIC COMPARISONS

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Background: *Tritrichomonas* spp. infections occur in bovines as a sexually transmitted disease affecting fertility, in cats as a cause of chronic large bowel diarrhoea, and asymptomatically in pigs and in some other hosts. In bovines, *Tritrichomonas foetus* has serious economic consequences and is officially regulated in many countries. The widespread occurrence of a very closely related parasite in cats therefore raised concerns.
Objectives: We conducted an epidemiological study into the *T. foetus* situation in Switzerland and applied genomic approaches to elucidate the differences between the different *Tritrichomonas* isolates from various hosts.

Material and Methods: Bulls and aborted foetuses were screened for *T. foetus* infection with *in vitro* culture (InPouch®) and PCR. Positive results were confirmed by sequencing. Genomic comparisons of bovine, feline, and porcine *T. foetus* / *T. suis* isolates were done by whole genome sequencing.
Results: In the epidemiological survey, we did not find *T. foetus* in the assessed animals. This confirms that *T. foetus* is not occurring in the Swiss bovine population, at least not in a higher prevalence than 0.3% (detection limit of the study). The whole genome sequencing revealed important differences between bovine and feline *T. foetus* isolates. On the other hand, bovine and porcine isolates were genetically very close, supporting older studies that suggested renaming *T. suis* as *T. foetus*.
Conclusion: The studies demonstrated that *T. foetus* in bovines in Switzerland seems to be absent and the parasite occurring in cats is most probably not posing a threat to eradication efforts of bovine tritrichomonosis.

Funding sources: Federal Food Safety and Veterinary Office, State Secretariat for Education, Research and Innovation

ORAL PRESENTATIONS

ANATOMICAL DISTRIBUTION OF *Toxoplasma gondii* IN NATURALLY AND EXPERIMENTALLY INFECTED LAMBS

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Consumption of raw or undercooked meat containing *Toxoplasma gondii* tissue cysts is one of the main source of contamination for humans world-wide. Among various species intended for human consumption, lamb appears as a risk factor of human toxoplasmosis. The present study focused on a detailed anatomical distribution of *Toxoplasma gondii* in naturally and experimentally infected lambs using fresh and frozen samples of various pieces of meat, from a public health perspective. Ranking the edible parts intended for human consumption, according to the parasite burden, and therefore, the risk for *T. gondii* contamination in human, was a first objective. A second objective was to evaluate the impact of freezing, as imports arrive mainly frozen. Viable *Toxoplasma gondii* tissue cysts or *T. gondii* DNA were present in all meat samples. High level of DNA parasite was observed in skeletal muscles and more particularly in edible portions such as quadriceps femoris, intercostal, deltoid and biceps femoris muscles with a significant difference in parasite burden between fresh and frozen samples ($p < 0.0001$) or natural and experimental infection ($p < 0.0001$). These results suggest that lamb should be thoroughly cooked or frozen before consumption. Further investigations need to be done in order to confirm the above mentioned differences in more animals and in different breeds.

Cryptosporidium SPECIES AND SUBTYPES IN SWEDISH RODENT POPULATIONS – CONCERN FOR ZOONOTIC TRANSMISSION

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Background. *Cryptosporidium* is a protozoan parasite and causes disease in animals and humans globally. Rodents may carry zoonotic pathogens including *Cryptosporidium* spp. They often live in close proximity to humans and are found where food is handled, stored or where food producing animals are farmed,

increasing the potential for disease transmission of zoonotic species. The prevalence and genetic diversity of *Cryptosporidium* spp. in rodent populations of Sweden was investigated.
Material and Methods. A total of 230 faecal and intestinal samples were collected and comprised several rodent species including: European brown rat, *Rattus norvegicus* (n=164), house mouse, *Mus musculus* (n=33), yellow-necked mouse, *Apodemus flavicollis* (n=26), field vole, *Microtus agrestis*: (n=5) and wood mouse *Apodemus sylvaticus* (n=2). All samples were collected either in urban environments, including kitchens of households and restaurants or on farms with food producing animals. *Cryptosporidium* spp. were detected by immunofluorescence microscopy and PCR, which targeted the 18S rRNA gene for species identification and *gp60* for subtype analysis. These targets were subsequently validated using Sanger sequencing.
Results. In total 73.1% (170/230) of the samples were positive. From the 18S positive samples, only 30% (51/170) were positive following *gp60* amplification. Sequencing of the 18S gene revealed many species, including zoonotic *C. parvum*, most common in European brown rats. Several other species of *Cryptosporidium* were found, including *C. ratti*, Rat genotype (IV) and (V), Vole Genotype (III), *C. occultus*, *C. tyzzeri*, *C. ditrichi*, *C. apodemus*, *Cryptosporidium* sp. (UK-E4) and *C. muris*. Sequencing highlighted high percentage of co-infection with several *Cryptosporidium* species in some samples, leading to inconclusive or unreadable sequences.
Conclusion. Rodent populations are dynamic in nature and are capable of moving between farms and urban areas making them important facilitating hosts in the transmission of *Cryptosporidium* spp., especially considering the findings of the zoonotic *C. parvum* in different rodent species.

ASSESSING THE ROLE OF NEUTROPHILS IN VECTOR-TRANSMITTED *Trypanosoma brucei* INFECTIONS

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Background. Human African Trypanosomiasis (HAT), also known as sleeping sickness, is a tsetse fly transmitted parasitic disease indigenous for the African continent. Trypanosome transmission occurs during the blood feeding of infected flies, resulting in the inoculation of parasites in the skin. The drugs that are currently available to treat this disease all have their limitations and to date an effective vaccine is lacking, endorsing the need for novel treatment strategies. Instead of focusing on the parasite, strategies to prevent parasite transmission are gaining interest as they are not subject to drug resistance. Therefore, insight into the early immunological events upon the bite of infected tsetse flies is required. Neutrophils are rapidly recruited to the dermal infection site but their role in vector-transmitted *Trypanosoma* infections is largely unknown.
Material and Methods. The response of neutrophils to a range of parasitic stimuli was evaluated using flow cytometry, bioluminescent imaging and genetically modified mouse models.
Results. These experiments showed that exposure of human neutrophils to naive tsetse fly saliva and live bloodstream form (BSF) parasites significantly prolonged neutrophil survival *in vitro*. The prolonged survival induced by tsetse fly saliva was shown to be mediated by TLR4 and P2X1 activation. Additionally, BSF parasites induced neutrophil extracellular trap (NET) formation and degranulation of azurophilic granules. Trypanosomes are thus responsible for neutrophil activation, however limited cell death in the presence of neutrophils and low levels of parasite phagocytosis show that the parasite is not hampered by activated neutrophils. Neutrophil depletion experiments in various genetically modified mouse models in combination with bioluminescent imaging showed an impact on dissemination and systemic parasite levels.
Conclusion. Parasite factors and tsetse salivary components influence neutrophils survival and activation, potentially influencing the skin immunological environment during the early phase of infection with an ensuing impact on systemic parasite dissemination**.**

DIVERSITY OF ECHINOCOCCUS AND OTHER TAENIIDS
Organizer / Moderator: Thomas Romig

INVITED LECTURES

Echinococcus PHYLOGENY AND NOMENCLATURE: AN UPDATE

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Few decades ago, *Echinococcus* was thought to contain four species only, one of them (*E. granulosus*) obviously highly polymorphic. In contrast, gene sequence analysis led to the current recognition of nine different species, five of them belonging to the *E. granulosus* (sensu lato) cluster. However, intraspecific variance and reproductive barriers are still insufficiently known, and two of the species show a complex genetic structure that may lead to the recognition of additional species: *E. granulosus* sensu stricto contains two closely related and globally distributed genotypes (G1 and G3); differences of lifecycles and disease manifestation have, however, not been convincingly demonstrated. A more distant genotype, G-Omo, is present in eastern Africa and may – based on genetic distance from G1/3 – in future have to be separated from *E. granulosus* s.s.. The most diverse species – as recognized here - is *E. canadensis*, whose three major genotypic lineages (G6/7, G8, G10) are clearly separated. G6/7 is globally distributed, usually transmitted in a domestic lifecycle involving pigs or camels and is responsible for the second largest number of human cases of cystic echinococcosis after *E. granulosus* s.s.. Subdivisions of *E. canadensis* into two or three species have been proposed based on genetics, geography and host range, but a consensus has not yet been reached due to gaps of knowledge. Here we provide a summary on the current taxonomic concepts with accepted and debated issues, put the genus *Echinococcus* in a phylogenetic context with other taeniid genera and highlight the relevance of taeniid taxonomy for epidemiology and public health.

THE TRANSMISSION OF *Echinococcus* SPECIES: HOW ECOLOGY EXPLAINS MOST OF IT

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Parasitic transmission occurs in many ways, but the ecological contexts are always critical in determining its dynamics. This is particularly true for complex life-cycle parasites, and even more relevant for those transmitted through a predator-prey relation, as *Echinococcus* spp. These species require a predator as definitive host, and a prey as intermediate host, where the larval stages develop causing forms echinococcosis with different severity.

The frequency of encounter between the predators (definitive hosts) and their prey, as between the parasite eggs and the intermediate hosts, determine the transmission dynamics, but these frequencies are affected by many ecological processes and conditions. Parasitic propagules (eggs) are not shed randomly, but according to the predator territorial behaviour; moreover, several definitive host species have huge differences in body size and home range.

Similarly, the competent intermediate host species have diverse vagility, longevity and distribution, and present different susceptibility to infections of *Echinococcus* spp. Moreover, high temperature and low humidity affect the viability of *Echinococcus* eggs smoothing down transmission dynamics, thus limiting their distribution. Relatively low pathogenicity and long longevity of intermediate hosts may help the parasite to overcome long arid seasons.

Moreover, some *Echinococcus* species have several competent definitive and intermediate hosts that may compete with each other (e.g. spatial exclusion, intra-guild predation), and the frequency of predation on

competent intermediate host species can be affected by the availability of other competent or not competent prey, and increasing or diluting transmission. Parasites may also affect the prey ability to escape predation, affecting the trophic cascades.
The combination of all these processes and relations determines the dynamics of transmission and the distribution of *Echinococcus* spp. as well represented by the invasion of North America by European *Echinococcus multilocularis* strains, or their European distribution, or *E. multilocularis* limited global distribution if compared to other *Echinococcus* species.

DO WE KNOW THE REAL BURDEN OF HUMAN CYSTIC AND ALVEOLAR ECHINOCOCCOSIS IN EUROPE?

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Background. The neglected zoonoses cystic (CE) and alveolar echinococcosis (AE) affect worldwide poor pastoral and rural communities but also those of medium-high income countries, including Europe, where they should be managed as orphan diseases. Even if human AE and CE are notifiable in some European member states (MS), in practice, these parasitic diseases are largely underreported in Europe.
Material and Methods. Data are presented on: 1) official ECDC/EFSA statistics at EU level on "echinococcosis" case definition; 2) CE case-series reported in the literature; 3) active search of CE carriers by means of ultrasound population-based surveys (cross-sectional study).
Results. Official statistics: In 2019, 751 confirmed human "echinococcosis" cases were reported in the EFSA/ECDC "EU One Health 2019 Zoonoses Report". The EU notification rate was 0.18 cases/100,000 population. Eg accounted for 73.5% (408 cases) and Em for 26.5% (147 cases). Clinical observations: Irrespective of the previous figure, case-series reported in the scientific literature recorded the presence of around 39,000 CE hospitalizations for a period of around 15 years only in Italy, Spain and France. Hidden burden: 24,693 people were screened by ultrasound during 2014-2015 in Bulgaria, Romania and Turkey. Based on the adjusted prevalence and the reference rural population size in 2015, it was estimated that 7,872 individuals may be infected with abdominal CE in rural Bulgaria and 37,229 in rural Romania.
Conclusion. Collection of accurate epidemiological and clinical data will give a reliable picture of the burden of these diseases in Europe, providing a statistically supported case series for future evaluation of efficacy and effectiveness of interventions. With the aim of improving surveillance of CE and AE, we encourage international agencies to lobby the European Commission to champion new health policies for the notification of human and animal CE and AE.

Funding source: MEME project is supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement № 773830: One Health European Joint Programme. https://onehealthejp.eu/jrp-meme/

ORAL PRESENTATIONS

MOLECULAR EPIDEMIOLOGY OF *Echinococcus multilocularis* IN EUROPE: A PRELIMINARY PICTURE

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Background. Alveolar echinococcosis has been ranked as the third most relevant parasitic foodborne disease worldwide. In Europe, the prevalence of the causative agent, *Echinococcus multilocularis* (Em), appears to be on the rise, driven by the presence and movement of wild and/or domestic hosts. However, baseline studies on the distribution of this pathogen have not yet been performed at the European level (EFSA, 2021).
Objectives. The goal of this study is to draw a first map of the genetic diversity of Em in Europe. The European Union Reference Laboratory for Parasites (EURLP) is coordinating this study in collaboration with several National Reference Laboratories.
Material and Methods. From January 2020 to January 2021, a collection of Em biological material was made available to the EURLP for molecular characterization. A multilocus typing scheme based on five complete mitochondrial genes (COB, ATP6, NAD2, NAD1 and COX1) was applied. The sequence of these genes was concatenated to generate a multiple alignment. The population structure (haplotype network) and the genetic distance between populations (pairwise fixation index, Fst) were investigated.
Results. The final multiple alignment consisted of 4968 positions and included 86 isolates from Austria (4), Croatia (12), Czech Republic (15), France (16), Hungary (4), Latvia (5), Poland (14), Slovakia (10) and Switzerland (6). Fifty-two haplotypes were detected, of which 12 were shared by multiple isolates. The Fst ranged from 0 to 0.5, suggesting varying levels of genetic differentiation among populations.
Conclusion. This preliminary analysis found no support for a clear geographic distribution pattern, since both unique and common haplotypes were present in almost all the populations investigated. Additional isolates are being analysed to determine the presence of ancestral Em haplotypes from which rarer haplotypes may have been derived by mutational events.

Funding source: This work was supported by the European Commission's Directorate-General for Health and Food Safety (DG SANTE) - European Union Reference Laboratory for Parasites, grant agreement № SI2.801980.

INDIVIDUAL PATTERNS OF *Echinococcus multilocularis* INFECTION IN FOXES IN AN ENDEMIC AREA OF ALVEOLAR ECHINOCOCCOSIS

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Background. Key parasite transmission parameters are difficult to obtain from wild animals, as they are often elusive. For *Echinococcus multilocularis*, the causative agent of alveolar echinococcosis (AE), red fox is responsible for most of the environmental contamination and the parasite spreading in Europe. Reliable

assessment of epidemiological parameters in this main host may help to predict AE dispersal patterns, enabling the design appropriate measures. Using faecal genotyping and molecular parasitological analyses, we assessed individual *E. multilocularis* prevalence in foxes in an AE endemic area.

Material and Methods. This study was conducted from March 2017 to January 2020 in a rural town in the Eastern part of France. In total, 386 fox faeces (of which 79 were previously tested positive for *E. multilocularis*) were collected noninvasively during two-month sampling intervals. From these, 180 faeces including all positive samples were genotyped and sexed for 14 microsatellite and one sex loci using a multiple-tubes approach. Individual *E. multilocularis* prevalence in foxes defined as the percentage of unique genotypes with at least one positive scat, was compared to the global faecal prevalence.

Results. We successfully genotyped 124 faeces (68.9%) corresponding to 45 unique individual foxes (27 as male and 18 as female) of which 26 were associated with at least one scat tested positive for *E. multilocularis*. Number of faeces per fox ranged from 1 to 15 (average =2.8). Overall individual *E. multilocularis* prevalence in foxes was 57.78 % (95% CI: 42.15 – 72.34) and was significantly higher than the faecal prevalence (20.47%, 95% CI: 16.55 – 24.84).

Conclusion. The individual *E. multilocularis* prevalence in foxes reported in this study was similar to others obtained previously through fox post-mortem examination in the same study area. Combining faecal genotyping and parasitological analysis allow to avoid potential biases associated with parasite prevalence estimates from copro-samples of unidentified animals.

GIARDIA
Organizers / Moderators: Marco Lalle & Christian Klotz

INVITED LECTURES

FROM INFECTION TO CLINICAL GIARDIASIS AND BEYOND

Kurt HANEVIK[1,2]
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Only a few cysts of Giardia is needed to establish infection in humans. The incubation period is variable, but often lasts around one week. The infection may be asymptomatic or give severe diarrhoeal and systemic symptoms, even if Giardia trophozoites are not invading the gut mucosa. Intestinal trophozoite numbers are high during initial infection, then decrease and may be eradicated by the host immune system within 2-4 weeks or with the aid of antibiotic treatment. However, in some individuals Giardia establishes a chronic infection lasting more than 2 months. Increasing treatment failure to nitroimidazole treatment has been seen over the last decade.
Even when successfully eradicated, recent studies have shown that early Giardia infection may lead to reduced linear growth in infants in low-income countries, while adults in high-income countries may develop post-infectious functional gastrointestinal disorders following giardiasis.

IN VIVO AND IN VITRO MODELS FOR GIARDIASIS: WHAT'S NEW

Christian KLOTZ
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Giardiasis is a widespread, multifactorial gastrointestinal disease caused by *Giardia duodenalis*. The pathophysiological mechanisms leading to giardiasis are still largely obscure, partly due to the lack of adequate model systems. In this presentation, I will briefly highlight current in vivo and in vitro models and discuss their respective advantages and disadvantages for studying disease mechanisms. A focus will be set on new research avenues based on infection models using human stem cell-derived organoids. This technology aims at mimicking the events in the human tissue much closer than other models. I will present first results of our own research using this technology and put them in the context of previous data using other model systems.

ADVANCES IN *Giardia* GENETICS AND GENOME MANIPULATION

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Giardia is a common single-celled microaerophilic intestinal parasite causing diarrheal disease and significantly impacting global health. Double diploid trophozoites have presented a formidable challenge to the development of molecular genetic tools to interrogate gene function. The divergence of *Giardia* proteins and the high percentage of proteins lacking homology to those in other eukaryotes have compounded these difficulties, slowing drug target validation and development. *Giardia* has been amenable to genetic manipulation for over 20 years. Plasmids introduced by electroporation are maintained as episomes and linear dsDNA can be integrated. Here I discuss new genetic approaches for evaluating gene function in *Giardia*, including CRISPR-based methodologies for knockdowns and knockouts. Robust and reliable molecular genetic approaches to interrogate protein function will be fundamental toward evaluation and identification of druggable targets. These new genetic tools are key toward understanding *Giardia's* unique molecular and cellular biology and pathogenesis.

DRUGS AND DRUG RESISTANCE IN *Giardia*: WHAT WE DON'T KNOW YET

Marco LALLE
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Giardia is a widespread protozoan parasite causing the commonest parasitic diarrheal disease-affecting humans. Although Giardia infections are largely asymptomatic, they often result in severe and chronic diseases and can be followed by post-infectious sequelae. The armamentarium of currently approved antigiardial drugs is still limited and restricted to compounds with a broad antimicrobial activity. The increased prevalence of treatment-refractory giardiasis, particularly with nitroimidazoles (ie, metronidazole), is alarming and poses a further challenges for an effective management of giardiasis.

Aim of this lecture is to provide an overview on current knowledge regarding biological mechanism behind "drug-resistance" in Giardia and recent advances in development, identification and functional characterization of repurposed and new drugs, including also non-pharmacological alternatives. Potential knowledge gaps in the field and future research directions are discussed.

CLINICAL AND TROPICAL PARASITOLOGY
Organizers / Moderators: Maria Antoniou & Miloš Korać

INVITED LECTURES

SURVEILLANCE OF ALVEOLAR ECHINOCOCCOSIS IN FRANCE: REPORT OF THE NATIONAL REFERENCE CENTRE FOR ECHINOCOCCOSIS

Jenny KNAPP, Florent DEMONMEROT, Séverine LALLEMAND, Carine RICHOU, Solange BRESSON-HADNI, Anne-Pauline BELLANGER, Laurence MILLON, and Members of the French National Reference Center for Echinococcoses - FrancEchino Network
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Background. Alveolar echinococcosis (AE) is a liver cancer-like disease due to the infection by the larval stage of the parasite Echinococcus multilocularis, necessitating long care management with few therapeutic options. We present recent data from the French alveolar echinococcosis registry
Material and Methods. From 1982, the French National Reference Centre for echinococcosis has implemented a registry to collect epidemiological and clinical data from all AE declared cases, with the patient's consent. All data are now recorded using the CleanWebTM online software. We considered 3 periods: periods A (1982-1999), B (2000-2009) and C (2010-2018).
Results. AE is a rare disease in France (average annual incidence 0.032/100000 inhabitants). However the average number of incidental cases is increasing (14.2 incident cases/year before 2000, 35.3 incident cases/year for the period C). In total 853 cases have been recorded.
We observed a rising number of cases collected outside the historical endemic area for AE (15% of incident cases in period C, versus 7 % before 2010) and a rising number of cases diagnosed in young people (20% of patients < 45 years-old in the last five years).
Patients were less often symptomatic at diagnosis in recent period (42 % in period C versus 55% before 2000). A context of immunosuppression conditions concerned 25% of the cases in the last 10 years. In total 89.7% of patients received benzimidazole therapy (95% in period C), with a decrease in the time interval between diagnosis and initiation (from an average of 10 to 2 months). Surgical treatment was available for 33.4% of patients in the period C. Liver transplantation decreased from 10.7 to 1% over time. The 10-year survival rate was better for women than for men on the same periods.
Conclusion. The organization of surveillance activities by the National Reference Centre allows an accurate recording of epidemiological and clinical data from AE patients. The care management and prognosis improve over time in the French population.

DISSEMINATED STRONGYLOIDIAIS IN CASES WITH DIFFERENT PRESENTATIONS

Nadia EL-DIB
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This communication is about the detection of 3 cases with different presentations detected in Kasr Al Ainy Medical School of Cairo University. The first case was presented with hypereosinophilic syndrome and congestive heart failure and was diagnosed by detecting larvae in stools after a saline purge. MRI on the brain showed some whitish patches representing abscesses, which could explain some acute neurological problems. The second case was presented with acute asthmatic attack and haemoptysis. The patient had a history of infection with TB which was treated five years ago. Asthmatic attack was treated with cortisone, which made the case deteriorated with development of haemoptysis. Sputum samples were stained with Ziehl neelsen stain and examined microscopically. Larvae of St*rongyloides stercoralis* were detected in sputum. The use of this stain was excellent for staining and identification of larvae of *S. stercoralis*. The Third case was of a patient that was submitted to a kidney transplantation operation. Both donor and recipient were free from parasitic diseases. After the surgery, the patient was given immunosuppressant drugs as well as corticosteroids. Kidney

functions began to deteriorate with rise in creatinine value. Urine examination showed bacterial infection. Kidney biopsy showed the presence of larvae in glomeruli. All the three cases were not detected except after the occurrence of complications due to Strongyloidiasis.

PSEUDOPARASITISM – AN ISSUE FOR PATIENTS AND PARASITOLOGISTS ALIKE

Dušan LALOŠEVIĆ[1,2]
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Today, many patients are seen in clinical practice who believe they have parasites. Under the influence of, sometimes criminal, advertising of antiparasitic drugs, mostly of plant origin, and pseudo-treatment for body detoxication, many people receive wrong information and believe that they have worms. Providing correct scientific and medical advice for those patients may turn out to be a challenge for parasitologists without experience in comparative parasitology, and sometimes without psychological training. Such patients include people who collect invertebrates in their rooms, beds, toilets, etc.; "pedant coprologists" who examine their stool in detail and collect non-digested food as „worms", and also patients with real health problems. We here report a series of interesting clinical cases, including: a) A man with live worms in sputum, "parasites" about 10 mm long collected only when he brushed his teeth, identified as dipteran larvae that invaded his toothbrush; b) A young woman with a worm in her nose, which proved to be an adult miriapod about 15 mm long, represents accidental pseudoparasitism or „Münchausen syndrome"; c) Many patients who have removed material from stool, believing that non-digestable plants or food of animal origin represent „worms". In our climate conditions of South Europe, and given that classical intestinal parasites are extremely rare, cases of myiasis may be also problematic for diagnosis in parasitological laboratory.

ORAL PRESENTATIONS

FATAL *Strongyloides stercoralis* HYPERINFECTION SYNDROME FOLLOWING HEART TRANSPLANTATION

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Background. *Strongyloides stercoralis* causes chronic, mostly asymptomatic infections; however, hyperinfection syndrome with dissemination may occur in immunosuppressed patients, especially those on corticosteroids.
Case report. A 62-year-old Caucasian female was admitted to the ICU for acute respiratory failure. Her medical history was significant for past HBV infection and a heart transplantation 2.5 months prior. Approximately 1.5 months after transplantation, increased troponin and segmental contraction abnormalities were observed. Because of a suspected transplant rejection, methylprednisolone dosage was increased with little improvement. Furthermore, repeated heart biopsy excluded organ rejection. The patient developed a macular rash that resolved after antihistaminic treatment but abdominal petechiae quickly followed. Four days prior to ICU admission, her pulmonary function deteriorated. Chest CT scan showed diffuse bilateral ground-glass opacities, prompting bronchoscopy and PCR testing for *Pneumocystis jirovecii*. All microbiology tests were negative except for *Klebsiella pneumoniae*-ESBL, isolated from blood cultures, and increased beta-D-glucan (267.8 pg/ml). No eosinophilia was found. Her condition deteriorated, necessitating intubation. The diagnosis of strongyloidiasis was first suspected by a pathologist, who identified the presence of isolated filariform *S. stercoralis* larvae in the skin biopsy of the petechial rash. Subsequent serology testing was positive for the presence of *Strongyloides* IgG. Microscopic examination of bronchoalveolar lavage (BAL) sample revealed numerous larvae, whereas none were detected in stool or in the previous BAL sample (tested for *P. jirovecii*).

In contrast, both BAL and stool samples were PCR-positive for *S. stercoralis*. Ivermectin (200 µg/kg per day) was immediately administered; however, the patient developed irreversible shock and died 2 days later. Interestingly, pretransplant bloodwork revealed eosinophilia of 14.7%.
Conclusion. Asymptomatic *S. stercoralis* infections are not uncommon in the Balkan region, therefore careful consideration is needed before transplantation. Corticosteroids may promote development of *Strongyloides* hyperinfection syndrome while obscuring typical laboratory findings, prolonging the time to diagnosis and treatment.

FIRST REPORT OF QUALITATIVE AND QUANTITATIVE DIFFERENCES OF *Echinococcus granulosus* IMMUNOREACTIVE PROTEINS BETWEEN "RELAPSED" AND "NON-RELAPSED" CE-PAEDIATRIC PATIENTS AND INVESTIGATION OF THE MOST PROMISING EARLY PROGNOSTIC CANDIDATES

Eya BEN SALAH[1,2,3], Coralie BARRERA[2,3], Wahiba SAKLY[1], Sana MOSBAHI[4], Thierry BALLIAU[5], Nathalie FRANCHE[3], Bruno GOTTSTEIN[6], Sabrine BEN YOUSSEF[4], Mongi MEKKI[4], Hamouda BABBA[1], Laurence MILLON[2,3]

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Background. The clinical management of cystic echinococcosis (CE) is still hampered by the high rates of post-surgical recurrences that frequently cannot be early detected with available radiological and serological tools.
Objectives. We aimed to assess the value of proteins extracted from *E. granulosus* protoscolex as candidates for the early prediction of CE post-operative outcomes.
Material and Methods. We applied a proteomic approach based on immunoprecipitation (IP) of *E. granulosus* protoscolex antigens with pediatric CE patients' plasma collected at 1-month and 1-year post-surgery followed by LC-MS/MS. We compared the proteomic content of IP eluates from young patients who relapsed within the first-year post-surgery (RCE) to cases with no detectable relapses until 3 post-operative years (NRCE). Selected proteins were recombinantly produced and subsequently assessed for their prognostic performance by ELISA.
Results. A total of 305 protoscolex-derived immunoreactive proteins were identified, 59 of which were significantly more abundant in IP eluates from RCE than NRCE for both follow-up time-points. We selected four protoscolex-proteins as CE promising prognostic candidates: cytoplasmic malate dehydrogenase (Eg-cMDH), citrate synthase (Eg-CS), Annexin A6 (Eg-ANX A6) and severin (Eg-SVN) (**Figure 1**). ELISA results showed that IgG antibodies against the four markers were significantly lower at 1-year post-surgery than 1-month in NRCE, in contrast to the RCE group that showed either stable or higher IgG levels. The Eg-cMDH and Eg-CS showed the best prognostic performance with respective probabilities of being "relapse-free" of 83% and 81% if a decrease of IgG levels occurred between 1-month and 1-year post-surgery.

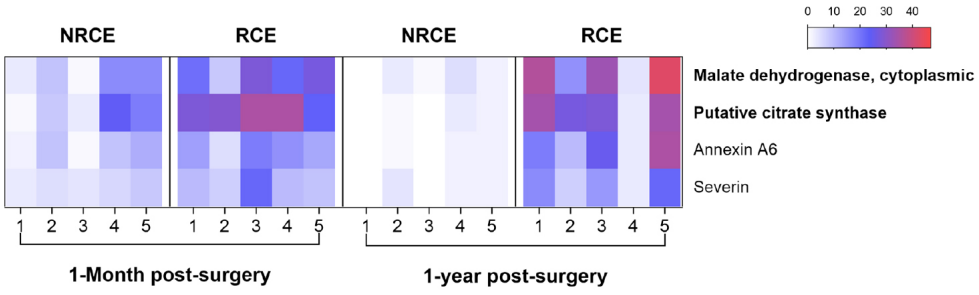

Figure 1. Differential expression of the selected immunoreactive proteins in NRCE versus RCE patients in 1-month versus 1-year postsurgery. Quantification represented by the heat-map tool was based on the spectral counting of each protein. NRCE. «Non-relapsed» CE patients, RCE. «Relapsed» CE patients.

Conclusion. This is the first study of its kind to report both quantitative and qualitative differences of immunoreactive proteins between “relapsed” and “non-relapsed” CE patients. The Eg-cMDH and Eg-CS showed the best performance as early predictors of CE post-surgical outcomes. Further assessment of these candidates is needed, especially in an adult patient cohort.

PROTOZOA IN FOOD & ENVIRONMENT – METHODS USED IN DIFFERENT ENVIRONMENTAL MATRICES
Organizer / Moderator: Isabelle Villena

INVITED LECTURES

DETECTION OF *Toxoplasma gondii* IN ENVIRONMENTAL MATRICES: CURRENT METHODS AND FUTURE DIRECTIONS FOR MITIGATION OF TOXOPLASMOSIS

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Toxoplasmosis is a prevalent infection in humans and animal populations worldwide. The disease is caused by *Toxoplasma gondii*, a zoonotic protozoan parasite that has a robust environmental stage – the oocyst. Three primary transmission routes can lead to infection with *T. gondii*: congenital transmission in-utero, horizontal transmission via consumption of parasite tissue cysts in undercooked or raw meat, and accidental oocyst ingestion through environmental matrices including contaminated water and foods such as shellfish and fresh produce. Of the three transmission routes, environmental transmission is the least understood and most difficult to mitigate. A major obstacle for accurate characterization and prevention of environmental transmission of *T. gondii* is the lack of standardized, sensitive and specific methods for detection of oocysts in the environment. This presentation will highlight current knowledge on methods that have been applied to detect *T. gondii* in soil, water, shellfish and fresh produce. Current limitations as well as opportunities for advancing the field of environmental transmission of *T. gondii* will be addressed.

DETECTION OF *Toxoplasma gondii* IN FOOD AND ENVIRONMENTAL MATRICES: UPDATE ON METHODS AND FUTURE DIRECTIONS FOR FOOD SAFETY

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Toxoplasma gondii is a zoonotic protozoan parasite that can cause morbidity and mortality in humans, domestic animals, and terrestrial and aquatic wildlife. The environmentally robust oocyst stage of *T. gondii* is fundamentally critical to the parasite's success, both in terms of its worldwide distribution as well as the extensive range of infected intermediate hosts.
Toxoplasma infections have been reported on every continent, and in terrestrial as well as aquatic environments. The remarkable resistance of the oocyst wall enables dissemination of *T. gondii* through watersheds and ecosystems, and long-term persistence in diverse foods such as shellfish and fresh produce. This parasite, as other protozoan parasites, is well identified as emerging foodborne pathogens by Food and Agriculture Organization of the United Nations and the World Health Organization. Moreover, until 2016, no standardized methods were available to detect Toxoplasma (oo)cysts in food.
To evaluate the risk of protozoan parasites in food, efforts must be made towards exposure assessment to estimate the contamination along the food chain, from raw products to consumers. We proposed to highlight reported methods to detect and estimate viability of these pathogens in vegetable and shellfish.

Funding source: Research program on Toxoplasmosis is funded by UMT Protorisk, National Reference Centre on Toxoplasmosis, University of Reims Champagne Ardenne and Reims Hospital.

Toxoplasma-FREE FOOD PRODUCTION IN BRAZIL, CURRENT SITUATION AND FUTURE PERSPECTIVES

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According to the World Health Organization, toxoplasmosis is amongst the leading foodborne parasitic diseases affecting >10.3 million persons worldwide. Changes in *Toxoplasma gondii* outbreaks profile has been observed in Brazil that are associated with new standard of healthy eating and the higher socioeconomic levels of people affected. Fruits and vegetables were the most described transmission vehicles in outbreaks. Brazil is a very fast-growing agricultural producer, with output expected to rise by more than 40% from now to 2026/27. The food and water sanitary control model in Brazil is shared among three entities from the public administration: The Ministry of Health, through both: The National Agency for Sanitary Supervision (ANVISA), the Surveillance Health Secretary (SVS) and the Ministry of Agriculture, Livestock and Supply (MAPA). Brazil outlined strategic sanitary measures and became a competitive country in the international meat market. Many challenges are posed for the production of toxoplasma-free food production in Brazil in the context of the agribusiness. However, we can be optimistic to reach sanitary goals even under the unfavourable current scenario, because the country has recent legislation for organic production and to inspect handcrafted products. In addition, and most important are the willingness to cooperation projects involving the public power sphere such as universities, research centres and the private sector to strengthen sanitary surveillance actions, certifications of good distribution and/or storage practices. For the effective implementation of public policies in this sector, the diversity of cultural and socio-economic realities in the country must be considered, in order to achieve a high standard of quality and competitiveness in agribusiness. Furthermore, there are still important challenges in relation to the quality of irrigation water, investment in basic sanitation, and the necessity for qualification of human resources to carry out the inspection and control activities.

ORAL PRESENTATIONS

ENVIRONMENTAL CONTAMINATION WITH *Toxoplasma gondii* OOCYSTS: A SYSTEMATIC REVIEW

Nadia María LÓPEZ UREÑA[1‡], Umer CHAUDHRY[2‡], Rafael CALERO BERNAL[1], Davide MESSINA[2], Francisco EVANGELISTA[2], Martha BETSON[2], Marco LALLE[3], Pikka JOKELAINEN[4], Luis Miguel ORTEGA MORA[1], Gema ÁLVAREZ GARCÍA[1]
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‡ Contributed equally

Background. *Toxoplasma gondii* is a major foodborne pathogen capable of infecting all warm-blooded animals, including humans. Although oocyst-associated toxoplasmosis outbreaks have been documented in the past few years, the relevance of the environmental transmission route remains poorly investigated. No systematic review on *T. gondii* contamination of all relevant environmental matrices, focused on detection rates and methods, but also on sampling strategies and their limitations, is available to date.
Objectives. To provide a comprehensive systematic review of the existing literature on *T. gondii* contamination of soil, water, fresh produce and bivalves, and identify knowledge gaps and limitations related to sampling strategies, recovery and detection methods.
Materials and Methods. A systematic review on the occurrence of *T. gondii* oocysts in soil, water, fresh produce and bivalves was carried out following PRISMA guidelines. Studies published up until the end of 2020 were searched in public databases (PubMed, Web of Science and Scopus) and screened. The bibliographies of the selected articles were examined to identify additional studies.
Results. A total of 103 out of 3,201 articles were selected. Among them, 13 articles focused on the analysis of two or more matrices, and 34 articles reported only on soil, 40 on water, 23 on fresh produce (vegetables/fruits) and 22 on bivalves. *Toxoplasma gondii* oocysts were detected in all types of matrices worldwide, with

overall detection rates ranging from 1% (7/700) to 78.1% (150/192) in soil, 5.4% (4/74) to 58.7% (27/46) in water, 0.26% (3/1,171) to 50% (13/26) in fresh produce, and 0.07% (1/1,396) to 46.3% (19/41) in bivalves using different PCR methods.

Conclusion. *Toxoplasma gondii* oocysts have been detected in environmental matrices across the world. The high heterogeneity in study designs might influence the detection rates. Therefore, sampling guidelines and standardisation of procedures could help to obtain more accurate and comparable results in future studies.

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MOLECULAR METHOD FOR DETECTION OF *Toxoplasma gondii* OOCYSTS IN LEAFY-GREEN VEGETABLES: METHOD SELECTION, VALIDATION AND SOP DEVELOPMENT

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Background. *Toxoplasma gondii* is a zoonotic pathogen with up to 60% of acquired infections associated to foodborne transmission. Consumption of unwashed raw fresh produce contaminated with the environmentally resistant *T. gondii* oocysts is one of the possible sources of infection. However, the relative importance of fresh produce consumption for human infections is unknown, partly due to lack of standardized detection method(s).

Objectives The aim of our work was to establish a standard operating procedure (SOP) for molecular detection of *T. gondii* contamination in leafy green salads.

Material and Methods. An extensive literature review and multi-attribute assessment of the molecular methods described and currently used to detect *T. gondii* oocysts was conducted. Based on the available literature, a comparative experimental work, using artificially spiked salad, was performed at two partner institutes for all the key analytical steps of the SOP. Effects of different equipment, DNA extraction kits, qPCR reagents and platforms were evaluated.

Results. A rapid and efficient DNA extraction method, relying on mechanical oocysts lysis combined with a specific yet highly sensitive qualitative multiplex qPCR assay, was selected showing an overall limit of detection of 10 *T. gondii* oocysts/30 g of salad. The SOP was drafted and, supported by video tutorials, was readily implemented at several TOXOSOURCES partner laboratories.

Conclusion. The method showed to be reliable in terms of robustness, reproducibility, repeatability, as well as availability and costs of reagents and equipment. Following validation by a ring trial, the SOP will be used for a multicenter pilot survey on ready-to-eat salads.

Funding source: This work was done as part of TOXOSOURCES project https://onehealthejp.eu/jrp-toxosources/, supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement № 773830: One Health European Joint Programme.

HOW TO IMPROVE RISK ASSESSMENT CAPACITY FOR FOODBORNE PROTOZOAN PARASITES IN THE EU: EVALUATION, VALIDATION, AND STANDARDIZATION OF A MOLECULAR METHOD FOR DETECTION OF *Cryptosporidium* spp. OOCYSTS IN READY-TO-EAT SALAD

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Background. *Cryptosporidium* is an important pathogen responsible of outbreaks associated with consumption of contaminated fresh produce. Lack of sensitive, validated, and standardized procedures hamper the detection of this parasite in food. The IMPACT project aims to increase the European-level capacity for risk assessment of foodborne protozoa on ready-to-eat (RTE) salad leaves, using *Cryptosporidium* as a model parasite.
Objectives. Evaluation, establishment, optimization and implementation of a standard operating procedure (SOP) for the molecular detection of *Cryptosporidium* spp. oocysts in salad leaves among consortium partners and validation of the SOP by a ring trial.
Material and Methods. Data from an extensive literature review, an online questionnaire and a qPCR protocol previously developed at CRU were combined to define the most suitable procedures for the key analytical steps. Comparative and iterative experiments were conducted at two partner institutes using baby spinach artificially contaminated with *C. parvum* oocysts. Variables, such as different equipment, DNA extraction kits, qPCR reagents and platforms were evaluated.
Results. An IMS-free oocyst recovery step was adapted from ISO 18744:2016. Bead-beating based DNA extraction methods followed by qPCR showed the highest performance (limit of detection of 10 *Cryptosporidium* oocysts/30 g of baby spinach). A draft SOP, supported by video tutorials, was implemented in consortium partner laboratories and confirmed the method performance in terms of sensitivity, robustness, reproducibility, and repeatability. Feedbacks obtained from implementing laboratories were considered for revision of the SOP.
Conclusion. The approach we undertook to define our SOP highlights the importance of validating a procedure by comparative work and capacity building. Following the final validation step by inter-laboratory ring trial, the present SOP has the potential to be become an ISO standard, useful not only for the detection of *Cryptosporidium* as a contaminant of fresh produce, but also for other (oo)cyst-forming protozoan parasites, such as *Toxoplasma*, *Cyclospora*, and *Giardia*.

Funding source: Partnering Grant Project Grant Agreement № GP/EFSA/ENCO/2018/03 – GA03 IMPACT: Standardising molecular detection methods to IMprove risk assessment capacity for foodborne protozoan Parasites, using *Cryptosporidium* in ready-to-eat salad as a model.

BEWARE, *Cryptosporidium parvum* OOCYSTS REMAIN INFECTIVE AFTER HOMEMADE COTTAGE CHEESE PRODUCTION

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Background: *Cryptosporidium* spp. are potential contaminants of food. Community-wide foodborne outbreaks of cryptosporidiosis are on the rise and have been reported as food choices, and eating habits have changed dramatically around the world. For numerous reasons, raw, unpasteurized milk consumption is a growing trend; raw milk and dairy products made from it can pose severe health risks. In November 2017, consumption of an organic, unpasteurized cottage cheese led to a cryptosporidiosis outbreak in France. Oocysts can contaminate foods due to poor hygiene conditions during transformation or preparation through food handlers, surfaces or equipment. Consequently, recommendations for manufacturers of dairy products are crucial considering the low infective dose that can lead to human cryptosporidiosis. This study was conducted to evaluate *C. parvum* infectivity recovered from experimentally infected yoghurt and cottage cheese (made from cow, sheep and goat's milk).
Methodology: Milk were seeded with 105 oocysts, and dairy products (yoghurt and cottage cheese) were prepared using a "yoghurt maker" device in which temperature and humidity were monitored during the assays (Testo). *Cryptosporidium* recovery was based on oocyst separation from the sample matrix using immunomagnetic separation with some modifications. The impact of milk processing on oocyst infectivity

was assessed using cell culture and real-time quantitative PCR (cc-qPCR). In a 24-well plate, wells with or without a monolayer of HCT-8 epithelial cells were inoculated with recovered oocysts.
Results and conclusion: The data analysis showed that the manufacturing temperatures used during the manufacture of (i) the yoghurt kill *Cryptosporidium* oocysts, and (ii) of the cottage cheese only reduces the infectivity suggesting that outbreaks linked to "homemade" yoghurt are likely due to post-manufacturing contamination while homemade cottage cheese can be due to contamination of the milk during the process. Manufacturers of dairy products must be subject to special hygiene considerations.

FOCUSING ON WATERBORNE AND FOODBORNE CRYPTOSPORIDIOSIS OUTBREAKS THAT HAVE OCCURRED IN FRANCE IN RECENT YEARS (2017-2020)

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Background. Currently, *Cryptosporidium* is well recognized as an important foodborne pathogen, ranked fifth out of 24 among foodborne parasites in terms of importance and a cause of many cryptosporidiosis outbreaks worldwide. In France, where very few outbreaks have been reported before 2017, data recently obtained by the Expert Laboratory of the Cryptosporidiosis National Reference Center (CNR-LE-Cryptosporidioses) have shown that outbreaks are in fact common and frequently underreported.
Objectives This presentation is aimed at reporting the characteristics of outbreaks that have been detected in France during the period 2017-2020, with emphasis on 3 major events.
Material and Methods. We propose to summarize investigations done by the CNR-LE for cryptosporidiosis regarding outbreaks occurred in France from 2017 to 2020.
Results. From 2017 to 2020, a total of 11 cryptosporidiosis outbreaks occurred in France. Three of them remain without any identified origin. Among the 8 remaining outbreaks: 6/8 were due to water contamination (5 from drinking water and 1 from recreational water), one was due to direct contact with infected calves and one was due to consumption of contaminated dairy products. Among these outbreaks, three of them exceeded the hundreds of cases. Drinking water network contamination with *Cryptosporidium* oocysts was implicated in two of them and, for the last one, consumption of a contaminated white cheese in a college refectory was incriminated.
Conclusion. Recent results obtained by the CNR LE-Cryptosporidioses have revealed the multiannual occurrence of *Cryptosporidium* outbreaks in France. Waterborne outbreaks have been more frequently detected, while foodborne outbreaks which are more difficult to spot were likely underreported. Present data call for improving the detection, characterization and notification of human cryptosporidiosis cases in France, especially in immunocompetent patients.

PARASITE TAXONOMY, SYSTEMATICS AND PHYLOGENY IN THE MOLECULAR ERA
Organizer / Moderator: Aneta Kostadinova
Co-moderators: Roman Kuchta & Isabel Blasco-Costa

INVITED LECTURES

INSIGHTS INTO THE SYSTEMATICS, EVOLUTION AND ECOLOGY OF ANISAKID NEMATODES IN THE GENOMIC ERA

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Anisakid nematodes are the major endoparasites of marine organisms across the globe. In addition to the zoonotic role exhibited by some species, knowledge on their systematics, population genetics, genomics and transcriptomics are also of interest from ecological and evolutionary perspectives. Over the years, a panel of molecular markers has been developed to create a multilocus sequencing and genotyping analysis of anisakids to investigate their molecular systematics, phylogeny, discover sibling species, investigate the existence of hybridization and/or introgression phenomena between closely related species, and estimate genetic variability and to infer their population genetic structure. In recent years, Next-Generation Sequencing (NGS) approach has facilitated the mining process of molecular markers, genotyping DNA microsatellite loci (SSRs) in species of the genus *Anisakis*. Based on the availability of partially whole-genome resource of *Anisakis* species, molecular tools based on single nucleotide polymorphisms (SNPs) have been also implemented. Thus, wider and robust tools were discovered for genotyping the three sibling species of *A. simplex* (s.l.) complex, in order to understand their hybridization events in sympatric areas, and their genetic structure in both definitive and intermediate/paratenic hosts from different water basins. The multi-genotyping approach provided not only useful diagnostic markers for these parasite species, but also resulted in the finding of sex-linked loci, improving the knowledge on their biology. Wide collection of population genetics data, elaborated by Bayesian methodologies, have thus dramatically raised the possibility to characterise anisakid species from different hosts and geographical areas. Additionally, knowledge on genetic/molecular bases that regulate mechanisms involved in the differential hosts' adaptation developed by anisakid species in the course of evolution, have been recently increased by transcriptomic approaches. Here, the major results recently achieved in the "omics era" in the genomics and transcriptomics of anisakids are reviewed, with their major impact of our understanding of biological, ecological and evolutionary aspects of these fascinating parasites.

EXPLORING PARASITIC FLATWORM PHYLOGEOGRAPHY WITH ddRAD DATA

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Elucidating the spatial genetic variation of species and teasing apart species complexes has always been a challenge to taxonomists. To date, commonly employed molecular markers have serious limitations. Genome-wide markers are suited to address such challenges at the right level of resolution, although have not yet been applied to Platyhelminthes. We present an example using *Proteocephalus longicollis* (Platyhelminthes, Cestoda) with the goal to examine its phylogeographic structure across populations of recent postglacial origin in the sub-Arctic and the Alps regions. We developed a customised protocol for double digest Restriction-site Associated DNA sequencing (ddRADseq) of genomic data of cestodes and trematodes. ddRAD reads were mapped on a newly obtained reference genome and Single Nucleotide Polymorphism calling was performed and used for population genetic analyses and investigating the spatial structure and influence of environmental factors to parasite differentiation. Over 700 specimens were analysed from two sub-Arctic

lakes and four lakes in the Alps. Genomic differentiation was highest between the sub-Arctic and the Alpine regions but divergence between the two sub-Arctic populations was almost as high. *P. longicollis* showed genetic structuring between lakes but not within lakes among coexisting host species. Thus, postglacial diversification of *P. longicollis* did not follow the pace of its definitive host, *Coregonus* species (Teleostei, Coregonidae), which have diverged into multiple sibling species inhabiting the lakes in the last 15K years. The contribution of different spatial and environmental factors will be discussed. *Proteocephalus longicollis,* and possibly other parasites with complex life-cycles, are subject to multiple constrains that may enhance as well as limit the spatial differentiation of their populations. By using fine-scale genomic approaches, we can now gain insights into the consequences of recent ecological events for the genetics of parasite species, which will help to anticipate future shifts in the distribution and differentiation of species under climate change.

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Spirometra TAPEWORMS IN EUROPE IN THE MOLECULAR ERA: A NEGLECTED HUMAN PARASITE OR A MATTER OF CONCERN?

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The assignment of species identity to the causative agents of sparganosis, a relatively neglected foodborne and waterborne disease, has been problematic or completely ignored by previous surveys. The current phylogenetic analysis indicates there are at least 6 well-defined molecularly well-defined lineages of *Spirometra* corresponding to separate species with a clear geographical pattern behind them. Two sympatric species have been found in Europe for the first time: (i) a more common *S. erinaceieuropaei*, probably restricted to Europe, but confirmed only in wildlife so far; and (ii) *S. mansoni*, a common causative agent of sparganosis in Asia and Oceania. Samples reported from Asia and Africa as *S. decipiens* (species endemic to the New World) and *S. ranarum* (invalid species) are in fact *S. mansoni*. Molecular data are currently essential for precise determination of a pathogen's identity, allowing an understanding of the spectrum of its natural hosts and, consequently, the epidemiology. While it is known that several species of *Spirometra* can infect humans, the importance of different species in the etiology and variable pathology of sparganosis remains completely unknown thanks to the lack of initiative and limited opportunities to carry out accurate pathogen identification using molecular data and phylogenetic tools. The etiology of tissue helminthoses, in general, would benefit if parasite tissues from clinical cases were to be routinely preserved in absolute ethanol and if collaboration between clinicians and taxonomists/parasitologists were to be increased. Improved general awareness of this zoonosis among clinicians, its presence in the natural environments in Europe, and recognition of the potential for transmission to humans will, in our opinion, help avoid future misdiagnoses, may enhance treatment, and would improve the estimation of the actual number of human cases.

Funding source: This work was supported by the Czech Science Foundation (project № 19-28399X); the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences (RVO: 60077344); and the National Science Center, Poland (project № 2016/21/B/NZ8/02429).

VARIABILITY IN THE ACANTHOCEPHALA

Omar M. AMIN
Institute of Parasitic Diseases

Unique and unusual features in the many species of acanthocephalans described and/or studied by Amin from fish, amphibians, reptiles, birds, and mammals, in various parts of the world including South America, Vietnam, Japan, the United States, the Middle East, and North and East Africa, are described. The presentation is in five parts. (1) An introductory section dealing with the classification, general morphology, ecology, and life cycles of the Acanthocephala. (2) Unusual anatomical features of taxonomic or of questionable taxonomic importance addressing variations in the proboscis, proboscis hooks, male and female reproductive organs, and lemnisci. Newly described structures including (a) Para-receptacle structure (PRS) and hoods in certain

species as well as a new order of Acanthocephala from Vietnamese birds, are also featured. (3) Structural and functional relationships explaining the relationship between the metamorphosis of the giant nuclei in Eoacanthocephala and worm reproductive cycle. (4) Host-parasite relationships elucidating the relationships between worm anatomy and biology during worm growth. (5) Curiosities in reviews and revisions highlighting taxonomically based zoo-geographical patterns and trends in the genera *Neoechinorhynchus*, *Polymorphus*, and *Pallisentis*. A comprehensive treatment of the acanthocephalans of South America and those marine forms off the Eastern United States is also included here.

ORAL PRESENTATIONS

CESTODES IN AFRICAN WILDLIFE: DARK TAXA AND CRYPTIC SPECIES

Sonja DUMENDIAK[1], Ali HALAJIAN[2], Ortwin ASCHENBORN[3], Yitagele TEREFE[4], Ute MACKENSTEDT[1], Thomas ROMIG[1], Marion WASSERMANN[1]

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Background. While cestodes with direct relevance to human and livestock health have received broad attention in parasitological research, helminths of wildlife are far less thoroughly investigated. This is especially the case for tropical regions (e.g. sub-Saharan Africa), and for cestodes morphologically resembling Dipylidiidae, Davaineidae or Mesocestoididae. In addition, description of species by morphological traits alone is likely to have led to cryptic species complexes in need of molecular resolution.

Material and Methods. A total of 332 cestode samples (adults, eggs and metacestodes) were opportunistically collected from 116 land mammals (18 species) in Ethiopia, Namibia and South Africa, e.g. from road-killed animals or in the context of other surveys. Samples were analysed using molecular (partial sequences of *cox1*, *nad1, cob* & *18S-rRNA*) and morphological methods.

Results. Preliminary results affirm that a high percentage of the analyzed cestode species, especially those with wild carnivore definitive hosts (Table 1), had not yet been recorded on a molecular level and could not be identified to species level.

Several of these species can be assigned to the genera *Taenia, Mesocestoides* and *Dipylidium*, while some could not even be matched to any of the genera deposited in GenBank. Phylogenetic analysis reveals a cryptic clade whose species might have been misidentified in the past due to morphological similarities to Dipylidiidae.

Conclusion. In collecting genetic information from various cestode specimens gathered from African wildlife hosts, a large amount of 'dark taxa' are being discovered, thereby revealing how little we know about the true diversity of cestodes even in terrestrial mammals.

Table 1: Presence of cestode species found in carnivores of Ethiopia, Namibia and South Africa and the ratio of species that could and could not be identified through matching *cox1* and/or *nad1* sequences deposited in GenBank.

Host species	# Hosts	# Identified cestode species	# Unidentified cestode species	% Unidentified
Acinonyx jubatus	5	0	5	100%
Canis mesomelas	5	2	0	0%
Caracal caracal	2	0	2	100%
Civettictis civetta	1	0	1	100%
Crocuta crocuta	8	1	5	83%
Felis catus familiaris	11	2	4	67%
Felis lybica	1	1	2	67%
Genetta genetta	2	1	2	67%
Genetta maculata	5	1	1	50%
Ichneumia albicauda	1	0	1	100%
Leptailurus serval	3	0	4	100%
Panthera leo	9	3	2	40%
Panthera pardus	3	3	1	25%

Funding source: We acknowledge partial financial support by Deutsche Forschungsgemeinschaft Project RO 753/3-1 and KE 282/9-1.

DIGENEA LIFE CYCLES IN THE SUBTIDAL COMMUNITIES OF THE WHITE SEA

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Background. Complex life cycle is the most fascinating feature of the Digenea; however, it challenges biodiversity research on these parasites. Even in well-studied regions like the Northern Atlantic and adjacent Arctic, for the majority of digenean species life cycle data either lack or are doubtful.

Objectives. In our research we focused on the Digenea in the subtidal communities of the White Sea which use fish and marine mammals as the definitive hosts. We aimed to revise their diversity and to reconstruct or verify their life cycles.

Material and Methods. To describe the digenean life cycle stages a complex of morphological methods was used, including histological mounts and sections, scanning electron microscopy and confocal microscopy. To ensure morphological species identification and to link life cycle stages we analysed sequences of 28S and 18S rDNA fragments, ITS1, 5.8S rDNA, ITS2 and mitochondrial ND3 and COI genes.

Results. Two digenean species were found in the White Sea for the first time, and one more species (*Derogenes* sp.) was new to science. The first intermediate hosts were discovered for eight digenean species from six families. Among them – the first reconstructed life cycle of the Brachycladiidae, parasites of marine mammals. For five more species life cycles were verified (including stages in the first and second intermediate hosts). The majority of the subtidal digeneans in the White Sea utilizes gastropods of the families Buccinidae and Naticidae as the first intermediate hosts.

Conclusion. The data obtained contribute both into the knowledge on biodiversity of the White Sea and to the discussion on life cycle evolution within studied digenean taxa.

Funding source: The reported study was funded by Russian Science Foundation project № 19-74-10029.

EXPLORING PARASITE DIVERSITY ACROSS THE AFRICAN CONTINENT: A NEW GENUS OF GYRODACTYLIDAE (MONOGENEA) FROM LAKE KARIBA, ZIMBABWE

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Background. The family Gyrodactylidae currently contains 24 valid viviparous only genera. The African continent has a rich diversity of fish and amphibians in its inland water systems that serve as hosts for seven gyrodactylid genera.

Material and Methods. In August, 2011, eight gyrodactylid parasites were collected from the gills of two specimens of bulldog, *Marcusenius macrolepidotus*, from Lake Kariba, Zimbabwe. Morphometric evaluation and 18S rDNA sequencing of the collected specimens was performed.

Results. Morphometric and molecular features confirmed that the specimens represented a new genus of this family. The attachment apparatus consists of a single pair of large slender hamuli with prominently flattened roots that are connected by a simple, thin dorsal bar. The ventral bar is small and possesses a thin lingulate membrane. There are sixteen marginal hooks of one morphological type, but of three different sizes, with large falculate sickles that are proportionately equal in length to that of their handles. The two largest pairs of marginal hooks are positioned closest to the opisthaptoral peduncle, while the neighbouring four pairs of medium-sized marginal hook sickles are situated along the lateral margins of the opisthaptor. The remaining two pairs, and smallest marginal hooks, are positioned along the posterior margin of the opisthaptor. The male copulatory organ consists of a muscular pouch armed with approximately 30 gracile spines. Phylogenetic analyses constructed on partial sequences of the 18S rDNA using Maximum Likelihood

and Bayesian Inference using a GTR+I+γ phylogenetic model placed the new genus within the lineage of solely African genera.

Conclusion. The study demonstrates that the African continent still hides a rich diversity of yet to be discovered parasites. Parasites from five genera formed a well-defined African clade, all of which possess a non-bulbous MCO, representing quite unusual diversity when compared to the bulbous MCO typical seen in the genera *Gyrodactylus* and *Macrogyrodactylus*.

Funding source: This work is based on the research supported in part by the National Research Foundation of South Africa (Grant № 101054).

MITOCHONDRIAL GENOMES OF NEGLECTED GROUPS OF PARASITIC NEMATODES: EVOLUTIONARY ANALYSES AND COMPARATIVE INFORMATIVITY

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Background. Permanently broadening knowledge of mitochondrial genomes is one of the powerful methods to infer the phylogenetic relationships between nematodes. Not all the groups of parasitic nematodes are studied with these methods, and the nematodes parasitic in invertebrate hosts are between such neglected groups.

Material and Methods. DNA from the frozen nematodes samples was isolated using the QiAmp Micro Kit (Qiagen) according to a standard protocol. DNA library preparation was implemented using the NEBNext Ultra II DNA Library Prep Kit for Illumina. Sequencing was performed on Illumina HiSeq 4000 system with a 150 bp read length. Obtained nucleotide data were analysed with several methods, including Maximum Likelihood and Bayesian Inference. The Gene Arrangement was also compared with published data for other rhabditid nematodes.

Results. The circular mitochondrial genomes of 6 parasitic nematodes were annotated. Mitogenomes of *Heth initiensis, Synoecnema hirsutum, Severianoia pachyiuli, Blatticola blattae, Dicelis lovatiana* and *Alloinema* sp. contains all 36 of the typical metazoan genes: 22 tRNA genes, 2 rRNA genes and 12 protein-encoding genes. Gene atp8 is lacking.

Conclusion. Mitochondrial data analysis is supporting a hypothesis of polyphyletic origin of the Drilonematoidea (parasites of earthworms), demonstrating the closer relationships of *Synoecnema* (Ungellidae) vs. *Dicelis* (Drilonematidae) with diferent genera of Acrobelidae. Thelastomatid nematodes of the genera *Blatticola* and *Severianoia* are resembling the oxyurids of vertebrates. Previously reported close relationships of *Alloionema* with Strongyloididae was confirmed in our study.

Funding source Work was supported by the Russian Science Foundation grant 19-74-20147.

COMBATTING ANTHELMINTIC RESISTANCE IN RUMINANTS (COMBAR) – COST ACTION CA16230
Organizers / Moderators: Laura Rinaldi & Smaragda Sotiraki

INVITED LECTURES

COST ACTION COMBAR: COMBATTING ANTHELMINTIC RESISTANCE IN RUMINANTS IN EUROPE

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Background. Anthelmintic resistance (AR) is a growing concern in the control of helminth infections in ruminant livestock worldwide.
Objectives. The COST Action COMBAR aims at coordinating research at the European level to advance knowledge in this area, and to develop and promote new solutions for parasite control.
Material and Methods. COMBAR is a network uniting 199 researchers from 34 countries with expertise in diagnostics, vaccine development, targeted selective treatment strategies, bioactive forages and other complementary control tools with the aim to integrate the various disciplines and propose new control options. Collaboration with agricultural economists helps to understand the financial impacts of helminth infections and AR, while the adoption of social sciences helps to understand and overcome the socio-psychological barriers to the uptake and maintenance of sustainable control approaches.
Results. Progressing discussions indicate the solution of AR will not be a single new drug but should be based on diagnosis before treatment approaches, understanding socio-economic drivers of treatment decisions and a broader panel of control options, including vaccines and nutraceuticals. This presentation will give an overview of COMBAR's research coordination, capacity building and dissemination activities.
Conclusion. Through joint collaboration, COMBAR has the ambition to pave the way towards a transnational, multi-actor initiative connecting researchers, farmers, veterinarians and industry to develop required solutions for AR and promote the uptake of sustainable approaches.

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ROADMAPS FOR THE COORDINATION OF GLOBAL RESEARCH INTO HELMINTH INFECTION CONTROL IN FARMED RUMINANTS

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Background. Helminth infections in ruminants are the cause of commonly witnessed and economically very important diseases around the world. With new challenges threatening sustainable parasite control such as climate change, changing farming practices and anthelmintic resistance, there is a need to coordinate global research to deliver sustainable control solutions.
Objectives. To develop research road maps that identify the current knowledge and research gaps that hamper the timely delivery of needed control solutions.
Material and Methods. The STAR-IDAZ International Research Consortium coordinates global animal health

research to accelerate delivery of disease control tools and strategies against infectious diseases and has constructed generic research roadmaps for the development of candidate vaccines, diagnostic tests, therapeutics, and control strategies for priority animal diseases. The Livestock Helminth Research Alliance (LiHRA) in collaboration with the COST Action COMBAR established different working groups to complete these road maps for gastrointestinal nematode and liver fluke infections in ruminants.
Results. Six different roadmaps were created, spanning different fields of helminth research from diagnostics to control strategies. The road maps are published at https://roadmaps-public.star-idaz.net/#/WjW9Q). Recent and ongoing research projects are being mapped over the roadmaps, to identify research gaps and underfunded areas.
Conclusion. The developed roadmaps inform funders of research in targeting new research projects towards the pressing needs to address current challenges in helminth control. In particular, they underpin the development of integrated helminth control strategies considering local epidemiology, anthelmintic resistance, climate change and farm management.

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COMBATING ANTHELMINTIC RESISTANCE IN RUMINANTS: A SERBIAN PERSPECTIVE

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Heavy reliance on anthelmintics to control gastrointestinal nematodes (GIN) of grazing ruminants, led to the emergence of anthelmintic resistance (AR), a well known global problem to sustainable animal production, health and welfare. Ruminant producers in Serbia are seldomly aware of the serious losses that GIN can cause. Although resistance of *Trichostrongylus* spp. to ivermectin (IVM) was detected earlier in sheep, they rarely know about the existence of AR. To address the AR issue in a new manner, several Serbian researchers attended COMBAR training schools (TSs) and short term scientific missions (STSMs) in order to acquire new skills for improved diagnostics and control of GIN, such as the application of the Mini FLOTAC technique and the conduct of faecal egg count reduction tests (FECRTs) for monitoring anthelmintic efficacy. Using Mini FLOTAC, a set of small scale surveys was performed, to monitor GIN in grazing cattle (50 animals from 5 herds) and assess anthelmintic efficacy in sheep (11 farms tested for IVM, 3 farms tested for levamisole (LEV)) and goats (one farm tested for IVM, eprinomectin (EPR) and albendazole (ALB)). Results showed low levels of GIN infection in cattle (average 13 eggs per gram (epg), range 5-95 epg). In the goat farm, resistance to EPR and IVM was detected (percentage of egg reductions= 83 and 92%, respectively), while ALB retained full efficacy. Regarding sheep, AR to IVM was established in 8 farms (73%), with egg reductions ranging from 55 to 92%, while LEV showed full efficacy against GIN. An STSM supported the evaluation of essential oils from Serbian native plants against GIN using *in vitro* studies and showed promising results. Overall, COST Action COMBAR is contributing to sustainable parasite control in Serbia through training researchers in new research practices.

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POTENTIAL USE OF AGROINDUSTRIAL BY-PRODUCTS CONTAINING TANNINS FOR THE INTEGRATED CONTROL OF GASTRO INTESTINAL NEMATODES IN RUMINANTS

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Parasitic infections with gastrointestinal nematodes (GINs) still represent worldwide a major pathological threat associated with the outdoor production of various livestock species, in particular ruminants. Because of the widespread resistance to synthetic chemical anthelmintics, there is nowadays a strong impetus to explore novel approaches for a more integrated management of these parasitic infections. The use of nutraceuticals in the control of GINs is one of the alternatives which has been widely studied for 20 years. These studies on nutraceuticals have mainly relied on models of tannin containing legumes (e sainfoin, *Lespedeza cuneata* and some tropical legumes). These previous results have led to the identification of condensed tannins (CT) and other related polyphenols (flavonoids) as main secondary metabolites explaining the AH effects. This basic knowledge on the main bioactive metabolites responsible for the AH properties of these Legumes has led to the identification and validation of other CT containing resources such as a wide range of agro industrial by products (BP) which were usually considered as industrial wastes. The diversity of CT rich BP resources, the pros and cons of their use for the integrated control of GINs and the current hudrles for on farm application will be presented.

MONITORING THE EFFICACY OF ANTHELMINTICS AND EARLY DETECTION OF DRUG RESISTANCE IN SHEEP FROM SOUTHERN ITALY

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Background. Anthelmintic resistance (AR) has become an urgent global issue in the control of gastrointestinal nematodes (GINs) of sheep worldwide (Rose Vineer et al., 2020 Parasite. 27:69).
Objectives. The aim of this study was to further investigate the current efficacy of benzimidazoles and macrocyclic lactones in sheep farms of southern Italy.
Material and Methods. From July 2020 to May 2021, 19 sheep farms located in southern Italy (Campania, Basilicata and Calabria regions) were selected to evaluate the efficacy of albendazole (ALB) and ivermectin (IVM) using the faecal egg count reduction test (FECRT) in accordance with the protocols established in the European COST Action "COMBatting Anthelmintic Resistance in Ruminants - COMBAR". For each tested drug, 20/40 sheep were sampled at D0 and sampled again 14 days (D14) after the anthelmintic treatment. The samples were analysed using the Mini-FLOTAC (Cringoli et al., 2017 Nat Protoc. 12(9):1723-1732). The efficacy was classified as 'reduced, 'suspected' and 'normal'. Coprocultures were performed at D0 and D14. From farms with FECR < 95%, an *in vitro* egg hatch test (EHT) was conducted.
Results. High efficacy (from 95.7% to 100%) was observed for ALB and IVM in 17 farms. The 'reduced' efficacy was observed only for ALB on Farm 1 (FECR = 75%) and 'suspected' efficacy on Farm 2 (FECR = 93.3%) with the predominant GIN genus *Trichostrongylus* and *Haemonchus* at D14. The EHT confirmed AR in both farms (Farm 1: 89%; Farm 2: 74%).
Conclusion. The continuous monitoring of AR in sheep farms in regions like southern Italy is essential, as anthelmintic efficacy is still high and the development of AR needs to be detected early to promptly respond with sustainable countermeasures of integrated parasite control.

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ORAL PRESENTATIONS

PRESENCE OF *Ostertagia ostertagi* AND *Fasciola hepatica* ANTIBODIES IN BULK TANK MILK FROM CATTLE HERDS IN GREECE

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Background. Milk yield in dairy cattle can be negatively affected by helminth infections acquired during pasture grazing.
Objectives The aim of this study was to estimate the exposure to *Ostertagia ostertagi* and *Fasciola hepatica* in dairy cattle herds across Greece, where access to grazing is limited, through measurement of antibody levels in the bulk tank milk (BTM).
Material and Methods. BTM samples (333 for *O. ostertagi* and 251 for *F. hepatica)* were collected from herds located in 22 different counties and tested using commercial antibody ELISA kits (SVANOVIR® *O.oster*tagi-Ab and *F.hepatica*-Ab). Moreover, performance and management data (farm size, nutrition forage type, access to pasture, anthelmintic treatments) were collected to determine possible relationships with anti-helminth antibody levels, expressed as optical density ratios (ODRs).
Results. In total 36.6% of the herds showed an *O. ostertagi* ODR > 0.5 indicating exposure to gastrointestinal nematodes. The ODR of these herds ranged from 0.50 to 1.44, suggestive of production losses on several of these herds. Herds with outside access and/or access to fresh hay showed significantly *O. ostertagi* ODRs than those without access. The analysis also showed significant correlations between *O. ostertagi* ODR and herd size (ODR decreased with increased herd size) but not with farm location. As regards *F. hepatica,* 1.6% of the herds tested positive with likely production losses (ODR > 0.6), having a ODR value ranging from 0.69-1.97. However, 13.2% of the herds had ODR values corresponding to contact with the parasite, ranging from 0.31 to 0.59. There was no correlation between the *F. hepatica* ODR and any of the measured management factors.
Conclusion. This study emphasizes that *O. ostertagi*-induced production losses should be considered on dairy farms in Greece even if the majority of which are housed indoors with limited outdoor access. Additionally, although *F. hepatica* prevalence is quite low, but it could be a re-emerging issue in specific habitats.

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MULTIPLEX REAL-TIME PCR ASSAYS FOR THE IDENTIFICATION AND SEMI-QUANTITATIVE ASSESSMENT OF STRONGYLE NEMATODES IN FAECES

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Background. The diagnosis of strongyle nematode (SN) infections in small ruminants is routinely based on morphological/morphometric analysis of parasite specimens recovered by coprological methods and followed by larval culture techniques. Such procedure is not only archaic but also demanding on time and labor, requiring a skilled expert. Nowadays, molecular methods are the cornerstone of reliable diagnostics for sustainable parasite control and accurate SN identification.
Material and Methods. Two multiplex real-time PCR assays for specific detection of five main and one invasive SN species, including an internal amplification control to avoid false negative results, were designed. The assays were optimized for analysis of DNA extracted from sheep faeces and verified for *Haemonchus contortus, Teladorsagia circumcincta, Trichostrongylus colubriformis, Nematodirus battus, Chabertia ovina*, and *Ashworthius sidemi*. Eggs per gram of faeces value was assigned to each of the detected species based on

a semi-quantitative evaluator which was established using a plasmid construct and a dilution series of sheep faeces with a known number of nematode eggs. The applicability of assays was tested on 44 individually collected faecal samples from three farms. Results were compared to those recorded using the Concentration McMaster technique and larval cultures.

Results. Assays showed great specificity to target species of SN and further clarified species identification obtained by larval culture. Also proved higher sensitivity in strongylid-type egg detection over faecal egg counts techniques by revealing three false negative samples, while showing moderate agreement in evaluation of infection intensity.

Conclusion. Multiplex assays proved to be rapid and accurate for analysis of the faecal samples with the focus on simultaneous and reliable species identification and semi-quantitative estimation of the number of eggs present. This approach increases diagnostic value and may add a high degree of precision to evaluation of anthelmintic efficacy, where it is important to identify species surviving after treatment.

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Organizers / Moderators: Santiago Mas-Coma & Bonnie Webster

INVITED LECTURES

GENETIC DIVERSITY OF *Schistosoma bovis*, *S. haematobium* AND THEIR HYBRID FORMS

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Background Schistosomiasis is a prevalent disease of humans, domestic livestock and wildlife caused by a variety of *Schistosoma* species. Two prominent species in Africa are *S. haematobium* and *S. bovis* causing urogenital human schistosomiasis and ruminant intestinal schistosomiasis respectively. Additionally, these two species are very closely related and are known to be able to hybridise and produce viable hybrid offspring, although pre- and post- zygotic barriers are at play to maintain species integrities in their natural settings. Despite this there is growing molecular data showing that large numbers of *S. haematobium-bovis* hybrids are excreted in the urine of many individuals in several sympatric African countries however, more advanced genetic interrogation is needed to determine the dynamics of this hybridisation and its consequences.
Material and Methods Here we used a *cox1* barcoding approach to investigate geographical or host structuring of *S. bovis* and *S. haematobium-bovis* hybrid populations using data available, published and unpublished, from samples from animals (cows, goats, sheep and rodents) and snails. Mitochondrial *cox*1 was aligned and analysed in MEGAX to inform relationship between samples and a haplotype network analysis was performed using PopART.
Results The phylogenetic analysis showed no structuring of the samples by hybrid or pure species status or geographically, suggesting that there has been no evolutionary processes occurring within these populations. However, the haplotype network analysis showed a strong clustering and a reduced genetic diversity of the *S. haematobium-bovis* hybrids compared to the *S. bovis* samples which also showed some low-level geographical structuring.
Conclusion Our analyses suggest that although *S. haematobium-bovis* readily exist there are strong selection pressures in place, within the hybridisation process with specific mitotypes observed in humans. This adds to our hypotheses that the hybridisation between *S. haematobium* and *S. bovis* is not random and prolific but more selective with species able to maintain their integrities despite some low-level hybridisation taking place.

USING CHOICE MODELLING TO IDENTIFY POPULAR AND AFFORDABLE ALTERNATIVE INTERVENTIONS FOR SCHISTOSOMIASIS IN UGANDA

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Background. Over 240 million people are infected with schistosomiasis, predominately in low- and middle-income countries. People become infected by direct contact with contaminated water, through activities such as bathing and fishing. Water becomes contaminated when human waste is not adequately contained. The main control strategy recommended by the World Health Organization (WHO) is praziquantel mass drug administration. However, coverage remains low in many areas and hotspots, where transmission is not reducing, remain. Additional interventions are needed to reach the ambitious WHO 2030 goals for schistosomiasis.

Objectives. We elicit community preferences towards alternative water access, sanitation and hygiene (WASH) interventions that would reduce individuals' risk of contracting, or transmitting, *Schistosoma mansoni* with the aim of identifying popular and affordable WASH-based interventions.
Materials and Methods. We administered a discrete choice experiment to understand community preferences for improved WASH interventions. We compared interventions that target behaviours that put oneself at risk versus behaviours that mainly put others at risk. We quantified what individuals are willing to give up in time and/or money.
Results. New sources of potable water and open defection fines were the highest valued interventions, closely followed by new latrines, five minutes' walk from their home. There was a strong negative preference for the status quo, indicating that whatever the interventions, people wanted to see something done. People were more willing to work to reduce risk to others, but willing to pay to reduce risk to themselves. However, a large portion of people ignored the payment vehicles, which is key for policy analysis.
Conclusions. People wanted to see a change to the current situation and were willing to work and/or pay for this. Infrastructure interventions that provide safe drinking water and latrines near homes were more popular, may therefore have higher uptake, and therefore be more sustainable, than other WASH-based interventions.

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IMPACT OF FASCIOLIASIS REINFECTION ON *Fasciola hepatica* EGG SHEDDING: RELATIONSHIP WITH THE IMMUNE-REGULATORY RESPONSE

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Background. In human fascioliasis hyperendemics, reinfection and chronicity are the norm. Control requires egg count techniques to assess the appropriate treatment dose for colic risk prevention. This study investigates how reinfection affects egg shedding and its relationship with the immune-regulatory response.
Material and Methods. The experimental design reproduced the usual reinfection/chronicity conditions in such areas and included *Fasciola hepatica* primo-infected Wistar rats (PI) and rats reinfected at 4 (R4), 8 (R8), and 12 weeks (R12), and negative control rats. In a longitudinal study (0-20 weeks post-infection, p.i.), serical IgG1 levels and eggs per gram of faeces (epg) were analyzed. In a cross-sectional study, the expression of the genes associated with Th1 (Ifng, Il12a, Il12b, Nos2), Th2 (Il4, Arg1), Treg (Foxp3, Il10, Tgfb, Ebi3), and Th17 (Il17) in the spleen and thymus was analyzed.
Results. In R8 and R12, transiently higher averages of epg and epg/worm in reinfected groups vs PI group were detected in the weeks following reinfection. Reinfected groups followed a IgG1 pattern similar to the PI group, but transiently higher averages were detected in post-reinfection weeks. Epg correlated with IgG1 levels, systemic Il10 and thymic Ifng, and Il10 expression levels.
Conclusion. Epg depends on the Th1 and Treg phenotype. Fluke burden by epg is likely to be an overestimation in cases of recent reinfection in low burden situations. Egg count techniques and the subsequent decision on the appropriate treatment dose for each patient to prevent colic risk is required.

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GENETICS AND GEOGRAPHY OF LYMNAEID VECTORS IN THE HIGHEST HUMAN FASCIOLIASIS HYPERENDEMIC: KEY POINTS WITHIN A ONE HEALTH CONTROL INITIATIVE

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Background. Fascioliasis is a snail-borne zoonotic trematodiasis emerging due to climate changes, anthropogenic environment modifications, and livestock movements. Highest prevalences and intensities were reported from four provinces of the northern Bolivian Altiplano, where preventive chemotherapy is ongoing. New strategies are now incorporated to decrease infection/re-infection risk, assessment of human infection sources to enable efficient prevention measures, and additionally a One Health initiative in a selected zone.
Material and Methods. 25 lymnaeid populations representative of the whole Altiplano, and 11 used for population dynamics studies, were analyzed by rDNA ITS2 and ITS1 and mtDNA cox1 and 16S sequencing.
Results. Lymnaeid populations proved to belong to a monomorphic group, *Galba truncatula*. Comparisons of transmission foci data from the 1990's with those of 2018 demonstrated an endemic area expansion. Altitudinal, northward and southward expansions suggest movements of livestock transporting *G. truncatula* snails, with increasing temperatures transforming previously unsuitable habitats into suitable transmission areas. Transmission foci appear to be stable when compared to past field observations, except for those modified by human activities.
Conclusion. For a One Health initiative, the control of only one *Fasciola* species and snail vector species simplifies efforts because of the lower transmission complexity. Vector monomorphism suggests uniformity of vector population responses after control measure implementation. Hyperendemic area outer boundary instability suggests a climate change impact. All populations outside previously known boundaries were close to villages, human dwellings and/or schools, and should therefore be considered during disease control planning.

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ORDERING RESERVOIR SPECIES PRIORITIES IN A ONE HEALTH CONTROL ACTION FOR HUMAN FASCIOLIASIS: A LARGE COMPLEXITY OF EXPERIMENTAL AND FIELD STUDIES

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Background. Preventive chemotherapy by means of mass treatments by yearly triclabendazole mono-dose campaigns is being implemented in human fascioliasis hyperendemic areas within the WHO control strategy. Surveillance interventions demonstrate that infections and re-ínfections of mainly children occur in between the yearly treatments. This is related to the high infection risk in such areas, which is due to the animal reservoir species assuring the *Fasciola hepatica* life cycle and consequent high transmission rates.
Material and Methods. A One Health strategy to complement preventive chemotherapy is now being followed to decrease the aforementioned human infection risk in the Bolivian Altiplano. One key axis concerns the experimental and field studies to assess the livestock reservoir species priorities to enable for the most efficient control activities according to the funds available for control, which in low-income countries are usually low for rural areas.
Results. The laboratory studies include the experimental follow-up of the development stages of egg and miracidium, lymnaeid snail vector infection, intramolluscan larval development, cercarial production, chronobiology of the cercarial shedding, vector survival to infection, and metacercarial infectivity of mammal host. Field surveys to assess prevalences, intensities and egg outputs further help in evaluating the potential role of the host species as a reservoir of the disease.

Conclusion. Significant results appear when comparing the different potential reservoir species. Sheep and cattle are the main contributors to transmission, followed by pig and donkey. South-American camelids are negligible, similarly as lagomorphs and rodents.

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ORAL PRESENTATIONS

NO PRE-ZYGOTIC ISOLATION MECHANISMS BETWEEN *Schistosoma haematobium* AND *Schistosoma bovis* PARASITES: FROM MATING INTERACTIONS TO DIFFERENTIAL GENE EXPRESSION

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Background: Species usually develop reproductive isolation mechanisms allowing them to avoid interbreeding. These preventive barriers can act before reproduction, "pre-zygotic barriers", or after reproduction, "post-zygotic barriers". Pre-zygotic barriers prevent unfavourable mating, while post-zygotic barriers determine the viability and selective success of the hybrid offspring. Hybridization in parasites and the underlying reproductive isolation mechanisms maintaining their genetic integrity have been overlooked.
Objectives: Using an integrated approach this work aims to quantify the relative importance of pre-zygotic barriers in *Schistosoma haematobium x S. bovis* crosses.
Material and Methods: *Schistosoma haematobium* and *S. bovis* are two co-endemic species cause schistosomiasis, one of the major debilitating parasitic diseases worldwide. They can hybridize naturally. Using mate choice experiments we first tested if a specific mate recognition system exists between both species. Second, using RNA-sequencing we analysed differential gene expression between homo- and hetero-specific pairing in male and female adult parasites.
Results: We show that homo- and hetero-specific pairing occurs randomly between these two species, and few genes in both sexes are affected by hetero-specific pairing.
Conclusion: We hence suggest that i) mate choice is not a reproductive isolating factor, and that ii) no pre-zygotic barrier except spatial isolation "by the final vertebrate host" seems to limit interbreeding between these two species.

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TRANSLATING FROM EGG- TO ANTIGEN-BASED INDICATORS FOR *Schistosoma mansoni* ELIMINATION TARGETS: A BAYESIAN LATENT CLASS ANALYSIS STUDY

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Background. Schistosomiasis is a parasitic disease infecting over 240 million people. World Health Organization (WHO) targets for elimination are based on faecal egg counts (FEC), without translation to the widely-used, urine-based, point-of-care circulating cathodic antigen diagnostic (POC-CCA). We aimed to standardise POC-CCA score interpretation and translate them to FEC-based standards, broadening diagnostic utility to inform progress towards elimination.
Objectives. To provide guidance on optimal interpretation of POC-CCA scores, generating analogous targets to those based on Kato-Katz egg counts.
Material and Methods. A Bayesian latent-class model was fit to data from 210 school-aged-children over four timepoints pre- to six-months-post-treatment. We used 1) FEC and the established POC-CCA scoring (Negative, Trace, +, ++ and +++), and 2) FEC and G-Scores (a new, alternative scoring method (G1 to G10)). We established the relationship between FECs and POC-CCA scores, and the score-associated probability of true infection. To establish antigen-based elimination targets, we conducted a simulation, parametrised with model estimates.
Results. True infection was associated with a POC-CCA score of ≥ + or ≥G3, however, POC-CCA scores cannot predict FECs because the POC-CCA cassette saturates at low FEC. Elimination targets can be identified by the distribution of POC-CCA scores in a population. ≤2% of a population with ++ and above, or ≤1% G7 and above indicates reaching the current FEC-based elimination targets.
Conclusion. The POC-CCAs lack resolution to discern between WHO FEC-based moderate- and high-intensity infection categories, limiting use in certain settings. Fluctuating POC-CCA sensitivity/specificity with treatment indicates a changing relationship between egg excretion and antigen levels (living worms). Population-level cut-offs for POC-CCA scores should be used for WHO elimination targets.

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CHANGES OF THE PEPTIDASE PROFILE DURING *Fasciola hepatica* EMBRYONATION EVIDENCED BY OMICS DATA

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Background. *Fasciola hepatica* is a widespread foodborne trematode that causes significant economic losses and severe liver conditions in humans. Its survival and reproduction are directly linked to the production of a wide repertoire of hydrolytic enzymes - peptidases, which have been thoroughly investigated in metacercariae, newly excysted juveniles and adults. In this respect, eggs of *F. hepatica*, have not yielded much scientific attention, even though the eggs of other trematodes play key roles in pathogenesis of the disease and immunomodulation of the host.
Objectives. We therefore aimed to expand our understanding of peptidases present in the embryonic stages of *F. hepatica*.
Material and Methods. We used a combined transcriptomic/proteomic approach which led to the identification of expressed and translated peptidases in developing eggs of different age. Additionally, the presence of different peptidase classes was verified by measuring the proteolytic activity in extracts from eggs.

Results. *F. hepatica* differentially regulates the expression and translation of peptidases throughout the embryogenesis. The proportion of cysteine peptidases diminishes during the egg maturation with progressively more metallopeptidase genes being transcribed as the egg matures. Number of cathepsins L and B isoforms were transcribed in developing eggs implying the role of this highly amplified group of peptidases in fasciolids.
Conclusion. Our integrated bioinformatic analysis can represent a data resource for further studies of *F. hepatica* miracidia formation as well as for comparative studies with other medically important trematodes, such as schistosomes.

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DNA MULTIMARKER CHARACTERIZATION OF *Fasciola gigantica* FROM ALGERIA

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Background. Fascioliasis is an emerging disease due to influences of climate and global changes. It is caused by two species of trematodes, *Fasciola hepatica* and *F. gigantica.* While *F. hepatica* prefers cool habitats of northern latitudes or altitude areas in tropical/subtropical regions and has an almost worldwide distribution, *F. gigantica* chooses warm habitats of lowland areas of only Africa and Asia. In Africa, *F. gigantica* is distributed throughout almost the whole continent except in the north-western Maghreb countries of Morocco, Algeria and Tunisia where only *F. hepatica* is present.
Material and Methods. Fasciolid flukes were collected from three Sidaouin sheeps in Timiaouine community, southern Algeria, a zone characterized by a hot desert climate typical of the hyper-arid Saharan zone. Molecular characterization of the fasciolid flukes was carried out using the sequences of the complete intergenic nuclear ribosomal DNA (rDNA) region, including the spacers ITS-2 and ITS-1 and the 5.8S gene, and the complete genes cox1 and *nad*1 of the mitochondrial DNA (mtDNA).
Results. Both rDNA and mtDNA multimarker sequences used for the molecular characterization and their comparisons with available sequences of the same markers in *F. hepatica*, *F. gigantica* and *Fasciola* sp. demonstrate that they belonged to the genetically 'pure' species *F. gigantica*.
Conclusion. This discovery represents not only the first finding of *F. gigantica* in Algeria, but also the first molecularly verified citation of this fasciolid species in the north-western Maghreb countries.

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LONG-TERM EFFECTS OF TEMPERATURE ON EMBRYONIC DEVELOPMENT AND HATCHING SUCCESS OF *Fasciola hepatica* MIRACIDIA

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Background. Fascioliasis is a helminthiasis caused by two liver fluke species of the trematode genera *Fasciola* of which *Fasciola hepatica* is the only one present in the Americas. The most common reservoirs of this parasite are cattle and sheep. This evidences the need for a One Health approach for its control, including long-term experimental studies.
Material and Methods. Faecal samples from naturally infected sheep and cattle from Bolivia and Mexico were used for the obtaining the *Fasciola hepatica* eggs. Bolivian eggs were embryonated at constant 20 °C and followed-up periodically through microscopic examination. Same process was executed with Mexican eggs after keeping them one year at 4 °C in darkness and with oxygenation.

Results. The established conditions allowed the embryogenesis of the eggs of *F. hepatica*, after one year preserved at 4 °C, following a gradual and normal kinetics until the complete development and exit of the miracidia**.** The egg hatching rate was lower after one year at 4 °C (0.3%), but light stimulation accelerated this process, up to a ratio of 4.6-10%. Fully developed miracidia were observed at days 24 and 10 in Bolivian and Mexican isolates, respectively.
Conclusion. The interruption of the embryogenesis of *Fasciola hepatica* eggs during one year at 4 ºC, shows that once the temperature of 20 °C is recovered, the embryogenesis process takes place, although the percentage of egg hatching is lower. Given the influence of climate features in the parasite cycle, climate change could modify the *Fasciola* endemic areas as well as its life cycle.

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FASCIOLIASIS IN PRESCHOOL AGE CHILDREN: UNEXPECTEDLY INVERSED GENDER RATIO REGARDING THAT IN SCHOOL CHILDREN AND ADULTS

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Background. Fascioliasis is a disease caused by *Fasciola hepatica* and *F. gigantica*. In human endemic areas, infection mainly concerns schoolchildren. Females appear more infected than males at all ages, whether in prevalences, intensities or both aspects. This gender distinction has been linked to a different male/female behaviour regarding infection sources, although a potential different hormonal physiology has also been tentatively evoked.
Material and Methods. A deep analysis has been made on this gender ratio aspect in preschool age children for the first time. This study has been performed at the occasion of the diagnosis of fascioliasis in five very small children (3 males and 2 females), including two with liver fluke infection having occurred from the very early age of only 5 months.
Results. The analysis comprised all cases on liver fluke infection in small children aged from only months up to 4 years, reported throughout the worldwide literature. The oldest report dates from 1856 in UK and the most recent from 2016 in Peru. A total of 38 past cases were found, including 3, 7, 12 and 16 cases of 1, 2, 3 and 4 years, respectively. They comprised 22 males, 8 females and 8 with unspecified gender.
Conclusion. The study showed an evident faster increase of infection in males than in females from 2 years onwards in children aged less than 5 years. This differentiation may perhaps be linked to a higher activity and more precocious curiosity of males in sucking or putting things into the mouth.

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THE IMPORTANCE OF SNAIL MOLECULAR XENOMONITORING FOR ACCURATELY IDENTIFYING SCHISTOSOMIASIS TRANSMISSION

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Snail xenomonitoring, detection of *Schistosoma* infected snail intermediate hosts, can inform on active transmission sites, risk of infection and also contamination of waterbodies with excreted eggs. However, levels of infected snails are usually low compared to the levels of individuals within a snail population. This is

particularly true when only emerging cercariae are used as a measure of infection. Moreover, as interventions to control human schistosomiasis increase the level of snails found infected decreases, sometimes to an undetectable level. This can be mitigated, to some level, by using molecular methods to detect the schistosomes within the snail, as they mature or even if they do not reach patency. Additionally, animal infected *Schistosoma* species are in high abundance and often account for the majority of snail infections within many endemic zones. This makes *Schistosoma* species identification a vital component to snail xenomonitoring programmes in relation to investigating human schistosomiasis transmission.

Here we will report on molecular snail xenomonitoring studies in Cote D'Ivoire, Niger, Tanzania, Zanzibar and Swaziland and the methods used to detect and identify the infecting *Schistosoma* species. The data shows that animal *Schistosoma* species are in abundance in many areas and that novel endemicities for two *Schistosoma* species, *S. bovis* (cattle species) and *S. kisumuensis* (rodent species) were identified in Zanzibar and Tanzania respectively. Additionally, molecular xenomonitoring supported a more detailed micro mapping of human schistosomiasis transmission and risk of infection. In conclusion molecular snail xenomonitoring is an important component of schistosomiasis transmission monitoring, whilst broadening our understanding of schistosome and snail epidemiology.

NITRIC OXIDE HINDERS THE INFECTION WITH THE NEUROPATHOGENIC SCHISTOSOME *Trichobilharzia regenti* IN MICE, PARTLY BY INHIBITING ITS VITAL PEPTIDASES

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Background. Avian schistosomes penetrate the skin of mammals and cause cercarial dermatitis. The immune system eliminates them soon by unknown mechanisms. Given that NO retards human schistosomes, it could also negatively affect the avian species.

Objectives. We examined the role of NO in the clearance of *Trichobilharzia regenti*, the neuropathogenic avian schistosome, in mice.

Material and Methods. *In vivo* methods involved diminishing NO production with aminoguanidine to assess the effect of NO on the course of infection, and immunohistochemistry and flow cytometry to examine the immune response and myelination. *In vitro* methods included schistosomula viability assays after treatment with NO donors, followed by electron microscopy. The NO effect on *T. regenti* recombinant peptidases was tested by fluorogenic assay.

Results. Cells producing inducible NO synthase (iNOS) surrounded the parasite 8 hours post infection in the skin and 3 days post infection (dpi) in the spinal cord. Production of NO did not lead to demyelination of the nervous tissue. Diminishing NO synthesis first stunted the parasite growth (3 dpi) but supported it later (7 dpi). Reduced NO production also increased the number of schistosomula isolated from the spinal cord. However, NO did not affect schistosomula viability or ultrastructure *in vitro*. Nevertheless, NO inhibited the activity of recombinant *T. regenti* cathepsins B1.1 and B2, the peptidases essential for parasite digestion and migration, respectively.

Conclusion. NO only stunts the growth and migration of *T. regenti* schistosomula, partly by suppressing the function of peptidases essential for their feeding and movement.

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HOST-PARASITE INTERACTIONS
Moderator: Alisa Gruden-Movsesijan

ORAL PRESENTATIONS

A NANOBODY-BASED *IN SITU* KNOCKDOWN APPROACH FOR FUNCTIONAL PROTEOMICS IN *Trypanosoma brucei*

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Background. African trypanosomiasis, caused by parasites of the genus *Trypanosoma*, is a neglected tropical disease affecting both humans and livestock. Although a substantial toolbox for research on these organisms already exists, essential genes are more difficult to investigate as genetic knockout and knockdown approaches result in lethal phenotypes. Camelid single-domain antibodies ('*Nanobodies*', Nbs) have proven to be useful tools for proteome-level studies, possessing a myriad of advantages compared to conventional antibodies, including a considerable smaller size (12-15 kDa) and the relatively easy integration with fluorescent proteins into so-called '*chromobodies*'.
Objectives. This work aimed to explore the use of chromobodies as *in situ* tools for functional proteomics studies in different subcellular compartments of *T. brucei brucei*, using glycolytic enzymes as proof-of-concept.
Material and Methods. The previously characterized Nb42 (an inhibitor of trypanosomal pyruvate kinase, PYK) was cloned in fusion with the mCherry fluorophore into the pLew100v5 vector and transfected into the *T. b. brucei* New York single marker (NYsm) strain for tetracycline-inducible cytoplasmic expression. As controls, PYK-specific (Nb42) and nonspecific Nb constructs (Nb-BCII10) were made to incorporate endoplasmic reticulum (ER) signal and/or retention tags. Transfections were validated by RT-qPCR, Western blotting, flow cytometry, and epifluorescence techniques. Several monoclonal lines with various levels of expression were selected by single cell sorting. Knockdown phenotypes were assessed by recording *in vitro* growth curves.
Results. *In situ* chromobody expression could be detected by the various techniques and could be targeted to specific subcellular localizations (cytoplasm versus ER). When targeted to the cytoplasm, the Nb42 chromobody caused a growth deficit correlating with the expression levels. No growth deficits were observed in the Nb-BCII10 and ER targeted constructs.
Conclusion. These results provide a proof-of-concept that *in situ* expression of chromobodies can be used as a novel targeted knockdown approach for functional proteomics studies in *T. b. brucei*.

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Fasciola hepatica JUVENILES: EXPLOITING THE FIBRINOLYTIC SYSTEM TO MIGRATE THROUGH HOST TISSUES

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Background. The fibrinolytic system of vertebrates is composed of plasminogen (PLG), the zymogen of the serine protease plasmin, an enzyme responsible for degrading fibrin clots. Owing to its broad range of substrates, plasmin activity can be exploited by different parasites for migration purposes. In the case

of fasciolosis, this mechanism could be of paramount importance during the early stages of infection by supporting the migration of *Fasciola hepatica* juvenile flukes, which appear in the small intestine and take a complex migratory route towards their definitive location, the intra-hepatic biliary ducts.
Objectives. The aim of this study was to investigate the interaction between the tegument antigens of *F. hepatica* newly excysted juvenile worms (FhNEJ) and the fibrinolytic system of their host.
Material and Methods. The FhNEJ tegument (FhNEJ-Teg) was extracted in vitro, and its capability to bind plasminogen and enhance plasmin generation were analysed by a combination of enzyme-linked immunosorbent, chromogenic and immunofluorescence assays. Two-dimensional electrophoresis combined with immunoblot and mass-spectrometry analysis were carried out in order to identify the potential plasminogen-binding proteins in FhNEJ-Teg, and the observed interactions were confirmed and validated by using FhNEJ recombinant proteins.
Results. FhNEJ bind human plasminogen at their tegument surface and enhance plasmin generation. Additionally, FhNEJ isoforms were identified in 33 protein spots as potential plasminogen receptors. These included canonical PLG receptors identified in other parasite species, namely enolase, annexin, glyceraldehyde-3-phosphate dehydrogenase and fructose-bisphosphate aldolase, as well as novel *F. hepatica* PLG-interacting proteins such as juvenile cathepsins L3, B2 and B3.
Conclusion. FhNEJ interact with the host fibrinolytic system, which represents a potential mechanism that the parasite has evolved to efficiently migrate through host tissues. Understanding host-parasite relationships at the early stages of fasciolosis could pave the way for the development of more effective treatment and control strategies against this global disease of humans and farm animals.

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EFFECT OF *Mesocestoides corti* AND *Taenia crassiceps* LARVAE ON MELANOMA TUMORS IN MICE

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Background. Several studies have shown that infection with helminths may affect the development of cancer. Some species like *Opisthorchis viverrini* or *Schistosoma haematobium* can promote the development or even be the causative agent of cancer. On the other hand, infections with other species, such as *Trichinella spiralis*, can reduce tumors and potentially have a protecting effect.
Objectives. Our work investigates the effect of infections by *Mesocestoides corti* and *Taenia crassiceps* in different strains of mice on the growth and metastasis of B16F10 melanoma tumors.
Material and Methods. *M. corti* and *T. crassiceps* larvae were used to infect BALB/c, C57BL/6J, or ICR mice which were then challenged with B16F10 melanoma cells administered intravenously, intraperitoneally, or subcutaneously. The effect of larval excretory-secretory products on the viability of melanoma cells *in vitro* was assessed via AlamarBlue assay. Flow cytometry was used to detect changes in peritoneal immune cell populations of *M. corti*-infected mice.
Results. Although an increase in metastatic activities was observed after intravenous administration of melanoma cells to *M. corti*-infected mice and no effect on subcutaneously localised tumors was noted, both tapeworms showed a strong suppressive effect on the size and number of tumors and metastases formed when the cells were administered intraperitoneally. In some cases, it led to the complete elimination of tumor cells. *In vitro* cultivation of B16F10 cells in the presence of larval excretory-secretory products led to a decrease in their viability. *M. corti*-infected mice showed a significant increase in peritoneal macrophages and granulocytes.
Conclusion. *M. corti* and *T. crassiceps* larvae are able to suppress the development and metastasis of B16F10 melanoma tumors in the peritoneum of mice. Although larval excretory-secretory products decreased the viability of melanoma cells *in vitro*, the observed effect in the murine peritoneum is most likely mediated by the the immune response modulated via tapeworm larvae.

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THE INTERACTION WITH THE HAEMOSTATIC SYSTEM OF THE HOST AS A POSSIBLE SURVIVAL MECHANISM FOR MIGRATING PARASITES: THE THIRD LARVAL STAGE OF *Ascaris suum* AS A MODEL

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Background. Migrating stages of parasites have been proposed as a selectively advantageous strategy facilitating their establishment in their host. The third larval stage of the nematode *Ascaris suum* (AsL3), the causal agent of porcine ascariasis, undergoes an extensive migratory route through the bloodstream of its host before establishing in its definite location to reach maturation. During this migration, AsL3 could interact with components of the haemostatic system, the mechanism responsible for maintaining blood fluidity within the vascular system in vertebrates. This system comprises two pathways, coagulation and fibrinolysis, which lead to the formation and degradation of blood clots, respectively. The coagulation cascade, in which the activated factor X (FXa) participates, culminates with the formation of fibrin, the protein that provides stability to the blood clot and is degraded by plasmin, the catalytically active enzyme of plasminogen, the key enzyme of the fibrinolytic system.
Objectives. The purpose of this study was to analyse the interaction between the cuticle and excretory/secretory antigenic extracts (AsL3C and AsL3ES) from AsL3 with the haemostatic system as a possible mechanism to facilitate the migration and survival of the parasite.
Material and Methods. Both the interaction with the coagulation cascade and the fibrinolytic system were analysed using different techniques based on ELISA and electrophoresis, chromogenic and anticoagulant assays and receptor-ligand proteomics and mass spectrometry.
Results. AsL3C and AsL3ES possessed anticoagulant potential since both antigenic extracts inhibited the coagulation cascade and FXa. In addition, AsL3C and AsL3ES bound plasminogen and enhanced plasmin generation, which revealed their pro-fibrinolytic potential. Three and 12 potential parasite proteins were identified as FXa inhibitors and plasminogen receptors, respectively.
Conclusion. These data suggest that AsL3 could control the formation of blood clots in its host, which could be used by the parasite as a mechanism to facilitate its migration and survival.

THE TEGUMENT OF *Fasciola hepatica* JUVENILES CONTAINS PROTEINS THAT INTERACT WITH LAMININ, A MAJOR COMPONENT OF THE INTESTINAL BASAL LAMINA

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Background. Fasciolosis caused by *Fasciola hepatica* is a major global infection of livestock and an emerging zoonotic disease that affects millions of people worldwide. The lack of an effective vaccine, coupled with an increase in the number of cases of resistance to the pharmacological treatment, highlight the need for a deeper understanding of host-parasite relationships at the early stages of infection. *F. hepatica* newly excysted juvenile worms (FhNEJ) appear in the duodenum and need to break through the gut wall to migrate towards their definitive location, the intra-hepatic biliary ducts. The first physical barrier that FhNEJ encounter is the intestinal epithelium, which is outlined by the basal lamina, a supporting structure rich in collagens and laminin. FhNEJ express proteases (mainly cathepsin L3) that can bind to and cleave collagen, but whether interaction with other basal lamina components also occurs remains to be elucidated.
Objectives. The aim of this study was to investigate the interaction between the tegument antigens of FhNEJ and laminin and fibronectin.
Material and Methods. The FhNEJ tegument (FhNEJ-Teg) was extracted *in vitro*, and its capability to bind to laminin and fibronectin was analysed by a combination of enzyme-linked immunosorbent and immunofluorescence assays. Two-dimensional electrophoresis combined with immunoblot and mass-

spectrometry analysis were carried out in order to identify the potential laminin-binding proteins of FhNEJ-Teg, and the observed interactions were confirmed and validated by using FhNEJ recombinant proteins.
Results. We discovered that FhNEJ bind laminin but not fibronectin at their tegument surface, and also identified 14 protein spots containing isoforms that potentially serve as laminin-binding proteins. Among these, cathepsin L3 stands out as the most prominent protease with laminin-binding properties identified in our study.
Conclusion. FhNEJ express proteins at their tegument surface with the ability to bind laminin, which could potentially serve as an anchoring mechanism before the onset of intra-organ migration.

Funding source: Project RTI2018-093463-J-I00 funded by MCIU, AEI and FEDER.

EXPLORATION OF THE PROTEOME OF HUMAN INTESTINAL EPITHELIAL CELL LINE (CACO-2) EXPOSED TO EXTRACELLULAR VESICLES OF *Anisakis simplex* L3 LARVAE – PARASITE-HOST INTERACTIONS OVERVIEW

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Background. *Anisakis simplex* is a parasitic nematode of marine organisms. Humans can be accidental hosts for this species. The finding that parasitic nematodes can release extracellular vesicles (EVs) was the breakthrough discovery. The secretion of EVs, as signal molecules, by parasitic nematodes has been poorly studied. This prompted us to characterize the proteome of host intestine cells exposed to EVs isolated from *A. simplex* larvae.
Material and Methods. The EVs of *A. simplex* L3 larvae were isolated by ultracentrifugation and added to the *in vitro* culture of the human intestinal epithelial cell line (CACO-2). Using TMT-based quantitative proteomics the proteome of the CACO-2 cells was characterized. Moreover, the concentrations of selected cytokines (IL-6, IL-8, IL-10) were determined.
Results. The results revealed in total 9,791 proteins, which were filtrated for further analysis. The final repository consists of 849 proteins, in a group of which, 144 were identified as differentially regulated. Furthermore, it was shown that CACO-2 cells responded to EVs with significant changes in cytokine secretion ($p<0.05$).
Conclusion. The obtained results will expand the existing knowledge about the role of EVs in the host-parasite communication.

Funding source: This work was funded by National Science Centre of Poland, grant № 2019/33/N/NZ6/01353. R. S. is also a recipient of a scholarship from the UE, grant № POWR.03.05.00-00-Z310/17. This work was also supported by the GAIN-Xunta de Galicia Project (IN607D2017/01) and the Spanish AEI/EU-FEDER PID2019-103845RB-C21 project.

MALARIA
Organizer / Moderator: Sanjeev Krishna

INVITED LECTURES

CHEMO-ATTENUATED LIVE *Plasmodium falciparum* IMMUNIZATION

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Potent protection against malaria can be induced by attenuated live-immunization with Plasmodium falciparum (Pf) sporozoites (SPZ). However, a better understanding of the critical processes involved in the establishment of protective immunity is needed. We explored the safety and vaccine efficacy of early chemo-attenuation of PfSPZ under atovaquone-proguanil (AP). AP caused early arrest of P. berghei liver stages. Despite the absence of replication, robust protection in mice correlated with parasite-specific effector-memory CD8+ T-cell responses. In a phase I clinical trial a single dose of AP prevented Pf infections in the liver of adult, human subjects who received three doses of 5.12×104 or 1.5×105 PfSPZ by direct venous inoculation combined with oral AP. However, only 2 of 8 (25%) and 2 of 10 (20%), respectively, were protected against controlled human malaria infection (CHMI) 10 weeks after the last vaccine dose, despite levels of IgG antibodies to the Pf circumsporozoite protein (PfCSP) comparable to those achieved in fully protected volunteers after immunization with 5.12×104 PfSPZ with chloroquine chemoprophylaxis active only against subsequent blood stages. We identify lower IgG recognition of the secreted liver stage-specific antigens LISP2 and LSA1 and the multi-stage antigen MSP5 as immune signatures of inferior vaccine efficacy compared to PfSPZ with chloroquine chemoprophylaxis. In conclusion, we show that immune signatures of liver stage antigens, but neither an established rodent malaria model nor concentrations of antibodies against the major surface protein of sporozoites, permit prediction of vaccine efficacy. Thus, this study provides a clear rationale for the development of live sporozoite vaccination protocols that boost exposure to Pf liver stage antigens.

WHEN THE TEST-LINE APPEARS: LATERAL FLOW TESTS FOR *Plasmodium vivax* LACTATE DEHYDROGENASE WITH INCREASED SENSITIVITY, LOWER COST AND GREATER AVAILABILITY

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Background. Lateral flow tests (LFTs) for *Plasmodium vivax* (Pv) infection are available but there is a need for more accurate, low-cost rapid diagnostic tests to guide mass drug administration (MDA) as part of malaria elimination strategies. Such tests are needed for targeted treatment, including guided vaccination. Plasmodium vivax (Pv) is widespread in Asia, Latin America, the Horn of Africa and Madagasca. Globally, around 2.5bn people are at risk of infection with Pv. The Mologic Centre for Advanced Rapid Diagnostics (CARD) investigated factors that limit the sensitivity of specific lateral flow tests for Pv lactate dehydrogenase (LDH) released from infected erythrocytes.
Objectives. To develop RDTs with increased sensitivity, lowest possible cost and wider mass availability, beyond those of tests already available.
Material and Methods. The study was carried out with a range of antibodies (including engineered recombinant antibodies) capable of specific Pv LDH detection in LFT systems. Each antibody was characterised in terms of binding kinetics and epitope specificity. Pv LDH is not strongly immunogenic in most species used for making antibodies. The epitopes available for differentiating Pv from other Plasmodium species are immunologically "subtle". More-sensitive indicator particles were evaluated and the potentials of higher-speed manufacturing processes evaluated.
Results. Antibody affinity was a key driver of sensitivity and the association rate (ka) the most important property. Engineered antibodies with more than one paratope per molecule (e.g. bi-valent, bi-specific etc.)

were the most effective. Performance also depended on the molecular structure of the multimerization domains. Various indicator particles were able to deliver improved sensitivity, and emerging manufacturing processes provided options for lower costs and wider accessibility.
Conclusion. Advances in antibody technology can provide improvements in LFT performance. Other developments can enhance sensitivity, enabling a new generation of Pv RDTs better availability, cost and performance.

Funding source: Bill and Melinda Gates Foundation

UNDERSTANDING CLIMATE AND ENVIRONMENTAL DRIVERS OF RURAL HOSPITAL ADMISSIONS FOR DIARRHOEAL DISEASE, MALARIA, PNEUMONIA, AND ASTHMA IN SOUTH AFRICA

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Background. Climate and environmental variables impact human health. In an era of climate change, there is a pressing need to understand relationships between climate and health, to best inform how such impacts are likely to change.
Objectives. We investigated time series of daily admissions from two public hospitals in Limpopo Province in South Africa, in relation to time series of meteorological and air quality ground records or satellite data from the same geographical area.
Material and Methods. We used wavelet transform cross-correlation analysis to monitor coincidences in changes of meteorological (temperature and rainfall) and air quality (concentrations of $PM_{2.5}$ and NO_2) variables with admissions to hospitals for gastrointestinal illnesses including diarrhoea, pneumonia-related diagnosis, malaria and asthma cases. We were interested to disentangle which changes in meteorological or environmental variables might be associated with underlying temporal variations of disease prevalence.
Results. We found preconditioning of prevalence of pneumonia by changes in air quality and showed that malaria in South Africa is a multivariate event, initiated by co-occurrence of heat and rainfall. We provided new statistical estimates of time delays between the change of weather or air pollution and increase of hospital admissions for pneumonia and malaria that are addition to already known seasonal variations. We found that increase of prevalence of pneumonia follows changes in air quality after a time period of 10 to 15 days, while the increase of incidence of malaria follows the co-occurrence of high temperature and rainfall after a 30-day interval.
Conclusion. Our findings have relevance for early warning system development and climate change adaptation planning to protect human health and well-being.

Funding source: SAMRC, South Africa; SATREPS (Science and Technology Research Partnership for Sustainable Development) Program of JICA (JAPAN International Cooperation Agency)/AMED (Japan Agency for Medical Research and Development), Japan; ACCESS (Alliance for Collaboration on Climate and Earth Systems Science) program of NRF (National Research Foundation) and DST (Department of Science and Technology), South Africa; Serbian Science Fund, Serbia.

DRUGS FOR THE TREATMENT OF MALARIA: WHAT IS IN THE CLINICAL DEVELOPMENT PIPELINE?

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The emergence and spread of *Plasmodium falciparum* strains not responsive to current artemisinin combination therapies in the Greater Mekong Region poses a threat for the successful control of malaria. Whereas the preclinical and early clinical development pipeline for antimalarials is richer than ever before, it is unclear how the imminent lack of new antimalarial drugs may be handled in the short run. Here we discuss novel therapeutic strategies and concepts for antimalarial chemotherapy.

ORAL PRESENTATIONS

AVIAN MALARIA: TROPICAL DEFORESTATION AND HOST SPECIFICITY

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The effects of environmental changes on parasite distributions are varied and despite potential consequences to ecosystem health, large-scale studies involving wildlife have been scarce. Here we present data of the effects of rapid deforestation on the prevalence and diversity of mosquitoes and avian blood parasites. In Cameroon, we have initiated a long-term study of mosquitoes, birds and avian malaria to determine how deforestation for the cultivation of palm oil plantations affects parasite transmission. For three years, we have collected samples from the same sites, pre- and post-deforestation. Sampling was done four times/year to account for seasonality. By analyzing over 2600 avian blood samples, and about 12000 mosquitoes, we find that habitat degradation leads to altered patterns of parasite prevalence and disruptions in parasite species dominance. The diversity of parasites, birds and mosquitoes changes significantly with deforestation. We also present data on how habitat may affect the evolution of lineage diversity and specialist vs. generalist strategies in avian malaria. Our work incorporates bioclimatic data to quantify differences among collection sites, and predict how microhabitat changes may affect the spread of infections. We have also initiated studies on genes involved in host pathogenicity, with the characterization of the transcriptomes of three parasites; *Plasmodium delichoni*, *P. homocircumflexum* and *Haemoproteus columbae*. We report orthologs of genes known for erythrocyte invasion, and host-specificity, including *msp-1*, *maebl*, *ama-1* and *ron-2*. With our long-term agenda to discern the interplay between habitat, vector ecology, and genetics on the host-specificity of parasites, we emphasize that influences of land use changes on parasite prevalence are complex, and will require the detailed study of the vector ecology, and habitat effects. Through time, our multidisciplinary approach will aid in predicting how habitat changes will influence future scenarios of host-parasite interactions.

EVALUATION OF A NOVEL REAL-TIME PCR ASSAY FOR THE DETECTION, IDENTIFICATION AND QUANTIFICATION OF *Plasmodium* SPECIES CAUSING MALARIA IN HUMANS

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Background. The entry of PCR-based techniques into malaria diagnostics has improved the sensitivity and specificity of the detection of *Plasmodium* infections. It has been shown that humans are regularly infected by at least six different *Plasmodium* species (*P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale wallikeri*, *P. ovale curtisi*, *P. knowlesi*), and that infections with *P. cynomolgi* might also be more common than previously suspected.

The MC004 real-time PCR assay for malaria diagnosis (MRC Holland) is a novel single-tube assay that has been developed for the purpose of simultaneously detection and discrimination of all *Plasmodium* species known to infect humans. Detection and identification of *Plasmodium* species relies on molecular beacon probe-based melting curve analysis. In addition, this assay might be used to quantify the parasitaemia of at least *P. falciparum* by calculating the level of parasitaemia directly from the Cq-value.

Material and Methods. The samples used in this study comprised reference samples, patient samples, and synthetic controls. The following analytical performance characteristics of the MC004 assay were determined: analytical specificity, limit of detection, the ability to detect mixed infections, and the potential to determine the level of parasitaemia of *P. falciparum*, including assessment of within-run and between-run precision.

Results. No false positive or false negative results were observed. The limit of detection of *P. falciparum* was 1 x 10^{-3} IU/mL (WHO standard). Mixed infections with *P. falciparum* and non-*falciparum* species were correctly identified. A calibration curve could be established to quantify the parasitaemia of at least *P. falciparum*. The within-run and between-run precision were less than 20% CV at the tested parasitaemia levels of 0.09%, 0.16%, 2.15% and 27.27%.

Conclusion. Based upon the analytical performance characteristics that were determined, the MC004 assay showed performance suitable for use in clinical settings, as well as epidemiological studies.

TRAVEL TO CAMEROON, FEVER, THROMBOCYTOPENIA AND MALARIA POSITIVE TEST: HOW MUCH WOULD YOU BET ON THE DIAGNOSIS?

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Background. We report the case of a 54-year-old female patient who was recently admitted to the hospital with fever, chills and sweats after a trip to Cameroon as a flight attendant.

The Case. In May 2021, she was hospitalized in a private clinic for history of chronic hyperthermia. She described no other symptoms and clinical exam was normal. A biological investigation showed the following results: CRP 90.6 mg/L; leukocytes 1 310 /mm^3; haemoglobin 9.6 g/dL; platelets 106 000/mm^3. Thick and thin blood smears along with Lamp PCR were negative for malaria but the malaria rapid diagnostic test (RDT-PALUTOP®+4 Optima) came out positive (Pan line only). She first received hydroxychloroquine and artemether/ lumefantrine and was referred to the University hospital of Nice because there was no improvement of her clinical condition. Although the RDT turned out positive again, Malaria's diagnosis was definitively ruled out based on both negative smears, and specific PCR.

The solution. After complete assessment, myelogram showed amastigotes of *Leishmania sp.* while *Leishmania* serology and blood PCR were positive. Molecular identification confirmed the diagnosis of *L. infantum* visceral leishmaniasis. The key to explain the false-positive malaria RDT was actually a cross-reactivity with the appearance of a rheumatoid factor (RF=16 IU/mL), probably due to the long evolution of the visceral leishmaniasis. One month after treatment, the patient was fine and RF and malaria RDT were negative again. Tests conducted since with patients without RF (negative malaria RDT) or with RF (positive malaria RDT) supported this hypothesis.

Conclusion. The cross-reactivity between RF and malaria RDTs have been well described in the literature but it is still a rare situation in our daily practice. This case is a helpful reminder for all not to rush into a malaria diagnosis with a positive RDT, under penalty of missing another important diagnosis.

AMINOQUINOLINES AFFORD RESISTANCE TO CEREBRAL MALARIA IN SUSCEPTIBLE MICE

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Background. Malaria is a parasitic infection that affects millions of people worldwide. *Plasmodium* parasites are developing resistance to nearly all conventional antimalarials, and *Anopheles* vectors are becoming resistant to insecticides, with no vaccine existing to date. Additionally, climate changes and migrations may favor further spread and the re-emergence of malaria even in areas considered free of the disease.

Material and Methods. Here we explored the activity of ten novel benzothiophene, thiophene and benzene aminoquinolines. *In vitro* testing was performed by the lactate dehydrogenase assay in chloroquine (CQ)-sensitive *Plasmodium falciparum* strain 3D7 and CQ-resistant (CQ^R) *P. falciparum* strain Dd2. *In vivo* activity was evaluated by a modified Thompson test using C57BL/6 mice infected with *Plasmodium berghei* ANKA strain.

Results. Nine of the ten compounds had a lower 50% inhibitory concentration (IC_{50}) than CQ against the CQ^R strain Dd2. Five of these compounds that were available for *in vivo* evaluation were shown to be nontoxic. All five compounds administered at a dose of 160 mg/kg/day for 3 days prolonged the survival of treated compared with untreated mice. Untreated control mice died by Day 7 with a mean parasitaemia of 15%. Among treated mice, a dichotomous outcome was observed, with a two-third majority of treated mice dying by Day 17 with a low mean parasitaemia of 5%, whereas one-third survived longer with a mean hyperparasitaemia of 70%; specifically, five of these mice survived a mean of 25 days, whilst two even survived past Day 31.

Conclusion. The significant antimalarial potential of this aminoquinoline series is illustrated by its excellent *in vitro* activity against the CQ^R *P. falciparum* strain and significant *in vivo* activity. Most importantly, compounds ClAQ7, ClAQ9 and ClAQ11 were able to confer resistance to cerebral malaria and afford a switch to hyperparasitaemia to mice prone to the neurological syndrome.

Funding source: This work was supported by grants №s III 41019 and ON172008 from the Serbian Ministry of Education, Science and Technological Development.

WILDLIFE PARASITOLOGY
Organizer / Moderator: Pikka Jokelainen

INVITED LECTURES

WILDLIFE AS SENTINELS AND INDICATORS FOR TRANSMISSION OF *Toxoplasma gondii*

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Toxoplasma gondii is a true One Health parasite: it can infect humans and numerous animal species, including wildlife, and it has an environmental stage that plays a major role in the epidemiology of toxoplasmosis worldwide. Wildlife populations have important roles in the overall transmission of *T. gondii.* Infected wildlife can be sources of *T. gondii* infections to other hosts, including humans consuming undercooked meat from hunted animals. Some wildlife species are particularly susceptible to severe toxoplasmosis, which can be an important cause of mortality. Wildlife can also serve as sentinels and indicators for presence of local *T. gondii* transmission and reveal patterns in environmental contamination with *T. gondii* oocysts. For example, samples from free-ranging animals that are hunted for human consumption have been investigated in epidemiological studies in northern Europe, revealing north-to-south increasing gradient in *T. gondii* seroprevalence in Fennoscandia. Genetic characterization of *T. gondii* has further highlighted the links for parasite transmission between land and sea environments. We will highlight key studies and ongoing investigations that demonstrate how oocysts shed in the feces of infected terrestrial felids can be a source of *T. gondii* infections for sea otters in coastal California.

Funding source: PJ is supported by TOXOSOURCES project, which is supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement № 773830: One Health European Joint Programme.

WILDLIFE AND REPTILES AS SENTINELS FOR ZOONOTIC VECTOR-BORNE DISEASES

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The Anthropocene has been an era where the world has experienced radical climate change and environmental modifications. These environmental factors, as well as the illegal wildlife trade have thinned the boundaries between wildlife and humans. This growing interaction has been, in certain way, monitored using animals as sentinels for zoonotic risk and overall human health that has gained worth under the concept of "One-Health". This integrative approach of human, animal and environmental health has demonstrated to be a powerful tool to mitigate and respond to pandemic threats in a globalized context. Wild and synanthropic animals represent important link between human and environmental health and the sentinel events given by anthropogenic pressures (i.e., deforestation, urbanization). Indeed, the COVID-19 pandemic is a paradigmatic example of health threats given by the increasing interactions of wild animals, emerging zoonoses and humans. To illustrate the usefulness of wild animals as sentinels, reptiles are discussed in different contexts, and types of interaction. These interactions are given by wild reptiles *sensu stricto* in environments in which they interact with human populations (i.e., parks, zoos, reserves, food source); synanthropic reptiles that were forested species and due to a myriad of causes (e.g., habitat loss) adapted to urban and peri-urban environments; invasive species of reptiles that bring to new geographical areas their vectors; and the international legal and illegal wildlife and pet trade that aids spread the distribution of vectors where they can thrive. Thus, monitoring reptile population health status can provide insights on the risks for human infection by bacteria (i.e., *Borrelia*, *Rickettsia*), protozoa (i.e., *Leishmania, Trypanosoma*) and viruses (i.e., Arboviruses, Crimean-Congo haemorrhagic fever). Furthermore, reptiles used as sentinels can provide information on the expansion and threat to autochthonous or allochthonous arthropod vectors (i.e., Acarina, Diptera). Thus, monitoring reptiles in different epidemiological contexts can be of great use for public health initiatives and policies.

AVIAN MALARIA PARASITES: ANNUAL VISITORS AND POTENTIAL THREATS TO WILD BIRDS

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Malaria parasites (genus *Plasmodium*) are widespread throughout the world and are highly diverse. More than 1,300 avian malarial parasite haplotypes that cause diseases of varying severity are currently identified. Most birds migrate to the southern regions annually and can get infected with malaria parasites in wintering areas. Birds infected with tropical malarial parasites serve as reservoirs at European grounds. As long as there are no suitable vectors in Europe, these parasites will not spread. However, things can change due to global warming and changes of environments, which could be suitable for invasive, new blood-sucking insect species. Despite extensive research on avian malaria pathogens, knowledge about haemosporidian vectors or virulence for the vertebrate host is very limited. More detailed studies of malarial parasites are necessary to understand the epizootiology of these pathogens, their potential to spread, and their effects on different avian populations.
During the presentation, we will provide information obtained from some experimental studies, showing that parasites transmitted in the tropics can have very different effects on birds that do not migrate before winter to the tropics. Depending on the species of *Plasmodium* parasite, very light infections may develop which often do not cause avian health problems. However, other malarial pathogens may develop high parasitemias that have an adverse effect on the health of the bird (weight loss, low hematocrit, hemoglobin values). In some cases, the disease can be lethal. It should also be emphasized that not only high levels of parasitemia cause death. This can occur even in low parasitemias, where malarial parasites adversely affect bird health through other mechanisms, disrupting blood cell production or causing cerebral paralysis.
New information on parasite biology and parasite-host relationships is particularly important for determining the virulence of *Plasmodium* parasites, for better assessment of malaria spread, and for epizootiological studies of vector-borne infections.

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ORAL PRESENTATIONS

GREY WOLVES AS SENTINELS FOR THE PRESENCE OF *Echinococcus* spp. AND OTHER GASTROINTESTINAL PARASITES IN FRANCE

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Background. Since the return of gray wolves to France through the Alps, the population is carefully monitored but very scarce information are available concerning their helminthofauna despite parasite species of public health importance as *Echinococcus multilocularis* and *Echinococcus granulosus sensu stricto* (ss).
Material and Methods. A large collect of fecal sample (911) and some intestines (n=15) of wolves was analyzed to evaluate the presence of *Echinococcus* species in the French wolf population but also to obtain a large overview of its helminthofauna by comparison of the one from dogs (n=57) and red foxes (n=79) fecal samples collected from the same areas. The diagnosis from feces was realized using a copro-PCR approach amplifying a large spectrum of parasite, when the intestines were analyzed by SCT.
Results. Similar occurrences of parasites species compared to other wolf European populations were obtained from fecal samples: *Taenia hydatigena* (7.2%), *T. krabbei* (2.4%), *E. granulosus ss* (2.4%). *Uncinaria*

stenocephala (2.4%), *E. multilocularis* (0.3%), *T. multiceps* (0.1%), *Toxascaris leonina* (0.1%) and *Baylisascaris procyonis* (0.1%). The three most abundant species were also found in intestines and most of the species were shared with dogs and/or red foxes. The detection of *B. procyonis* in wolf and *Mesocestoides canislagopodis* in red fox were unexpected.

Conclusion. Infection by *E. granulosus ss* are in accordance to the main localization of wolf pack in the transhumance area of sheep corresponding to the main focus of the parasite species in France without a significant role of wolf in the lifecycle. The detection of *E. multilocularis* at the Italian border extends the southern border of the known French endemic area. Further analyses concerning wolves will be required to specifically investigate the different topics of interests notably using molecular tools as NGS method to obtain a better association between diet and parasitism.

MOLECULAR CHARACTERIZATION AND PREVALENCE OF INTESTINAL PARASITES INFECTING NON-HUMAN PRIMATES IN COLOMBIA

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Background. 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 & Pérez-Ponce de León, 2018). Ecosystem transformation increases contact between humans and NHP (Trejo-Macías et al., 2007), leading to potential zoonotic parasite transmission.

Objective. This study aimed to assess the prevalence of intestinal parasites in free-ranging NHP living in five forest fragments in Colombia. A further aim was to molecularly characterize selected species of zoonotic interest.

Material and Methods. Fecal samples were collected from NHP immediately after defecation, and stored in 10% formalin solution and 96% ethanol. Faecal smears and flotation were performed (Botero & Restrepo, 2012). Samples microscopically classified as positive for *Blastocystis* sp. and Ascarididae were processed for molecular characterization (Mattiucci et al., 2016; Cavallero et al., 2013).

Results. 160 fecal samples were collected from primates *Alouatta seniculus* (n=46), *Ateles hybridus* (n=13), *Aotus griseimembra* (n=5), *Cebus versicolor* (n=20), *Saimiri cassiquiarensis* (n=73), and *Sapajus apella* (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, *Strongyloides* sp., *Trypanoxyuris* sp., Ancylostomatidae), and acanthocephalans were observed. *Ascaris lumbricoides* and *Blastocystis hominis* (ST8) were identified at species and subtype level, respectively, through molecular techniques.

Conclusion. The finding of parasites with zoonotic potential suggests epidemiological implications in NHP conservation and human health, particularly in wild-urban interface and in highly transformed ecosystems.

Toxoplasma gondii SEROPREVALENCE IN EUROPEAN WILDLIFE: A SYSTEMATIC REVIEW

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Background. *Toxoplasma gondii* is an important zoonotic parasite with major impact on both animal and human health. Amongst the various transmission routes, the meat of infected animals, including wild animals, appears to be a major source of infection in Europe.
Material and Methods. As a part of an extensive systematic literature review, we aimed to estimate *T. gondii* seroprevalence in a selection of commonly consumed European wildlife species. The systematic review was conducted and will be reported according to PRISMA guidelines. Study selection was performed independently by 20 scientists from 13 countries across Europe, using an online tool. We included peer-reviewed articles published in English since 2000, based on specified criteria including a list of host species.
Results. A total of 64 publications met the inclusion criteria for data extraction. These studies provide data on wild birds, wild ruminants and wild boars with proportions of seropositive animals of 4.2 % (112/2638), 18.8 % (2886/15390) and 28.9 % (4290/14833), respectively.
Conclusion. The data provide a comprehensive overview on *T. gondii* seroprevalence in wildlife species commonly consumed in Europe. The data will be analysed by Bayesian hierarchical modelling and used as input data in a multi-country quantitative microbiological risk assessment within TOXOSOURCES project.

Funding source: This work was done as part of TOXOSOURCES project, supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement № 773830: One Health European Joint Programme.

MODERATE TO SUBSTANTIAL AGREEMENT BETWEEN DIRECT AND INDIRECT DETECTION OF *Toxoplasma gondii* IN GAME

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Background. For *Toxoplasma (T.) gondii*, the causative agent of toxoplasmosis, one possible route of infection is the consumption of undercooked or raw meat containing tissue cysts. Although hunting and presumably consumption of game in Germany have increased in the past years, little is known about the occurrence of *T. gondii* in wild animals.
Material and Methods. In this study, sera of wild boar (*Sus scrofa*), roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*) and red deer (*Cervus elaphus*) from the German Federal State of Brandenburg were investigated for the presence of *T. gondii*-specific antibodies using a commercially available ELISA kit (ID. Vet, France). For direct detection of *T. gondii* tissue cysts, 50 g of heart muscle tissue were analyzed using an acid pepsin digestion (PD) and magnetic capture (MC). Direct DNA extraction of 5 g heart muscle tissue was additionally performed (DE). DNA extracts were analyzed using a qPCR targeting the 529 bp-repeated element.
Results. Seroprevalences of about 20% in wild boar, 11% in roe deer and 6% in red deer were observed.
12% wild boar, 6% roe deer, 2% of fallow deer and 2% red deer tested positive for *T. gondii* DNA in at least one direct detection method. MC qPCR detected the highest proportion of animals positive for *T. gondii* DNA, with PD qPCR showing similar results.
Serological and molecular detection showed moderate to substantial agreement. In total, almost 50% of the examined seropositive animals that were also investigated by qPCR showed positive results in at least one of the three direct detection method, indicating the actual presence of tissue cysts.
Conclusion. The indirect and direct detection results of *T. gondii* indicate that game may represent a relevant source of infection for humans, if proper heating (72°C, 2 min) throughout is not ensured before consumption.

IDENTIFICATION OF *Alaria alata* MESOCERCARIAE BY MALDI-TOF MASS SPECTROMETRY

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Background. *Alaria (A.) alata* mesocercariae (AM) have increasingly appeared as incidental findings during mandatory *Trichinella* testing of wild boars in many European countries. For AM detection, an *Alaria* spp.-specific PCR is available which is, however, time- and cost-intensive. Therefore, we developed a rapid, easy and cost-efficient MALDI-TOF assay for identification of AM in wild boar meat, which enables application in routine diagnostics.

Materials and Methods. In this study, a fast and methodically simple protocol for protein extraction of AM from different countries and host species was established and an AM-specific reference spectra database created as an on-going development of an already existing *Trichinella* spp. database.

Results. In total, 61 main spectra profiles (MSPs) from different host individuals were stored in an AM-specific MSP library. The cluster analysis of these 61 MSPs indicated possible variation within the *A. alata* species with a tentative association to the geographical origin of the host, but not the host species.

Conclusion. This MALDI-TOF assay allows a fast verification of AM isolates which is the next step in the development of a universal database for identification of several parasites isolated from meat.

INSIGHTS INTO MONITORING AND PREVALENCE STUDIES OF CIRCULATING ZOONOTIC PARASITES IN GERMAN WILDLIFE

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Background. Information on the prevalence of zoonotic pathogens in German wildlife is scarce and long term pathogen monitoring or surveillance projects including parasites, viruses, virus-likes, and bacteria did not exist previously. Here, we present data indicating presence of zoonotic parasites in an ongoing pathogen-monitoring project conducted by the German Federal Institute for Risk Assessment (BfR) as part of a One Health initiative study in defined wildlife habitats in Germany (2017-2021). Animals included in the study are herbivores and omnivores that are typically hunted as game and common carnivores in Germany such as raccoon dog and fox.

Objectives. A collection of prevalence/occurrence data for three parasites i) *Toxoplasma gondii*, ii) *Cryptosporidium* spp., and iii) *Alaria alata* mesocercariae from start of the project to current state in conjunction with climate change implications will be presented.

Material and Methods. During the hunting seasons 2017/18, 2018/19, 2019/20 and 2020/21 samples of heart muscle, foreleg muscle, fatty tissue, fascia, diaphragm, liver, tongue, tonsils and blood were collected and either tested for presence of antibodies and DNA, or isolated for i) *Toxoplasma gondii*, ii) *Cryptosporidium* spp., and iii) *Alaria alata* mesocercariae, and other pathogens (bacteria, viruses, circular DNAs).

Results. Our data suggests autochthonous circulation of all investigated pathogens with prevalences ranging from i) 5 – 25 % *Toxoplasma gondii*, ii) 0 – 31 % *Cryptosporidium* spp., and iii) 26 – 29 % *Alaria alata* mesocercariae.

Conclusion. Aside from recognition of circulation of parasites pathogenic to humans in German wildlife/game, acquired data further allows us to hypothesize a direct dependency of climate conditions-pathogen-host interactions where pathogens are also associated with the environment underlining this One Health approach.

PARASITE LOAD OF NEMATODE SPECIES IN *Apodemus flavicolis*: EFFECTS OF HOST SPLEEN SIZE, BODY MASS, BODY CONDITION AND SEX

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Background. In wild populations of small rodents, different intrinsic and extrinsic factors affect variation in parasite burdens. The energetic status of animals has a great impact on the immune system, while environmental factors can change the relationship between infection and the host body condition. We aimed to analyse the relationship between relevant morphological traits and parasitological parameters in populations of yellow-necked mice, *Apodemus flavicollis* (Melchior, 1834).

Material and Methods. Nematode burdens were assessed in 49 *A. flavicollis*, from 7 different localities in Serbia. Morphometric data were measured for all captured mice. Spleen mass was used as a proxy of immunocompetence. The host body condition, individual parasite load (IndPL), individual parasite species richness (IndPSR), prevalence, mean abundance and mean infection intensity were calculated.

Results. A total of 12 nematode species were recorded in *A. flavicollis*. The overall prevalence of infection was very high (100%). Individual parasite species richness (IndPSR) was significantly positively correlated with body condition (R^2=0.173; p=0.003), and the same pattern was observed in both sexes. Spleen size was also significantly positively correlated with body condition (R^2=0.344, p<0.005) and body mass (R^2=0.341, p<0.005). Contrary to expected, no parasitological indices (IndPSR and IndPL) were significantly related to spleen mass.

Conclusion. Animals exhibiting better body conditions are parasitized simultaneously with a higher number of nematode species (i.e. their parasite infracommunities are richer). This could prevent any of them to become overabundant in host.

ECTOPARASITE BAT FLIES (DIPTERA: NYCTERIBIIDAE) OF SCHREIBER'S BENT-WINGED BAT AND THEIR FUNGUS PARASITE

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Background. Schreiber's bent-winged bat, *Miniopterus schreibersii*, is one of the most common cavernicolous bat species in southern Europe, often heavily parasitized with different ectoparasites thanks to its gregarious nature, i.e. forming large colonies of many thousands of densely packed animals. Flies from Nycteribiidae family are highly specialized ectoparasites of bats that live on their pelage and feed on blood.

Material and Methods. We collected ectoparasites from 168 host specimens *M. schreibersii* from eight sites in Serbia and Bosnia and Herzegovina, aiming to characterize bat flies species assemblage and identify abundance patterns. Flies were identified morphologically, and cytochrome oxidase subunit 1 (COI) sequences were used to confirm nycteribiid species identification and to further explore their genetic diversity. During the morphological examination, flies were checked for the presence of hyperparasite fungi (Ascomycota: Laboulbeniales).

Results. Three bat fly species were identified: *Nycteribia schmidlii* (210), *Penicillidia conspicua* (71), and *P. dufourii* (5). Prevalence of ectoparasite infection ranged from 58.6% to 100%, with mean abundance and intensity of infection being 1.7 and 2.3, respectively. *Arthrorhynchus* sp. fungus was found in 15 specimens of *P. conspicua* fly (21%). We report 21 (*N. schmidlii*), 12 (*P. conspicua*) and three (*P. dufourii*) COI haplotypes in these fly species, as well as relatively high haplotype and nucleotide diversities.

Conclusion. The results presented here contribute to the knowledge of rarely studied bat ectoparasite fauna in central Balkans by revealing abundance patterns, adding new barcoding sequences to the reference base, and identifying cases of hyperparasitism on bat flies from Serbia and Bosnia and Herzegovina.

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THE MICROBIOME OF PARASITES AND ROLE IN DISEASES
Organizers / Moderators: Cinzia Cantacessi & Nolwenn Dheilly

INVITED LECTURES

DIVERSITY AND EVOLUTION OF VIRUSES OF PARASITIC FLATWORMS (PHYLUM PLATYHELMINTHES, GROUP NEODERMATA)

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Background. Viruses are abundant, ubiquitous, and can have profound effect on their host health and evolution. We recently discovered viruses within a cestode parasite and demonstrated that while most viruses are vertically transmitted, some are transmitted to parasitized hosts. Flatworm-associated viruses could contribute to parasite pathogenicity, and because parasites have such a close and intimate relationship with their hosts, parasites could constitute an underappreciated source of virus emergence.
Objectives To obtain an unbiased picture of parasite-associated virus evolution, and assess the spillover potential of viral biodiversity, a more comprehensive characterization of the virome of parasites is necessary. We have investigated the composition of the virome of parasitic flatworms that specialize in parasitizing vertebrates.
Material and Methods. We employed a viral metagenomics approach to identify viral sequences in 68 species of Platyhelminthes, including 30 Neodermatan parasites. Phylogenetic analyses were conducted to position these viruses relative to the known diversity of viruses.
Results. We found over 100 new viruses associated with Platyhelminthes. We revealed that the virome of flatworms changed dramatically during the transition to a parasitic lifestyle and identified novel undescribed taxa specific to parasitic flatworms. Some viral taxa were found only in a subset of closely related parasite species indicating a potentially recent acquisition while others were found in a broad range of Neodermatan parasites. Within most taxa, viruses cluster together based on their parasitic host phylogenetic relationship, revealing a close association and co-diversification. Parasitic flatworm viruses often had a basal position to other virus groups, the most striking example being the ancestral position of Rhabodviruses of trematodes and cestodes to vertebrate-associated viruses.
Conclusion. This body of work revealed that parasitic flatworms host a great diversity of viruses, that co-infection by multiple viruses is frequent, and that parasites should be considered a potential source of viral emergence. Clearly further studies are needed to test the role of viruses in flatworm diseases. The discovery of viruses specific of Neodermata offers new opportunities for the development of original control measures, diagnostic approaches, and therapeutic strategies.

Giardiavirus AND FRIENDS: GENOMIC AND FUNCTIONAL ANALYSIS EXPAND OUR KNOWLEDGE ON VIRUSES INHABITING THE PROTOZOAN PARASITE *Giardia duodenalis*

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The protozoan *G. duodenalis* causes giardiasis a globally distributed parasitic diarrheal disease. A small dsRNA viruses comprising two ORFs (capsid protein and RNA-dependent RNA polymerase), referred to as *Giardiavirus*

(GLV, *G. lamblia* virus), family *Totiviridae*, might inhabit the cytoplasm of many human and animal isolates of *G. duodenalis*. Only three, almost identical, GLV genomes have been sequenced but functional study done on a single GLV isolate. The presence of viral endosymbionts was linked with disease severity caused by some protozoan parasites but correlation between the presence of GLV and *Giardia* virulence is lacking. To expand the current knowledge on GLV infection, the characterization of several GLV strains from naturally infected G. duodenalis isolates was undertaken. Viral genomes from several *Giardia* isolates were high-throughput sequenced and viral proteins were characterized by mass-spectrometry. Biological properties of the identified viral strains were also investigated both in their original *Giardia* isolate and by experimental infection of a naïve *Giardia* isolate. Our sequencing and proteomic analyses indicate that: i) viral capsid protein translation starts at proline and ii) that translation of the RNA-dependent RNA polymerase (RdRp) occurs via a new +1/−2 ribosomal frameshift mechanism. Phylogenetic analysis support the occurrence of at least two GLV subtypes which display different phenotypes and transmissibility in experimental infections of a GLV naïve *Giardia* isolate. A new, unclassified viral sequence (designed GdRV-2), unrelated to *Giardiavirus*, encoding and expressing for a single large protein with an RdRp domain homologous to *Totiviridae* and *Botybirnaviridae*, was also identified. Our study provides new evidence on GLV genome organization and biology. In deep transcriptomic and proteomic studies reveal the potential diversity of viral infections in the protozoan parasite *Giardia* and strengthens the possibility that GLV, or other endosymbiont virus infections, cause alteration of particular *Giardia* phenotypic traits, including virulence.

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MICROBIOME INCEPTION: AN INTESTINAL CESTODE SHAPES A HIERARCHICAL LANDSCAPE OF DISTINCT MICROBIAL COMMUNITIES NESTED WITHIN THE HOST

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Background. Cestodes represent major health concerns for both human and animal populations. As intestinal parasites, cestodes share space with the gut microbiome of their primary hosts. There is growing recognition that such parasitic helminths can harbour an internal endomicrobiome, creating a system of nested microbiomes within the primary host. However, how the cestode and its associated microorganisms interact with the host gut microbiome remains unclear, as do the consequences of these interactions for host health.
Material and Methods. In the first such study performed in a cestode, we characterised the microbial communities in an intestinal parasite, a cestode of the genus *Eubothrium*, and its primary host, Atlantic salmon (*Salmo salar*). We sampled the host gut mucosa, the surface of the cestode (the tegument) and the cestode endomicrobiome in 30 sea-farmed, harvest-aged salmon and characterised the microbiomes with 16S amplicon and shotgun metagenomics sequencing.
Results. Cestode presence altered the salmon gut microbiome, with an increase in putative pathobionts and a decrease in the dominating commensal *Mycoplasma* phylotypes. The cestode also carried a distinct endomicrobiome, while the tegument included bacteria from both the cestode and the salmon microbiomes. Shotgun metagenomics revealed distinct *Mycoplasma* phylotypes in the cestode endomicrobiome with functional potential that differed from the *Mycoplasma* phylotypes abundant in the salmon gut.
Conclusion. Our results indicate that cestode infection is associated with gut dysbiosis in the salmon host, by simultaneously serving as a potential source of novel bacterial species, as well as a selective force benefiting putative pathogens. Our study highlights the importance of taking a hologenomic approach to understanding parasite infections, where the parasite and its associated microorganisms are considered as a holobiont nested within the host holobiont, with combined effects on the host microbiome and overall host health.

ORAL PRESENTATIONS

COMPARATIVE ANALYSIS OF HOST AND PARASITE MICROBIAL COMMUNITIES IN EXPERIMENTAL MODELS OF THREE LIVER FLUKE INFECTIONS

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Background. Three epidemiologically significant food-borne trematodes (*Opisthorchis felineus, O. viverrini, Clonorchis sinensis*) differ in the level of carcinogenic potential. The mechanisms of carcinogenesis by the liver flukes are studied fragmentarily, the role of host and parasite microbiome is an unexplored aspect.
Material and Methods. In order to characterize the microbial communities in adult parasites as well as in host bile and colon, the hamsters were infected with metacercariae of *C. sinensis* (South Korea), *O. viverrini* (Thailand) and *O. felineus* (Russia). We performed high-throughput sequencing (MiSeq, Illumina) of libraries constructed from V3 – V4 region of 16S ribosomal DNA isolated from adult worms and from colon faeces and bile from the hamsters. Furthermore, *ureA* and *cagA* genes of *Helicobacter pylori* were assessed by the real-time PCR method in the stomach, feces and bile of hamsters.
Results. As a result, 1,784,000 reads were assigned to 13,244 operational taxonomy units (OTUs) and, in turn, to 273 genera of Bacteria. Analysis revealed the significant phylogenetic diversity of the microbial communities among three liver flukes. Numerous bacterial species were identified in the bile of the infected animals, in particular, bile contains the same bacterial phyla as worms do. Prevalence of *H. pylori* and *ureA* gene copy number was significantly higher in the liver fluke-infected hamsters than in the uninfected ones.
Conclusions. The infection with any liver fluke significantly modified the bile and faecal microbiome, increasing the abundance of *H. pylori*. Mechanisms of host microbiome modification by the liver flukes are discussed.

Funding source: This work was supported by the Russian Science Foundation [18-15-00098].

EPITRANSCRIPTOMICS REGULATION OF STRESS SURVIVAL IN THE PARASITE *Entamoeba histolytica* BY QUEUINE FROM THE GUT MICROBIOTA

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Background. *Entamoeba histolytica* is a unicellular human parasite that causes amebiasis. The parasite resides in the colon and feeds on the colonic microbiota. The gut flora modulate the physiology of the parasite and affect its virulence through unknown mechanisms. Queuine, a modified nucleobase of queuosine produced by the gut bacteria leads to tRNA modification at the anticodon loops of specific tRNAs.
Objectives. To investigate the role of queuine on tRNA modification, stress-survival and virulence of *E.histolytica*. To investigate how queuine is salvaged by the parasite from the gut microbiota.
Material and Methods. Acid denaturing gel to detect queuine in tRNA. Biochemical approaches to characterize EhTGT and EhDUF2419. Reverse genetics.
Results. We found that synthetic queuine is efficiently incorporated into *E. histolytica* tRNAs by a tRNA-guanine transglycosylase (EhTGT) and this incorporation stimulates the methylation of C38 in tRNAAsp GUC. Queuine protects the parasite against oxidative stress (OS) and antagonizes the negative effect that oxidation has on translation by inducing the expression of genes involved in the OS response. On the other hand, queuine impairs *E. histolytica* virulence by downregulating the expression of genes previously associated with virulence. Silencing of EhTGT prevents incorporation of queuine into tRNAs and strongly impairs methylation of C38 in tRNAAsp GUC, parasite growth, resistance to OS, and cytopathic activity. We have also found that queuine is salvaged by the parasite from bacteria and that EhDUF2419, a protein with structural similarity to DNA glycosylases is involved in this process.

Conclusion. Our study highlights the importance of bacterially derived products in shaping the physiology of the parasite. The fact that queuine inhibits the virulence of *E. histolytica* may lead to new strategies for preventing and/or treating amebiasis by providing to the host queuine directly or via probiotics. Biochemical and genetic characterization of EhDUF2419 is in progress.

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MIGRANTS AND MIGRATING PARASITES
Organizer / Moderator: Zeno Bisoffi

INVITED LECTURES

SCREENING OF MALARIA IN MIGRANTS

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Malaria causes high morbidity and mortality with 229 million clinical cases and 409 000 deaths in 2019, the majority in Sub-Saharan Africa. Moreover, children and adults living in malaria endemic areas are often infected with persistent low-level asymptomatic infections as a result of partial immunity. In addition of serving as reservoir for transmission, asymptomatic infections may become symptomatic, cause anemia, as well as have long-term effects on cognition, risk of lymphoma and accelerated telomere shortening. Children and pregnant women are at a specific risk of developing symptomatic and severe malaria, and anaemia; and asymptomatic infections during pregnancy may lead to low birth weight and other severe pregnancy complications.
Migration from countries in Sub-Saharan Africa where malaria is endemic has increased over the last years. Despite high parasite prevalence in many of these countries, malaria testing is not included in the health screening offered to newly arrived migrants in any European country, and evidence-based guidelines are needed. Preliminary results from an ongoing study where we offer a malaria test at the Asylum health facility in Stockholm, Sweden, show a high parasite prevalence by PCR (20%) in adults and children arriving from Sub-Saharan Africa. In addition, other studies have reported 3-30% parasite prevalence in migrants from endemic areas and high incidence of febrile malaria in certain migrant groups. To further guide a potential screening program, our preliminary findings need to be confirmed in a larger population; and methods for screening, time period and targeted high-risk groups need to be assessed. Also, an in-depth cost effectiveness analysis of introducing screening of malaria in migrants is needed. Further evidence on the benefits of preventing acute and long-term effects of malaria in migrants, both on a societal and individual level, will inform whether to include malaria in health screening programs.

Funding source: Swedish Research Council, Stockholm County Council

STRONGYLOIDIASIS IN MIGRANT POPULATIONS: IS SYSTEMATIC SCREENING THE BEST STRATEGY?

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Strongyloidiasis is one of the more prevalent infections in migrant populations with estimations of a pooled prevalence of 12.2% in migrants from endemic areas residing in non-endemic areas. Immunosuppressed individuals, increasing in high-income countries are at higher risk of severe complications from unrecognised chronic *S. stercoralis*. The disease may remain undetected for long periods of time due to lack of healthcare provider awareness, the unspecific presentation, and the ability of the helminth to reproduce indefinitely in the host, being the infection lifelong if untreated. For these reasons, routine screening for strongyloidiasis in migrants at high risk of exposure, particularly immunosuppressed migrants, has been recommended as a strategy to prevent severe complications.
Serological testing, the most sensitive diagnostic methods is not widely available in most clinical settings yet, which is a major drawback when considering implementing screening programmes.
A recent cost-effectiveness study evaluated possible public health interventions to address and prevent strongyloidiasis in migrants from endemic areas living in the European Union(EU). Six strategies were evaluated against a base-case scenario where no specific intervention is undertaken. Presumptively treating migrants

prior to immunosuppression in the hospital setting was the most cost-effective strategy. This strategy was found to be cost-saving compared with current clinical practice, with a negative ICER. However, this strategy should target migrant populations before the immunosuppression is established. In addition, ivermectin is not easily accessible in European countries what could entail supply side problems.
Presumptively treating for *S.stercoralis* in patients prior to immunosuppression seems to be a right strategy although the heterogeneity of health system characteristics, availability of ivermectin and the acceptability of this strategy, particularly in individuals at higher risk of developing severe side effects should be also considered.

IMAGING IN PARASITIC DISEASES: WHEN, HOW AND WHY

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Imaging techniques have to be considered an integral component of the possible diagnostic tools for parasitic infections. Especially point-of-care ultrasound (POCUS) has today a well-defined role in the diagnosis and clinical management of parasitic infections, while other "heavy" imaging techniques (CT, MRI, PET) are of primary importance in defined conditions. Depending on the parasite involved, imaging can be the diagnostic reference standard or aid in the clinical staging of the infection or provide important information towards the correct diagnosis if applied in the context of a differential diagnosis workup. Again, depending on the parasite involved, imaging techniques might be useful for screening or for diagnosis and/or follow-up in a clinical setting. This presentation will provide an overview of the current role of imaging techniques in the screening, diagnosis and clinical management of selected parasitic infections, with a particular focus on POCUS, to provide clinicians with practical concepts for the request and interpretation of imaging exams in this field.

INTESTINAL PARASITIC INFECTIONS IN MIGRANTS PASSING THROUGH SERBIA

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Background. Since 2015, Serbia has been part of the migrant route, with an average detention of migrants of 23 days. According to this, Serbian Ministry of Health established document of Ordinance on medical examinations of asylum seekers upon admission to the Asylum Center or other facility for accommodation of asylum seekers. One of the health parameters monitored in migrants was a parasitological stool examination.
Material and Methods. A 3875 stool samples from 19 Asylum Centres in Serbia were examined in the period from June 2015 to July 2021, from people who come from Afghanistan, Syria, Pakistan and other countries. From each migrant, fresh stool specimen was examined on the day of the specimen collection. After formalin - ethyl acetate sedimentation concentration technique, direct smear examination was performing with Lugol's liquid, examined under a microscope at 400 x magnification.
Results. Intestinal parasites were detected in about 5% of investigated samples. Most frequently protozoa *Giardia lamblia* were diagnosed, followed by geohelminth *Ascaris lumbricoides*. Protozoa *Entamoeba hystolytica/dispar* were rare finding, while helminths such as *Trichuris trichiura*, *Hymenolepis nana*, *Taenia spp*. and *Enterobius vermicularis* were often diagnosed.
Conclusion. Given that Serbia has been part of the migrant route for years, monitoring of infectious agents in migrants is necessary to be carried out continuously. In that way, the health system of Serbia is ready to react in the suppression of certain infections, in this case intestinal parasitosis, and in their diagnosis and treatment in case they appear in the local population.

TOXOPLASMA GENETIC DIVERSITY
Organizer / Moderator: Marie-Laure Dardé

INVITED LECTURES

ANALYSING THE GENETIC DIVERSITY OF *Toxoplasma gondii* IN AN EUROPEAN COUNTRY VIA HUMAN SAMPLES

Marie-Laure DARDE[1,2,3], Karine PASSEBOSC[1,2], Marie-Fleur DURIEUX[2,3], Farid BOUMEDIENE[3], Lokman GALAL[3], Aurélien MERCIER[3], and the Network of the French National Reference Center for Toxoplasmosis
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Toxoplasma gondii is now well known for having a spatial structure of its population. France is a cosmopolitan country with multiple origins of migrating populations. It also covers a large geographical area due to the overseas departments, from the Americas to the Indian and Pacific Oceans. The French National Reference Center for Toxoplasmosis, that receives strains or DNA samples for genotyping, has made it possible to capture the diversity of isolates circulating in patients in France, and to show how this survey can contribute to the knowledge of both local and global geographical distribution of strains. Out of a total of 2124 samples (2006-2019), a full 15-microsatellite (MS) genotype was obtained for 1344 samples (63.3%). Nine hundred and twenty nine corresponded to unique genotypes and 236 were distributed among 83 genotypes using 15-MS (2 to 12 isolates per clone). Clones were more frequent in insular environments, but we also detected Type II epidemiological clones in France circulating over a few months. This large collection has made it possible to detect new genotypes or new clonal lineages in regions where the *Toxoplasma* genetic diversity was previously unexplored, such as the French Polynesian or La Réunion islands. The diversity of countries of origin of African patients has broadened our knowledge of the population structure on this continent, despite the uncertainties about the exact locations of their infection. In a number of cases, the presence of an unusual genotype in France (i.e. different from Type II) can be explained by the consumption of imported food, but this is not always the case, raising the question of the circulation in France of uncommon native genotypes, such as HG16 or HG12 usually found in North America. The associated clinical data are used to try finding an association with genotypes.

Funding source: The National Reference Center for Toxoplasmosis is funded by Santé Publique France.

MASSIVE INTROGRESSIONS OF *Toxoplasma gondii* DOMESTIC ALLELES IN THE AMERICAS COINCIDE WITH THE RECENT INTRODUCTION OF THE DOMESTIC CAT

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Toxoplasma gondii, a cyst-forming apicomplexan parasite of virtually all warm-blooded species, is the etiologic agent of toxoplasmosis, a disease causing substantial public health burden worldwide. Its wide range of host species and its global occurrence probably complicate the study of its evolutionary history, and conflicting scenarios have been proposed to explain its global spread. By analysis a global set of 156 genomes and by providing the first direct estimate of *T. gondii* mutation rate, we show that major Old World domestic clonal

lineages have spread from Europe and Africa to the Americas in the last few centuries and hybridized with New World specific clades. These events coincide with the recent expansion in the New World of the domestic cat and of a number of rodent species, the main hosts of *T. gondii* in the domestic environment. By combining environmental and functional data to selection inference tools, we identify the top candidate genes under selection in these hybrid populations of North and South America. We show that a unique domestic allele inherited from the recently introduced Old World lineages has been selected in these emergent domestic populations in the New World. The selection of this domestic allele is most parsimoniously explained by local adaptation to the domestic ecotype and to transmission by domestic cats.

ORAL PRESENTATIONS

GENOME-WIDE SINGLE NUCLEOTIDE VARIATION IN *Toxoplasma gondii* TYPE II ISOLATES FROM EUROPE

Pavlo MAKSIMOV[1], Simone CACCIO[5], Maike JOERES[1], Franz J. CONRATHS[1], Bretislav KOUDELA[2,3], Radu BLAGA[4], Mercedes FERNÁNDEZ-ESCOBAR[6], Rafael CALERO-BERNAL[6], M. Luis ORTEGA-MORA[6], Pikka JOKELAINEN[7], Gereon SCHARES[1]

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Background. *Toxoplasma gondii* is a highly prevalent protozoan parasite that globally infects a broad range of animals, including humans. A better knowledge of the genetic diversity and population structure of *T. gondii* may help to understand the many transmission routes and sources of infection. There are limited data on genome-wide comparisons of field isolates belonging to the same genotype or lineage. Therefore, the aim of the present study was to assess genome-wide genetic diversity among *T. gondii* type II isolates from Europe, where this lineage appears predominant.

Material and Methods. Whole genome sequences of 4 European type II field isolates were assessed by whole genome sequencing (WGS) and highly polymorphic regions identified. These regions showed a considerable number of single nucleotide polymorphisms (SNPs), insertions and deletions (INDELS) relative to a T. gondii reference genome (strain ME49), available in a public data base (ToxoDB).

Results. At least 95% of the reads for each *T. gondii* European field isolate were mapped to the reference genome. The mapped reads covered over 99% of the type II reference genome with a read depth of > 20 per base. The total number of SNPs varied between ~4000 and ~11000.

Conclusion. This study demonstrates considerable genetic variation among European type II isolates and provides new insights into the population structure of *T. gondii* in Europe.

Funding source: This work was part of TOXOSOURCES project, funded by the European Union's Horizon 2020 Research and Innovation programme under grant agreement № 773830: One Health European Joint Programme.

VIRULENCE AND UNDERLYING MECHANISMS OF FOUR DISTINCT LINEAGE III VARIANT GENOTYPES OF *Toxoplasma gondii*

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Background. Strains of *Toxoplasma gondii* lineage III circulate globally and tend to occur more frequently in animals. The archetype (ToxoDB#3) has been extensively used as an experimental model and has been shown to be of low to intermediate virulence, yet the virulence of variant strains is largely unknown as are the underlying mechanisms.

Objectives. The virulence of four strains representing distinct lineage III genotypes isolated from animals in Serbia was examined in vitro and in murine models of infection.
Material and Methods. Tachyzoite proliferation rate and lytic capacity were assessed in vitro on Vero cells. Moreover, the expression level of ENO2 was determined after 10 days of in vitro cultivation. In vivo, the main parameters assessed were cumulative mortality, as well as survival post RH challenge. Expression levels of IFN-γ, IL12 and IL10 were determined in the brain and spleen of infected Swiss Webster females in early (day 7) and late (day 42) infection, and 30 days after RH challenge.
Results. Genotypes EQ40 and K1 were shown to be intermediately virulent and G13 and EQ39 (ToxoDB#54) of low virulence. Lytic capacity as well as ENO2 expression were shown to be higher in intermediately virulent genotypes. In addition, IFN-γ production in early infection varied significantly. However, immunization with all four lineage III genotypes protected from lethal outcome after RH challenge.
Conclusion. The results demonstrate that *T. gondii* lineage III comprises genotypes of varying degrees of virulence. Enhanced lytic capacity, which elicits strong IFN-γ secretion and inflammation in vivo, appears to be an underlying virulence mechanism. Although variable virulence is manifested by distinct cytokine expression levels, it does not impact the effectiveness of immune mediated protection against re-infection with the acutely virulent RH strain.

Funding source: This work was supported by grants (project № III 41019 and contract № 451-03-68/2020-14/200015) from the Serbian Ministry of Education, Science and Technological Development.

HOW RODENT INVASION AND *Toxoplasma gondii* GENETIC DIVERSITY IN WEST AFRICA ARE LINKED: A COMPARISON BETWEEN TWO AFRICAN COUNTRIES, SENEGAL AND BENIN

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Background. By being the privileged preys of felids, rodents and other micromammals have an important role in the transmission of the parasite *Toxoplasma gondii*. Moreover, studies have shown that commercial roads may have introduced new lineages from a different land by the mean of micromammal invasion. In Benin (West Africa), maritime exchanges brought new invasive rodents (*Rattus rattus*, *Rattus norvegicus* and *Mus musculus*) on the territory of autochthonous species, thus questioning the prevalence and genetic diversity of *T. gondii* circulating among these hosts.
Material and Methods. For this study, 632 animals were captured in various neighbourhoods of Cotonou in Benin, including the port: *R. rattus* (n=234), *R. norvegicus* (n=77), *M. musculus* (n=102), *Crocidura sp.* (n=179), *Mastomys sp.* (n=28), *Cricetomys sp.* (n=7) and *Praomys sp.* (n=5). Organs of tropism for *T. gondii* (heart and brain) of each micromammal were dissected for DNA extraction before *Toxoplasma* qPCR. Positive samples with a sufficiently high parasite DNA quantity were genotyped by 15-Microsatellites genotyping.
Results. An overall *T. gondii* molecular prevalence of 15.2% (IC 95: [12.39; 17.99]) was found, with differences according to various factors (species, organs, neighbourhoods). Among the positive samples, seven genotypes were obtained: all were from the Africa 1 lineage, a highly virulent lineage for laboratory mice (*M. musculus*).
Conclusion. The nearly exclusive presence of a mouse virulent lineage in Cotonou could explain why certain species are restricted to certain neighbourhoods. As the invasion by micromammals such as the mouse is an ongoing process in Benin, the *T. gondii* genetic diversity could evolve in the future, as observed in Senegal.

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HELMINTH IMMUNOMODULATION AND INTERACTIONS WITH THE MICROBIOME
Organizers / Moderators: William Harnett & Ljiljana Sofronić-Milosavljević

INVITED LECTURES

THE ANTI-INFLAMMATORY PARASITIC WORM PRODUCT ES-62 MODULATES THE GUT MICROBIOME

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ES-62 is the major secreted protein of *Acanthocheilonema viteae*: it is immunomodulatory by virtue of multiple phosphorylcholine residues attached to *N*-type glycans, such that it exhibits protective effects when tested in a range of mouse models of chronic human conditions associated with aberrant inflammatory responses. These models include collagen-induced arthritis (CIA), a model of rheumatoid arthritis, where subcutaneous ES-62 administration significantly reduces the appearance of joint damage, and also high calorie diet (HCD)-accelerated ageing, where ES-62 prevents the development of comorbidities of ageing such as liver fibrosis and impaired gut health. ES-62 can interact directly with cells of the immune system and the protective effects of the helminth product in these models can be correlated with resetting of homeostatic immunoregulation. In CIA, for example, ES-62 interferes with production of IL-17, a cytokine known to be pathogenic in this model: interestingly, given the impact of the microbiome on TH17 responses, we recently discovered that the protective effects of ES-62 in CIA are lost when mice are treated with a cocktail of broad-spectrum antibiotics. We therefore investigated the effect of the helminth molecule on the gut microbiome, and found that whereas induction of CIA resulted in outgrowth of pathogenic bacteria that were associated with impaired gut health in the mice, ES-62 prevented this. Intriguingly, ES-62 was also found to "fine-tune" the microbiome composition, promoting potentially more health-inducing species even in the absence of CIA induction. Finally, the ES-62 mediated-protection against impaired gut health in the HCD-accelerated ageing model also correlated with prevention of potentially pathogenic changes in the gut microbiome. Overall therefore, our data reveal a previously unappreciated role for manipulation of the gut microbiome, in ES-62-protection against disease development in certain mouse models of human inflammatory diseases.

Funding: Arthritis Research UK; Biotechnology and Biological Sciences Research Council, UK

INTESTINAL NEMATODES RELEASE ANTIMICROBIALS AND BENEFIT FROM MICROBIOTA-DRIVEN HOST IMMUNE REGULATION

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Intestinal nematodes account for the vast majority of parasitic worm infections with about a quarter of the world's population being infected, in particular in tropical and sub-tropical areas. These infections are rarely life-threatening. Still, the chronic course and insufficient immune protection against reinfection causes significant morbidity. Furthermore, intestinal helminth infections lead to huge economic losses in farming. Intestinal parasitic nematodes live in intimate contact with the host microbiota which poses the question of an interaction of intestinal nematodes with the microbial environment. We used two intestinal nematode infection, namely *Ascaris suum* infection of pigs and *Heligmosomoides polygyrus* infection in mice to study the triangle relationship between the intestinal nematodes, the host immune response and the gut microbiota. First, we asked, if the anti-nematode immune and regulatory responses are altered in mice devoid of gut microbes. Our data show that *H. polygyrus*-infected germ-free mice developed increased small intestinal Th2 responses coinciding with a reduction in local Foxp3+RORgt+ regulatory T cells and

decreased parasite fecundity. Secondly, we studied if the small intestinal parasites produce factors with antimicrobial activity. The antimicrobial activities against gram-postive and gram-negative bacterial strains were assessed by the radial diffusion assay, while effects on biofilm formation were assessed using the crystal violet biofilm assays. In addition, bacterial neutralizing activity was studied by an agglutination assay. We found native, unconcentrated nematode excretory/secretory (ES) products from intestine-dwelling *A. suum* L4-stage larvae and from adult *A. suum* and *H. polygyrus* worms to display broad-spectrum antibacterial activity. Additionally, adult *A. suum* ES products interfered with biofilm formation by Escherichia coli, and caused bacterial agglutination. In addition, ES products from different *A. suum* life stages were analyzed by mass spectrometry and several proteins and peptides with known and predicted roles in nematode immune defense were detected. These results indicate that *A. suum* uses a variety of factors with broad-spectrum antibacterial activity to affirm itself within its microbe-rich environment in the gut. In conclusion, intestinal nematodes release factors with antimicrobial activity and benefit from microbe-induced regulatory cells potentially confounding more efficient anti-parasite immune responses.

Funding source: German Research Foundation: GRK 2046

NEW DELIVERY SYSTEM FOR *Trichinella spiralis* ANTIGENS – ACCELERATED APPROACH TO AUTOIMMUNITY TREATMENT

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Trichinella spiralis possess the potential to induce tolerance and restrain excessive immune responses, thus exerting beneficial effect on the outcome of experimental autoimmune encephalomyelitis (EAE), the animal model of human disease multiple sclerosis. *Trichinella* achieves this by continuous release of its excretoy-secretory (ES L1) products in the form of a complex mixture of individual components and extracellular vesicles (TsEVs), preferentially exsosomes. Our previous results suggested that the complex modulation of the host immune response is achieved by ES L1 products or its individula components, which induce the maturation of dendritic cells towards tolerogenic status. A new delivery system, based on nanomaterials as carriers of ES L1, was designed to resemble what happens in nature, ie. spontaneous release of *Trichinella* products over an extended period of time using a less invasive route of administration than all previously described for the treatment of autoimmune diseases. PLGA biodegradable nanofibers loaded with ES L1 (PLGA-ES L1), were used as subcutaneous implants. The tretment proved to be safe with no side effects, successful in delivering parasite products and mitigating the course of relapsing/remitting EAE in Dark Agouti rats. The mechanisms we examined included events at the systemic level and target tissue, spinal cord, that explain the shift from the pro- to anti-inflammatory responses and success of treatment. PLGA-ES L1 treatment also prevents the EAE-induced disruption of intestinal epithelial barrier. It diminished the inflammation by lowering the expression of TNF-α in gastrointestinal tract (GIT) and succesfully maintain the expression of mRNA for claudin, the most important tight junction protein in GIT. Obseved protective effect was acompained with the re-establishment of gut microbiota diversity that was disturbed in EAE-induced animals.

Funding source: Institutional financing agreement № 451-03-9/2021-14/ 200019

HOW DO *Schistosoma* EGGS BREAK THE GUT?

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The trematode parasite *Schistosoma mansoni* is a potent modulator of the immune system of the mammalian host. *S. mansoni* has evolved with humans to chronically infect the host for up to 10 years while adult worms reside in the mesenteric vasculature with eggs being excreted in the faeces. The eggs laid in the mesenteric blood vessels must perforate the intestinal wall to achieve egg translocation to the faeces. This egg excretion process involves the formation of a granulomatous cellular infiltrate around the egg. To achieve egg excretion the parasite induces a state of disease tolerance whereby the intestine is chronically ruptured by the parasite egg granulomas without perforation of the gut leading to frank local intestinal or systemic inflammation, such as sepsis. This process of egg excretion is immune mediated, involving CD4+ T cell populations. In this presentation the immunological basis of egg excretion by *S. mansoni* will be presented. The role of the intestinal microbiome in *S. mansoni*-infected mice, using male+female egg-laying or male worm-only infections, will be considered in context of regulation of intestinal inflammation and gut homeostasis. The presentation will deliver insight on how the *S. mansoni* has adapted to the chronically infect the host so that the egg can break the intestinal barrier daily for years without killing the host.

6th INTERNATIONAL WORKSHOP ON ARCTIC PARASITOLOGY – IWAP 6.0e
Organizer / Moderator: Antti Oksanen

INVITED LECTURES

CHANGING CLIMATE, CHANGING PARASITES IN THE ARCTIC

Antti OKSANEN
Finnish Food Authority (FINPAR), Oulu, Finland

Arctic parasites may have originated from temperate region ancestors sooner or later during the last 3 million years (Plio-Pleistocene to current). There is evidence suggesting that some reindeer (*Rangifer tarandus*) parasites originate from host switch during the Pleistocene, when lion, hyena, mammoth, aurochs, reindeer and rhinoceros coexisted in Central and Southern Europe. The closest relatives of the cyst-forming coccidian *Besnoitia tarandi* and the pentastomid *Linguatula arctica* are regarded as typically tropical parasites.
While endoparasites of arctic homeothermic animals live in conditions of constantly comfortable temperature, the free-living stages and those in invertebrate intermediate hosts have had to cope with the ambient environment with very low temperature, especially during the winter.
The current rapid climate change is fastest in the circumpolar areas, where biodiversity generally is lower than at lower latitudes. It forces also parasites to face new challenges and opportunities. The very typically arctic parasite, *Ostertagia gruehneri* of the reindeer, has in Canadian studies been found to have, at the free-living larval stage, a developmental high temperature threshold which already is often exceeded in the summer on the tundra soil surface, even though air temperature would be considerably lower. Warming may thus reduce the transmission rates, and perhaps also the abundance of this abomasal nematode. Some novel vector-borne reindeer nematode parasites (particularly the primarily roe deer *Capreolus capreolus* parasite *Setaria tundra*) clearly benefit from warmer climate allowing larval development in the insect vector during warm summers, which causes peritonitis outbreaks in naïve reindeer calves.
While climate change can be expected to unfavour arctic species in competition with their temperate region relatives, it is not the only change ongoing, which is possibly the reason the arctic *Trichinella nativa* species thrives in Finland apparently even better than before.

ORAL PRESENTATIONS

HELMINTH COMMUNITIES OF TELEOST FISHES AS INDICATORS OF ECOLOGICAL CHANGES IN THE ANTARCTIC MARINE ECOSYSTEMS

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Background. Nowadays, the ecological changes caused by global warming and anthropogenic factors are especially pronounced in Polar Regions. Parasitic organisms are biologically related to various groups of invertebrates and vertebrates and may be used as indicators of the state of marine ecosystems.
Objectives. This study investigated the composition and structure of the parasite communities of Antarctic fishes in their relation to the ecological state of marine ecosystems.
Material and Methods. During 2014–2021, more than 250 specimens of teleost fishes of 6 species were examines at the Ukrainian Antarctic Station “Akademik Vernadsky”, Argentine Islands, West Antarctica. More than 30,000 helminth specimens were collected and identified.
Results. In total, 31 helminth species were identified and assigned to five taxonomic groups: Monogenea (1 species), Digenea (10), Nematoda (5), Cestoda (4), and Acanthocephala (11). *Notothenia coriiceps* was

parasitized by 26 helminth species, *N. rossii* – 14, *Parachaenichtys charcoti* – 27, *Chaenocephalus aceratus* – 23, *Trematomus bernacchii* – 16, *Harpagifer antarcticus*–6. The helminth community was presented by 3 groups of species: dominant, background and rare. The analysis of the helminth communities in *N. coriiceps* revealed slight but statistically significant temporal changes in helminth assemblages and fish infection parameters during last decades. Cestode *Diphyllobotrium* sp., acanthocephalan *Metacanthocephalus rennicki*, and trematode *Neoleoburia antarctica* were found to make the most significant impact on the dissimilarity. Six helminth species can be considered as the potential indictors of the ecological changes in marine ecosystems of the West Antarctica.

Conclusion. This study revealed high species richness of the helminth communities in teleost fishes near Argentine Islands. Some of the changes in the helminth community during last decades are attributed to the changes in marine ecosystems in West Antarctica.

Funding source: This study was supported by the National Research Foundation of Ukraine (project № 2020.02/0074).

CHANGING MORPHOLOGY OF *Moniezia* spp. EGGS

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Background. *Moniezia* is a tapeworm inhabiting primarily small intestine of wild and domesticated ruminants (Cervidae, Bovidae). Live animal diagnosis is based on finding eggs in the animal faeces. Therefore, *Moniezia* egg morphology description is essential. Despite being widespread and long-known, *Moniezia* eggs are still described in a controversial way.

Objectives. We made an attempt to discover the real shape of *Moniezia* eggs at the different stages of their development and to determine the factors affecting and not affecting it.

Material and Methods. Eggs were collected from adult helminths (obtained from sheep, goats, reindeer and roe deer) and from the host faeces in Russia and Finland during 2018-2020. Morphology was studied using light and electronic scan microscopy, and 3D-scanning. Saturated sodium chloride solution, Darling's solution and other flotation-for solutions were used in our experiments along with water.

Results. Immature *Moniezia* eggs are spherical. They can be found both in proglottids and in faeces. Mature *Moniezia* eggs are of a complex shape, which can be simplistically called parallelepiped. Contrary to popular belief, flotation liquid (even hyperosmotic one) has no impact on round to angular transformation. Neither will storing of angular eggs in water (even for 3 months) not result in returning to spherical shape.

Conclusion. *Moniezia* eggs found in faeces and observed through the microscope on slides can seem both round (all kinds of early stages) and triangular (*M. expansa*) or square (other *Moniezia* species). Typical oncosphere hooks and host specificity can contribute to diagnosis if the egg shape is misleading.

HOST SPECIFICITY AND GENETIC DIVERSITY OF *Blastocystis* AND *Entamoeba* IN MUSKOXEN IN GREENLAND AS DETERMINED BY METABARCODING

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Background. Some single-celled intestinal parasitic genera are shared between human and non-human hosts. Some genera exhibit extensive genetic diversity, and efforts continue to identify the role of various species and lineages of for instance *Entamoeba* and *Blastocystis* in health and disease.

Objectives. We set out to identify the host specificity and genetic diversity of *Blastocystis* and *Entamoeba* in muskoxen sampled in different regions of Greenland.

Material and Methods. Faecal samples from 243 muskoxen sampled in North-East, West, and South Greenland were submitted to DNA extraction and metabarcoding using universal primers and ILLUMINA sequencing. Sequence reads were subject to quality trimming, read pairing, and chimera filtering and annotated to species level using the software BION. *Entamoeba*- and *Blastocystis*-specific sequences were clustered using Clustal Omega and consensus sequences subject to BLAST queries in the NCBI Database.

Results. Of the 243 muskox samples, 180 (74%) and 19 (8%) were positive for *Blastocystis* and *Entamoeba*, respectively. *Entamoeba* was found in 0/55 (0%), 4/120 (3%), and 15/68 (22%) samples in South, West, and North-East Greenland, respectively; for *Blastocystis*, the corresponding positivity rates were 44/55 (80%), 104/120 (87%), and 32/68 (47%), respectively. Of the adults and calves, 11% and 4% were colonised with *Entamoeba* and 72% and 41% were colonised with *Blastocystis*, respectively. The populations in the three regions did not differ in terms of age. Sampling time (winter *vs.* summer) did not appear to affect positivity rates. For *Blastocystis*, subtypes 10, 14, 21, 24-26 and at least one novel subtype were identified, often admixed. For *Entamoeba*, several novel lineages were observed, which are currently being analysed.
Conclusion. *Blastocystis* was about 10 times more common than *Entamoeba* and found in approximately 3 out of 4 animals. Multiple species/lineages of both parasites were seen, some of which were novel, and none of which were common in humans.

Funding source: The study was funded by The Danish State funding for Arctic Research (Grant № 80.19) and partly supported by The Greenland Fund (J. №: 2016-557).

ENDOPARASITES DETECTED IN FAECAL SAMPLES FROM MUSKOXEN (*Ovibos moschatus*) IN GREENLAND

Rebecca P.K. D.BERG[1,2,3], Pikka JOKELAINEN[4], C. Rune STENSVOLD[4] , Henrik V. NIELSEN[4], Susan KUTZ[5], Christian M.O. KAPEL[1]

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Background. The environmental changes caused by the continuous warming of the Arctic are affecting all parts of the ecosystem, including parasites. Despite the muskox (*Ovibos moschatus*) being one of the few large Arctic herbivores, baseline knowledge on parasites in muskox populations in Greenland has been limited.
Objectives. The present study aimed to estimate the prevalence of endoparasites in wild muskoxen from Greenland using a combination of methodological approaches.
Material and Methods. A total of 249 faecal samples were collected, during winter (n=80) and summer (n=68) 2017 and during summer 2018 (n=101), as droppings or rectally *post mortem*. The samples were analysed by centrifugal flotation and microscopy (n=241), 18S metabarcoding (n=241), the Baermann method (n=110), as well as specifically for *Giardia duodenalis* and *Cryptosporidium* by real-time PCR (n=233).
Results. By microscopy and/or metabarcoding, we detected *Eimeria* spp., strongyles, Anoplocephalidae, and *Trichuris* sp. in 96%, 84%, 24%, and 1% of the samples collected in summer, and 77%, 9%, 0%, and 0% of the samples collected in winter, respectively. Lungworm larvae were not detected. *Giardia duodenalis* and *Cryptosporidium* DNA were detected in 3% and 2% of the samples collected in summer, respectively; but neither were detected in winter samples.
Conclusion. Our results add to the knowledge on prevalence of parasites in large herbivores in the Arctic. The results suggest seasonal variation, which should be taken into account in planning future studies.

Funding source*:* The study was funded by The Danish State funding for Arctic Research (Grant no.: 80.19) and supported by The Greenland Fund (J. №: 2016-557). The work was also partly supported by the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement № 773830: One Health European Joint Programme (Joint Research Project PARADISE).

WHICH PARASITES WILL BE ABLE TO CROSS THE ARCTIC UNDER CONDITIONS OF CLIMATE CHANGES? AN EXAMPLE OF MARINE AND COASTAL BIRD DIGENEANS

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Background. Ongoing climate warming affects migration routes of marine and coastal birds. Some marine fishes and invertebrates, intermediate hosts of helminths, are spreading to high latitudes. This provides conditions for colonisation by helminths in the North Atlantic (NA) or North Pacific (NP) (depending on the native distribution).

Objectives. We tested capacity of several key taxa of digeneans infecting marine and coastal birds for trans-Arctic transfer.
Material and Methods. The material was intramolluscan stages, metacercariae and adult trematodes of the families Microphallidae, Gymnophallidae, Renicolidae, Notocotylidae and Himasthlidae. We sequenced 28S, ITS1 and ITS2 rDNA and cox1, nad1 and nad2 mitochondrial DNA for more than 300 samples and performed the phylogenetic analyses.
Results. Three major trends in the distributions of NA and NP sister species/populations were identified:
1. Sister species in NA and NP: *Parvatrema* spp., microphalids of the "*pygmaeus*" group, *Levinseniella* spp., *Renicola* spp.
2. Single species in NA and NP, but populations are relatively isolated: *Tristriata anatis*.
3. Single species and single population in NA and NP, with a chance for genetic exchange between its NP and NA parts: *Gymnophallus* spp., *Himasthla littorinae*, *Renicola roscovita*.
Option 1 – adults short-lived (except *Renicola* spp.), NA and NP populations/species of the first intermediate hosts (1IH) isolated by the Siberian seas.
Option 2 and Option 3a (*H. littorinae*, *R. roscovita*) – adults long-lived, 1IH distribuion ranges as in 1.
Option 3b (*Gymnophallus* spp.) – adults short-lived, 1IH with circumpolar distribution, including the Siberian seas.
Conclusion. If trans-Arctic migrations of birds become possible due to climate warming, exchange between NP and NA trematode faunas is realistic. It would first of all involve the species with long-lived maritae that have suitable potential 1IH both in NA and in NP.

Funding source: Russian Science Fund grant № 18-14-00170.

VERTICAL TRANSMISSION OF *Babesia microti* AND THE EFFECT OF CONCURRENT *Bartonella* spp. INFECTION ON ITS SUCCESS

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Background. Long-term studies in the Masurian Lake District (Poland) have shown a high prevalence (32-35%) of *Babesia microti* infection in voles, compared with infected ticks (3.4-5%) collected in the same area. These results suggested an alternative route of transmission, which was responsible for this disproportion, and allowed such a high prevalence to be maintained in the rodent population.
Objectives: The aim of this study was to determine if vertical transmission of *B. microti* occurs in reservoir hosts of the genus *Microtus,* and to verify whether co-infection with *Bartonella* spp. may have an impact on the effectiveness of vertical transmission of those pathogens.
Material and Methods. In total, 115 embryos were isolated from 21 pregnant females. Another 11 pregnant females were kept until they had given birth and weaned their pups. Blood smears and PCRs targeting the 550 bp 18S rRNA gene fragment were used for the detection of *B. microti*. Blood smears and PCRs targeting the *Bartonella*-specific *rpoB* gene fragment (333 bp) were used for the detection of *Bartonella*.
Results. *Babesia microti* DNA was detected in 61.4% of pregnant females. Vertical transmission was confirmed in 81% of the embryos recovered from *Babesia*-positive pregnant females. The DNA of *B. microti* was detected in embryonic tissues from 98% of *M. arvalis*, and 46% of *M. oeconomus Babesia*-positive females. Of the pups born in captivity, 90% were born to *Babesia*-positive dams. *Babesia microti* DNA was detected in 70% of *M. arvalis* and 83% of *M. oeconomus* pups. Congenitally acquired infections and co-infection with *Bartonella* had no impact on the survival of pups and did not affect the effectiveness of the vertical transmission of neither of the two pathogens
Conclusion. A high rate of vertical transmission of *B. microti* was confirmed for the first time in two species of naturally infected voles, *M. arvalis* and *M. oeconomus*.

Funding source: The study was financially supported by the National Science Centre, grants OPUS № 2014/13B/NZ7/02348 (MB) and 2011/03/B/NZ8/02212 (AB).

EVPC – EUROPEAN VETERINARY PARASITOLOGY COLLEGE SYMPOSIUM
Organizers / Moderators: Smaragda Sotiraki & Edwin Claerebout

INVITED LECTURES

ANIMAL LEISHMANIOSIS IN EUROPE: VERTEBRATE HOSTS OTHER THAN DOGS

Luís CARDOSO[1], Henk SCHALLIG[2], Maria Flaminia PERSICHETTI[3], Maria Grazia PENNISI[3]
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The parasitic disease leishmaniasis/leishmaniosis is caused by vector-borne protozoa of the genus *Leishmania* transmitted by female phlebotomine sand flies. Infection with *Leishmania infantum* can lead to severe disease in humans and dogs, with the latter acting as a primary reservoir for zoonotic transmission. Reservoirs of *Leishmania* should be abundant, infected at a high proportion, attractive and infectious to the sand fly vectors, and able to maintain infection year-round. Due to their high density, dogs seem to be the only domestic animal species that naturally maintain zoonotic populations of *Leishmania* parasites. However, an increasing number of reports suggest that *L. infantum* infections also happen in many other mammalian and even avian species, with potential transmission to humans from hosts/reservoirs that were previously not well identified. In addition, *Leishmania* spp. can also cause considerable disease in these animals and contribute to increase the risk of extinction of endangered wild species. This presentation provides an exhaustive list of around 50 domestic and wild vertebrates (other than dogs and humans) in which infections with or exposure to *Leishmania* parasites have been detected in European countries. Most cases are reported from the Mediterranean region. Domestic animals, in particular cats, pose a concern because of close contact with humans. The importance of cats as a reservoir of *Leishmania* spp. and not simply as an accidental host is gaining more and more ground. With the exception of hares (*Lepus granatensis*), the wildlife reservoir is less likely to contribute to zoonotic transmission. Nevertheless, a potentially large reservoir needs to be taken into account when developing prevention and control strategies for zoonotic leishmaniosis, within the scope of the "One Health" concept. Global warming and the movement of animals geographically expand the potential reservoir of zoonotic leishmaniosis caused by *L. infantum*, which is of considerable public and veterinary health concern.

Funding source: L.C. participation was funded by project UIDB/CVT/00772/2020 supported by the Portuguese Foundation for Science and Technology (FCT).

ORAL PRESENTATIONS

EVPC *QUOD EST ET QUO VADIT*
(PAST, PRESENT AND FUTURE OF EUROPEAN VETERINARY PARASITOLOGY COLLEGE)

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The mission of the European Veterinary Parasitology College is to advance the field of Veterinary Parasitology and to promote a high standard of training in our field. The European Veterinary Parasitology College aims to achieve this mission through continued scientific progress in teaching and research in Veterinary Parasitology, the establishment of the standards of training, experience and examination required to obtain

the qualification as a specialist in Veterinary Parasitology in Europe, the recognition of qualified specialists by suitable certification and other means in Europe, the development of continuing education programs in Veterinary Parasitology in Europe and the establishment of standards for the performance of clinical and laboratory procedures in Veterinary Parasitology.
Following an initiative taken at the 18th Congress of the World Association for the Advancement of Veterinary Parasitology (WAAVP), EVPC was established in 2003. The EVPC has been awarded definitive EBVS recognition during the 2013 EBVS Annual General Meeting in Brussels and was recertified in 2018. The College is incorporated under the laws of France as a non-profit organization which does not pursue commercial interests.
The future of the College is based on our high standard and alternative residencies that provide a continuous source of new diplomates and the need of Veterinary Parasitology expertise in practitioners and researchers.
The European Board of Veterinary Specialization (EBVS®) is a European organization focused on veterinary specialization in the Member States of the European Union and its neighbors and is composed of one voting representative from each of the 27 EBVS®-recognized veterinary specialist colleges, comprising more than 38 distinct specialties. The more than 4000 veterinarians active as European Veterinary Specialist™ are ready to serve the public, its animals, and the veterinary profession by providing high quality service in disciplines from anesthesia and analgesia to zoological medicine.

RE-ORIENTATION OF PARASITE-MANAGEMENT IN ADULT HORSES IN SWITZERLAND

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Background. The standard parasite management of horses based on regular anthelmintic treatments has resulted in a worrying expansion of resistant helminth populations, which may considerably impair control.
Objectives. The aim of the study was to obtain a retrospective (2010 – 2016) nationwide analysis of faecal egg count (FEC) data from the Swiss adult horse population, related to horse age and geographic region.
Material and Methods. Thirteen labs provided a total of 16'387 FEC data of horses, aged four to 39 years. In 2017 3'813 questionnaire feedbacks from owners covering equine management practices were analyzed.
Results. Independent of the annual sample size the yearly patterns of the FEC were very similar. Seventy-eight percent (N= 12'840) of the samples were negative and 90% (N=14'720) showed a FEC below or equal to 200 EPG. The annual mean strongyle FEC ranged between 60 and 88 eggs per gram (EPG) with a total mean of 75 EPG. With 222 EPG the mean FEC in the French part of Switzerland was significantly higher ($p < 0.05$) than in German-speaking region (60 EPG). Sixty-eight percent (N= 8'476) of the horses were dewormed without diagnosis, two percent (n=240) were not dewormed at all, whereas for 30% (n=3'721) the selective anthelmintic treatment (SAT) concept was applied. The SAT implementation rate differed significantly ($p<0.0005$) between regions, with 33, 20 and 25% for the German, French and Italian speaking areas, respectively. The rate of horses spending 16 - 24h on pasture per day was higher in the French-speaking region compared to the German speaking part ($p<0.0001$). In addition, pasture hygiene was practiced at a significantly lower rate in the French-speaking part compared to the German and Italian speaking regions (both $p <0.0001$).
Conclusion. The shift towards the SAT-strategy represents a very promising development with respect to mitigating the further spread of anthelmintic resistance in Switzerland.

THE CURRENT SITUATION OF *Angiostrongylus vasorum* IN ROMANIA

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Background. *Angiostrongylus vasorum* is a metastrongyloid nematode localized in the right heart and the pulmonary arteries of carnivores. The number of reports in Europe has recently increased, presumably because of a growing awareness among clinicians, animal owners and researchers. Previously, no surveys have been conducted to assess the prevalence and distribution of *A. vasorum* in wild and domestic canids in Romania, where awareness among veterinarians is limited or absent.

Objectives. The aim of the present study was to update the data on presence, distribution, prevalence of *A. vasorum* in wild and domestic canids in Romania.

Material and Methods. Between July 2016 and April 2017, 567 hunted red foxes; 12 wolves and 15 jackals from different counties of Romania were examined by necropsy for the presence of lungworms. Blood was sampled from a total of 1545 domestic dogs from 23 counties of Romania. Serum samples were tested for the presence of *A. vasorum* circulating antigens (AG) and specific antibody (AB) detection.

Results. The total prevalence of *A. vasorum* in foxes was 4.2%. The wolves and the jackals were both negative. Serological examination revealed the positivity of a total of 33 dogs (2.14%) for *A. vasorum* AG or anti AB against this nematode. A small part of the Romanian veterinarians is aware of the infection with *A. vasorum*.

Conclusion. Our results show that *A. vasorum* is present in Romania in domestic and wild canids. No clinical cases in domestic dogs were published yet.

Funding source: Bayer Animal Health Romania and Bayer Animal Health, POCU/380/6/13/125171

INTERNATIONAL PROJECTS IN PARASITOLOGY
Organizer / Moderator: Adriano Casulli

INVITED LECTURES

TOXOSOURCES: *Toxoplasma gondii* SOURCES QUANTIFIED

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TOXOSOURCES is a Joint Research Project of the One Health European Joint Programme that investigates the relative contributions of the transmission routes and sources of *T. gondii* using multidisciplinary approaches and multicentre studies. *Toxoplasma gondii* infection can be acquired by ingesting oocysts (environmental pathway) or tissue cysts (meatborne pathway). The cross-sectoral, international TOXOSOURCES consortium comprises more than 20 partner institutes, and the project runs 2020-2022 with a budget of 3 million EUR. The consortium has collected data for a multicenter quantitative microbiological risk assessment for *T. gondii*. A literature review supported the selection of a method to detect *T. gondii* oocysts in fresh produce, which will be applied in a multicentre study. The project also develops new methods. The consortium explores serology for detecting *T. gondii* infections caused by oocysts, and an unprecedented effort of Whole Genome Sequencing of *T. gondii* isolates from across Europe was used to identify polymorphic marker regions for the establishment of a new typing method to detect within-genotype variation. The key outcomes of TOXOSOURCES will include quantitative estimates of the relative contribution of the main sources and transmission routes to *T. gondii* infections in Europe.

Funding source: TOXOSOURCES has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement № 773830.

MULTI-CENTRE STUDY ON *Echinococcus multilocularis* AND *Echinococcus granulosus* s.l. IN EUROPE: DEVELOPMENT AND HARMONIZATION OF DIAGNOSTIC METHODS IN THE FOOD CHAIN (MEME PROJECT)

Adriano CASULLI[1,2] on behalf of MEME project[3]
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Background. MEME is an international multicentre collaborative project that started in January 2020 and aims to fill research gaps highlighted by international agencies for the detection and control of zoonotic parasites *Echinococcus multilocularis* (Em) and *Echinococcus granulosus sensu lato* (Eg), causing alveolar echinococcosis (AE) and cystic echinococcosis (CE), respectively.

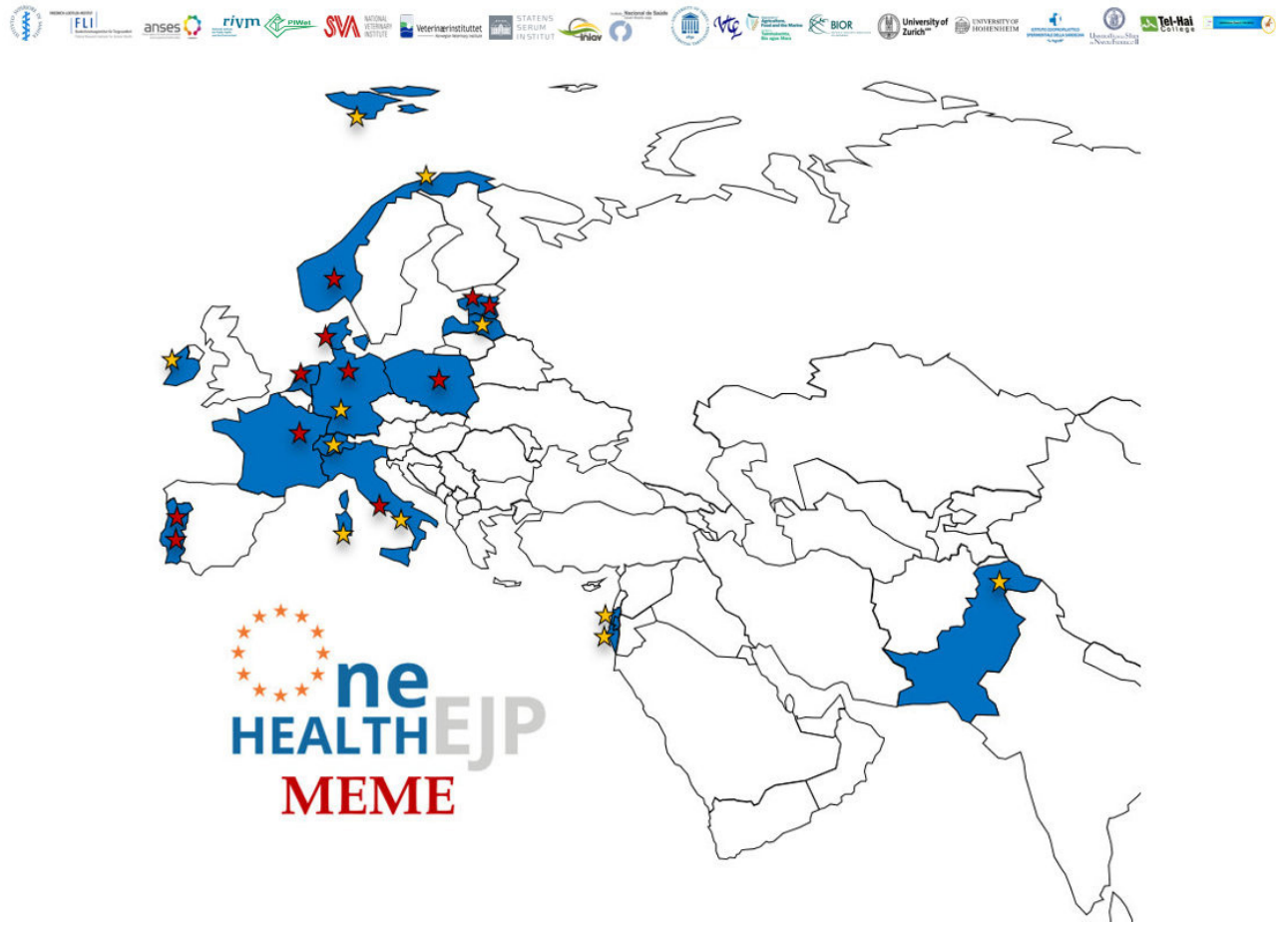

Figure 1. Countries (in blue) and centres (red star = funded partners; yellow star = external partners) participating in the MEME project.

Material and Methods. MEME focuses on standardization, harmonization and validation of existing parasitological and molecular methods, and the development and comparative assessment of innovative molecular tools to detect Em and Eg in the food chain. Production of epidemiological data on the presence of Em/Eg eggs in the food chain will focus on vegetables for human consumption and on canine faeces in selected endemic countries.

Results. During the first year of the project, MEME generated the key protocols and standard operating procedures to feed the main tasks in the project. Results on detection methods have already been published: 1) Comparison of two DNA extraction methods and two PCRs for the detection of Em in stool samples; 2) Bayesian Analysis of three methods for diagnosis of CE in sheep; 3) Microsatellite investigations of Eg cysts; 4) Species detection of Eg by novel probe-based real-time PCRs; 5) Validated method based on PCR-RFLP and multiplex PCR assay for the identification of Eg species; 6) Identification of Eg G1/G3 by SNPs assays. The project is now collecting biological samples for producing epidemiological evidence on the presence of these parasites in different matrices.

Conclusion. MEME will provide a comprehensive set of integrative activities to harmonize procedures, improve the detection and produce epidemiological data on potential pathways of transmission of Em and Eg.

Funding source: MEME project is supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement № 773830: One Health European Joint Programme. https://onehealthejp.eu/jrp-meme/

A BRIEF EXCURSION INTO PARADISE

Simone M. CACCIO'[1,2], Christian KLOTZ[3], Anton AEBISCHER[3], Marco LALLE[1], Emma OSTLUND[4], Anna Rosa SANNELLA[1], Paolo VATTA[1], Gregory KARADJAN[5], Yannick BLANCHARD[5], Isabelle VALLEE[5], Frits FRANSSEN[6], Joke VAN DER GIESSEN[6], Jeroen ROELFSEMA[6], Marieke OPSTEEGH[6], Pikka JOKKELAINEN[7], Rune STENSVOLD[7], Henrik NIELSEN[7], Jacek SROKA[8], Jacinto GOMES[9], Martha BETSON[10], Judit PLUTZER[11], Ioana BUJILA[12], Maria HELLMER[13], Barbora ZALEWSKA[14], Anne MAYER-SCHOLL[15], Rebecca DAVIDSON[16], Karin TROELL[4]

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Background. The PARADISE project primarily focuses on the zoonotic pathogens *Cryptosporidium parvum* and *Giardia duodenalis*, which can cause diarrhoeal disease in humans and animals, worldwide, and have been associated with food- and water-borne outbreaks in Europe and elsewhere.
Objectives. The main aims include the development of new tools for the genetic characterization of isolates and new strategies for enrichment of these pathogens from complex matrices.
Material and methods:.The project is organized into three research-oriented work packages. WP2 activities are focusing on NGS-based genomics and metagenomics, WP3 focuses on development and validation of new molecular typing schemes, and WP4 explores the use of nanobodies, aptamers and hybridization probes for new enrichment strategies.
Results. The generation of many new *Cryptosporidium parvum* and *Giardia duodenalis* whole genomes has allowed a rationale design of novel typing schemes with improved resolution. Detection of foodborne parasites in complex matrices is being explored using amplicon-based and shotgun metagenomics, as well as with protocols to capture parasite-specific DNA sequences. Nanobodies and aptamer technologies are being optimized to design novel enrichment protocols.
Conclusions:.This project will place Europe at the forefront in the fields of comparative genomics and metagenomics, and will have a large impact on the molecular epidemiology and the detection of parasites/ parasitic DNA in complex matrices in a one health setting (human, animal, environment, food).

Funding source: The PARADISE project is supported by funding from the European Union's Horizon 2020 Research and Innovation Programme, under grant agreement № 773830: One Health European Joint Programme.

MOLECULAR-EPIDEMIOLOGICAL STUDIES ON PATHWAYS OF TRANSMISSION AND LONG LASTING CAPACITY BUILDING TO PREVENT CYSTIC ECHINOCOCCOSIS INFECTION (PERITAS PROJECT)

Adriano CASULLI[1,2] on behalf of PERITAS consortium[3]

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Background. Cystic echinococcosis (CE) is neglected parasitic zoonosis endemic in livestock-raising areas worldwide. While the life cycle of the parasite may be only interrupted by animal-focused interventions, humans are accidental dead-end hosts of the parasite. Therefore, the implementation of preventive measures to avoid ingestion of infective parasite eggs is imperative to reduce consistently and sustainably in the long term the burden of human infection. PERITAS is an international collaborative project which aims, for the first time, to elucidate through an integrated approach, the pathways of transmission of *Echinococcus granulosus sensu lato* (Eg) which are poorly understood and have never been systematically investigated.
Material and Methods. The study design is composed by two subsequent stages conducted in selected areas of Argentina, Chile and Peru. STAGE 1 is a Cross-sectional ultrasound-based prevalence study for the identification of high endemic clusters with active cyst stages of human CE where the subsequent case-

control study was implemented. STAGE 2 is a village-based case-control study in positive households, negative households (controls) and village common areas where sampling of environmental matrices (dog fur, dog faeces, soil, vegetables, shoes soles etc.) was conducted for the molecular identification of genotype/species of Eg and other helminths.

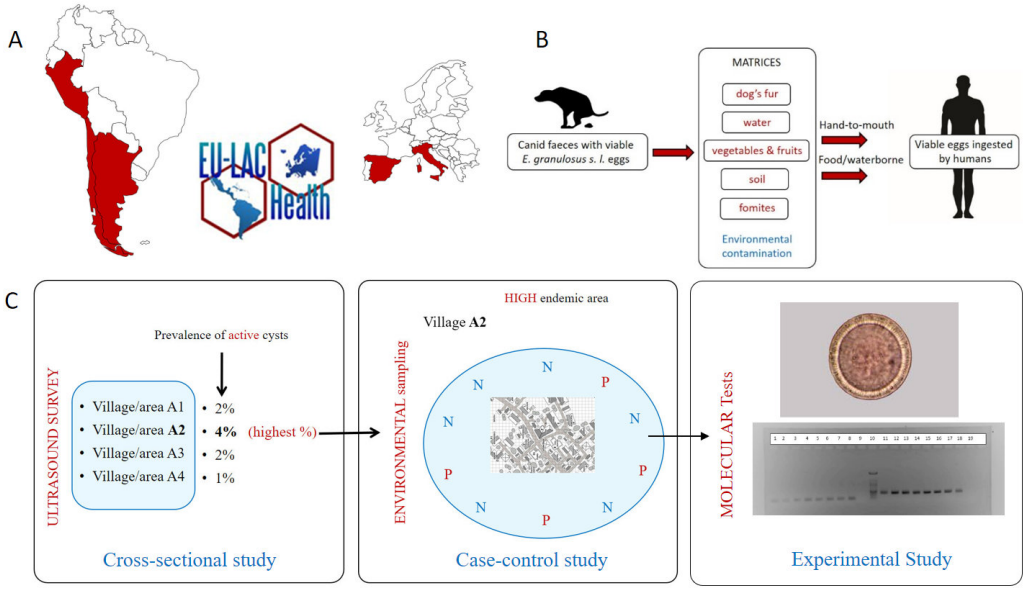

Figure 1. Outline of PERITAS project with participating countries (A), potential pathways of transmission of *E. granulosus* (B) and study design (C).

Results. In 2019, 2435 people were screened in the regions of Coquimbo (Chile), 581 in Rio Negro (Argentina), and 790 in Junin (Peru). Prevalence of human CE was 1.6%, 5% and 5%, respectively. COVID-19 pandemic disrupted scheduled surveys in 2020. Sampling of matrices were successfully conducted in 2020-2021. The analysis of the data will allow the identification of matrices contaminated by Eg eggs and of at-risk behaviours/ habits associated with odds of CE infection.

Conclusion. The direct applicability of project results will allow the implementation of more precisely-targeted human-centred control interventions and increasing the efficacy of control programs for the prevention of new human infections.

Funding source: This work is supported by EU-LAC Health project and National funding agencies of the participating institutions - PERITAS project (https://www.iss.it/en/web/iss-en/who-cc-peritas).

ORAL PRESENTATIONS

THE "EUROPEAN REGISTER OF CYSTIC ECHINOCOCCOSIS" (ERCE) BECOMING "INTERNATIONAL" (IRCE)

Francesca TAMAROZZI[1], Patrizia ROSSI[2,3], Paolo Vatta[2], Adriano CASULLI[2,3] on behalf of ERCE network

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Background. The real burden of human cystic echinococcosis (CE) is difficult to evaluate, since its notification is overlooked and under reported and its peculiar clinical features hamper the implementation of clinical trials and the issue of evidence-based guidelines. The European Register of CE (ERCE), is a prospective, observational, multicentre, online clinical register of patients with probable or confirmed CE caused by *Echinococcus*

granulosus sensu lato. Its aims are to gauge the burden of human CE at international level, bring CE to the attention of health authorities, and support collaborative research on CE.
Material and Methods. ERCE is currently undergoing restructuring to be expanded to the "International Register of CE" (IRCE), aiming to collect clinical data prospectively for the issue of evidence-based recommendations. IRCE will better comply with the General Data Protection Regulation (EU) 2016/679 (GDPR).
Results. The ERCE database was searched (24/06/2021) and data concerning patients' registration, follow-up, CE cyst details, and clinical management were analysed. 41 centres in 16 countries contribute to ERCE. 38 (93%) centres registered 2,515 patients.
Conclusion. ERCE has been constantly expanding and has successfully achieved the objective of highlighting the burden of CE in Europe and beyond. Under the auspices of the WHO, ERCE (becoming IRCE), appears a valuable starting platform to obtain a better picture of the epidemiology of clinical CE.

Funding source: This work is supported by EU-LAC Health project and National funding agencies of the participating institutions - PERITAS project (https://www.iss.it/en/web/iss-en/who-cc-peritas).

OIE COLLABORATIVE CENTRE ON FOODBORNE ZOONOTIC PARASITES SYMPOSIUM
Organizer / Moderator: Isabelle Vallee

INVITED LECTURES

OIE COLLABORATING CENTRE ACTIVITIES AT THE CANADIAN FOOD INSPECTION AGENCY'S CENTRE FOR FOOD-BORNE AND ANIMAL PARASITOLOGY

W. Brad SCANDRETT[1], Batol AL-ADHAMI[1], Kelly KONECSNI[1] Laura LALONDE[1], Vladislav LOBANOV[1], Patrick FRIES[1]

[1]Centre for Food-borne and Animal Parasitology, Saskatoon Laboratory, Canadian Food Inspection Agency, Saskatoon, Canada

The Centre for Food-borne and Animal Parasitology (CFAP) of the Canadian Food Inspection Agency (CFIA) is an ISO-17025-accredited national reference laboratory for federally-regulated parasites of animal and public health significance. It also designated as a World Organisation for Animal Health (OIE) Reference Laboratory for Trichinellosis and OIE Collaborating Centre for Food-Borne Zoonotic Parasites. An overview of these Collaborating Centre activities, including method development, surveillance, standards-setting, molecular characterization and provision to OIE member countries of proficiency samples and reference materials, pertaining to food-borne parasites such as *Trichinella*, *Taenia*, *Echinococcus*, *Cyclospora*, *Cryptosporidium*, *Toxoplasma* and *Giardia* will be presented. It is anticipated that this will facilitate increased collaboration, capacity and harmonization amongst the three OIE Collaborating Centres designated for this same specialty [in Canada (Americas Region), France (European Region) and China (Asia-Pacific Region)] that comprise the existing global network, as well as with other centres, laboratories and organisations, as relevant.

OIE COLLABORATING CENTER FOR FOODBORNE PARASITES IN ASIAN-PACIFIC REGION

Xuelin WANG, Mingyuan LIU

liumy@jlu.edu.cn; liumy36@163.com. or xuelin@jlu.edu.cn, Key Laboratory for Zoonoses, Ministry of Education, Institute of Zoonosis, Jilin University, 5333 Xian Road, 130062 Changchun, P. R. China

This center has the independent capabilities and skills to perform diagnostic tests and characterization for parasites transmitted by food: Helminthes (*Trichinella sp.; Cysticercosis, Clonorchis sinensis*), Protozoa (*Toxoplasma, Cryptopsoridium*). It also works to develop new diagnostic methods and produce reference reagents; it can provide training, expertise, scientific and technical support to laboratories in this field in China and other Asian-pacific region if necessary, advise for national or regional control police and related education for national or regional people as well.

OIE COLLABORATING CENTRE FOR FOODBORNE ZOONOTIC PARASITES (EUROPEAN REGION): OVERVIEW OF ACTIVITIES

Isabelle VALLEE, Karim ADJOU, Radu BLAGA, Pascal BOIREAU, Myriam THOMAS, Bruno POLACK, Grégory KARADJIAN[1]

Anses, Ecole nationale vétérinaire d'Alfort, INRAE, Anses laboratory for animal health, JRU BIPAR. Maisons-Alfort. France

OIE collaborating centres (CC) are designed by OIE (one CC by World region and thematic) to operate as a centre of research, expertise, standardization and dissemination of techniques. The scope of the OIE CC on Foodborne Zoonotic Parasites (FZP) - European region focused on the development of method for detection, identification of FZP to improve their diagnostic and/or to help public health decision makers. The OIE CC is involved in European projects, brings its expertise and assists OIE member countries upon request with the supply of reference material. Our recent activities focused on FZP such as *Trichinella, Toxoplasma,*

Cryptosporidium and *Giardia,* which are classified as having major importance worldwide and in Europe. Thus, results will be presented regarding 1/ the development of methods for identification of nematodes isolated from meat subjected to *Trichinella* official controls and how it allowed to identify other helminths in meat; 2/ the survival of *Toxoplasma* in pork delicatessen and 3/ the molecular characterization of zoonotic *Cryptosporidium* spp. and *Giardia duodenalis* in sheep in North Africa. This study is the first to report identification of both *Cryptosporidium* and *Giardia* from lambs. The presence of zoonotic *C. parvum* subtype families (IIa, IId) and *C. ubiquitum*, as well as the zoonotic *Giardia duodenalis* assemblage A, indicates that lambs can serve as a potential reservoir for zoonotic transmission.
The OIE CC in Europe is part of a network comprising two other OIE CC in the same scope (Americas and Asia-Pacific), which collaborate according to the needs at regional levels.

PREVALENCE OF *Clonorchis sinensis* INFECTION IN RESIDENTS AND FISH IN CHINA

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Clonorchis sinensis is one of the most important zoonotic parasites affecting public health in the northeast of China, especially in Jilin province. To investigate the infection status with *C. sinensis* in Residents and fish, stool samples and freshwater fish were collected in Jilin Province. The stool samples were individually examined by Kato-Katz thick smear method and the eggs of *C. sinensis* were detected. Statistical method was used to analyze the relevant data (gender, age, food habits about raw fish, drink) from questionnaires. The overall prevalence of clonorchiasis by gender and the age-prevalence was evaluated. The food habits about raw fish showed significant impact. The metacercariae of *C. sinensis* and *Metorchis orientalis* were detected in 10 species of fishes. The highest burden of these 2 trematodes were both *Pseudorasbora parva*. It was confirmed that *C. sinensis* is still high prevalent in residents and fish in Northeast of China.

NOVEL PERSPECTIVES FOR DIAGNOSIS AND TREATMENT
Moderator: Dušan Lalošević

ORAL PRESENTATIONS

DIFFERENTIAL PROTEIN EXPRESSION IN THE EARLY HOST-PARASITE INTERACTION IN FASCIOLOSIS: TOWARDS NEW VACCINE CANDIDATES

David BECERRO-RECIO[1], Judit SERRAT[1], Marta LÓPEZ-GARCÍA[1], Javier SOTILLO[2], Fernando SIMÓN[3], Krystyna CWIKLINSKI[4], John Pius DALTON[4], Javier GONZÁLEZ-MIGUEL[1], Mar SILES-LUCAS[1]

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Background. *Fasciola hepatica* is a worldwide-distributed parasite causing fasciolosis, a helminthiasis responsible for substantial economic losses in agriculture, as well as a relevant human health problem in endemic areas. As pharmacologic treatment is becoming progressively less effective, vaccines are a tractable and convenient strategy to combat the disease, although trials to date have not reached the desired effectiveness.

Objectives. This study aims to characterise the early host-parasite interface between *F. hepatica* Newly Excysted Juveniles (FhNEJ) and their mammalian host after a short time of stimulation, similar to that described for FhNEJ to pass through the intestinal wall. This is expected to define a variety of proteins relevant for the first close contact between both organisms.

Material and Methods. Both FhNEJ and Mouse Primary Small Intestinal Epithelial Cells (MPSIEC) proteomic profiles were analysed before and after co-culture using Sequential Window Acquisition of All Theoretical Mass Spectra (SWATH-MS), a label-free quantitative proteomic approach which stands out for its precision and reproducibility, and further analysed using different downstream bioinformatic techniques.

Results. A total of 300 differentially expressed proteins were detected between both organisms (171 belonging to FhNEJ and 129 from MPSIEC), highlighting the importance of processes such proteolysis, cytoskeleton reorganisation or energetic metabolism in *F. hepatica*, as well as gene transcription, vesicle transport, cell adhesion and response to oxidative stress in mouse intestinal epithelium.

Conclusion. The data obtained in this study is expected to provide a source of new molecular targets for future vaccines aimed at the *F. hepatica* juvenile stage, along with a deeper understanding of the early host-parasite interface, which serves as a starting point for future studies in the field.

Funding source. Projects AGL2015-67023-C2-2-R and PID2019-108782RB-C22 funded by MCIU, AEI and FEDER

THE ACHILLES' HEEL OF THE FOX TAPEWORM? - INVESTIGATION OF THE THREONINE METABOLISM OF *Echinococcus multilocularis*

Marc KAETHNER[1], Philipp GROSSENBACHER[2], Martin LOCHNER[2], Stefan SCHÜRCH[3], Clement REGNAULT[4], Daniel VILLALOBOS RAMIREZ[5], Britta LUNDSTRÖM-STADELMANN[1]

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Background. Alveolar echinococcosis (AE) is a severe zoonotic disease caused by the metacestode larvae of the fox tapeworm *Echinococcus multilocularis*. Treatment options are the surgical removal of all parasitic tissue, or lifelong treatment with benzimidazoles, since these drugs fail to kill the stem cells of the parasite. New drugs are urgently needed as treatment might fail and adverse side effects of benzimidazoles can lead to treatment discontinuation.

Objectives. Recent *in vitro* experiments elucidated that besides glucose, *E. multilocularis* metacestodes scavenge high amounts of threonine from the culture medium. This work aims to clarify the role of threonine metabolism in *E. multilocularis*. Threonine dehydrogenase (TDH) is known to metabolize threonine in other organisms, and the enzyme is actively expressed in *E. multilocularis* metacestodes. EmTDH is potentially an interesting future drug target against AE, as human TDH is a pseudogene.
Material and Methods. We currently trace $^{13}C_4$ L-threonine and its metabolites in *in vitro* cultured metacestodes to give new insights into how threonine is metabolized in *E. multilocularis*. Additionally, we investigate a potential effect of L-threonine on the growth rate of *in vitro* cultured *E. multilocularis* metacestodes, which will be tested using an automated script in ImageJ.
EmTDH will be recombinantly expressed and its activity will be measured by NAD+ reduction. Subsequently, we will treat EmTDH with quinazoline carboxamide inhibitors in a target-based approach and also against *E. multilocularis* by various established *in vitro* tests in a whole-organism based approach.
Results. A metacestode growth assay was developed to precisely measure the growth of individual metacestodes over time using an automated script in ImageJ.
Conclusion. The flux analysis with $^{13}C_4$ L-threonine will clarify the role of L-threonine concerning energy generation and purine synthesis in *E. multilocularis*. The newly developed metacestode growth assay allows the quantification of metacestode growth in the upcoming experiments.

DUAL INHIBITION OF THE *Echinococcus multilocularis* ENERGY METABOLISM

Sheena CHAUDHRY[1], Raphael ZURBRIGGEN[1], Stone DOGGETT[2], Britta LUNDSTRÖM-STADELMANN[1]
[1]sheena.chaudhry@vetsuisse.unibe.ch Institute of Parasitology, Vetsuisse Faculty, University of Bern, Switzerland;
[2]Oregon Health and Science University, Portland, Oregon, USA

Background Alveolar echinococcosis is caused by the metacestode stage of the zoonotic parasite *Echinococcus multilocularis*. Current chemotherapeutic treatment options rely on benzimidazoles, which have limited curative capabilities and occasionally cause severe side effects. Therefore, alternative treatment options are urgently needed. We know that the parasite relies on energy generation by two mitochondrial pathways functioning in parallel: The electron transfer chain (ETC) and the malate dismutation (MD)[1]. We have further investigated the energy metabolism of *E. multilocularis* as a potential target.
Methods 15 Endochin-like quinolones (ELQs) were repurposed for inhibition of the ETC: They were screened *in vitro* against two isolates of *E. multilocularis* metacestodes and isolated germinal layer cells by the phosphoglucose isomerase (PGI) assay and the CellTiter Glo assay respectively. For the most active ELQs, EC_{50} values against metacestodes were assessed by PGI assay, and IC_{50} values against germinal layer and mammalian cells were assessed by CellTiter Glo and Alamar Blue assay. Further focus was laid on ELQ-400, and it was shown with the Seahorse XFp Analyzer that cytochrome bc1 complex is a direct target of ELQ-400 in *E. multilocularis*. When tested under microaerobic conditions, ELQ-400 was not active against metacestodes and increased succinate levels could be measured. This suggested the switch to MD for energy generation.
Therefore, MD was also inhibited by the previously described experimental compound quinazoline[2], and effects on metacestodes were assessed by PGI assay and succinate measurements. Quinazoline alone did not induce any damage to the metacestodes under microaerobic conditions either, it reduced the production of succinate compared to control treated parasites (i.e. inhibited the MD), and it strongly improved the activity of the bc1 inhibitor ELQ-400 when applied in combination.
Conclusion Targeting the energy metabolism of *E. multilocularis* as a possible novel treatment approach can only be successful if both pathways are blocked simultaneously.

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ACTIVITY AND MECHANISM OF ACTION OF MEFLOQUINE DERIVATIVES AGAINST *Echinococcus multilocularis*

Roman MEMEDOVSKI[1], Reto RUFENER[1], Raphael ZURBRIGGEN[1], Sophie BRAGA[2], Manfred HELLER[2], Joachim MÜLLER[1], Marcus Vinicius Nora DE SOUZA[3], Britta LUNDSTRÖM-STADELMANN[1]

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Background. Alveolar echinococcosis (AE) is a zoonosis caused by the fox tapeworm *E. multilocularis*. AE is caused by the metacestode (secondary larval stage), exhibiting silent invasive growth primarily in the liver and metastatic potential. The current chemotherapeutic treatment is based on benzimidazoles, which have only limited curative capabilities and can cause severe side effects in some cases. Therefore, novel treatment options for AE are needed. Mefloquine, an antimalarial agent, had previously been shown to be active against various helminth species, and also showed activity against *E. multilocularis* metacestodes *in vitro* and *in vivo* (1). Mefloquine was shown to bind to *E. multilocularis* ferritin, an iron-storage protein, which sequesters ferrous iron (2). However, whether this interaction is relevant for the mechanism of action of mefloquine is not known.

Material and Methods. We here present a structure-activity relationship study of mefloquine derivatives and their physical interaction with *E. multilocularis* proteins. Three different assays were employed: the phosphoglucose isomerase assay (integrity of metacestodes) with 24 compounds, the Alamar blue assay (metabolic activity of metacestodes) with 17 compounds, and the motility-based activity assessment of protoscoleces with 15 compounds. Comparative affinity chromatography of mefloquine, one active (Mef-3) and two non-active mefloquine derivatives (Mef-13 and Mef-22) was performed, and bound metacestode proteins were analysed by LC-MS/MS.

Results. None of the derivatives showed higher activity compared to mefloquine in the three different assays described above. However, nine compounds caused limited physical damage in metacestodes and four of them slightly impaired the movement of protoscoleces at high concentrations. LC-MS/MS results showed 1`460 unique cell proteins, of which 22.7% and 16.2% bound exclusively to mefloquine and Mef-3, respectively. From the pool of proteins that bound exclusively to mefloquine, the top 20 proteins were those related to either energy production or cytoskeletal processes.

Conclusion. My results qualitatively show that mefloquine and Mef-3 may act on different targets within the metacestodes and that mefloquine may also harbour other important targets besides ferritin.

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GENETIC DIVERSITY IN THE METRONIDAZOLE METABOLISM GENES NITROREDUCTASE 1 AND 2 IN SUSCEPTIBLE AND REFRACTORY CLINICAL SAMPLES OF *Giardia lamblia*

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Background. The effectiveness of the antibiotic prodrug metronidazole against the tetraploid intestinal parasite *Giardia lamblia* is dependent on its activation/inactivation within the cytoplasm. There are several activating enzymes, including nitroreductase (NR) 1 that can metabolize metronidazole into toxic forms, while

NR 2 is inactivating it. Metronidazole treatment failures has been increasing over the last years, indicating genetic resistance mechanisms. Analyzing genetic variation in the NR genes in susceptible and refractory *Giardia* isolates may help identify potential markers of resistance.
Material and Methods. NR1 and NR2 genes from 7 clinical culturable isolates and 8 non-cultured samples from *Giardia* assemblage B were cloned into *Escherichia coli*. Vectors from successful transfections were sequenced and single nucleotide variations (SNVs) were analyzed to assess genetic diversity and determine individual alleles.
Results. A high, but similar, ratio of SNVs per gene length was found for both of the genes, 11.2% (NR1) and 10.7% (NR2). Of these, 4.3% (NR1) and 6.6% (NR2) were non-synonymous SNVs. The majority of the samples had 1 to 4 alleles of the genes. However, more than four alleles, were found in five isolates for NR1 six isolates for NR2. Three deletions were found in the MTZ inactivating gene, NR2, causing a frameshift mutation and a truncation in three of the presumed susceptible isolates. The dominant allele in one refractory isolate resulted in a truncation of the MTZ activating gene NR1.
Conclusion. The considerable variation, and findings of mutations leading to dysfunctional NR1 and 2 proteins in the present study of assemblage B *Giardia* show the potential for genetic markers for metronidazole susceptibility and resistance. Dysfunctional NR2 enzymes may lead to susceptibility towards MTZ, while dysfunctional NR1 enzyme may protect the parasite. Future studies, including functional laboratory experiments and clinically well-defined patient cohorts, are necessary to examine the importance of these genetic alterations.

COMPUTATIONAL IMAGE ANALYSIS REVEALS THE STRUCTURAL COMPLEXITY OF *Toxoplasma gondii* TISSUE CYSTS

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Background. *Toxoplasma gondii* is an obligate intracellular parasite infecting up to one third of the human population. The central event in the pathogenesis of toxoplasmosis is the conversion of tachyzoites into encysted bradyzoites. A novel approach to analyze the structure of *in vivo*-derived tissue cysts may be the increasingly used computational image analysis.
Objectives. This study aimed to quantify the geometrical complexity of *T. gondii* cysts by morphological, particle, and fractal analysis, as well as to determine if the structure is impacted by parasite strain, cyst age, and host type.
Material and Methods. A total of 31 images of *T. gondii* brain cysts of four type-2 strains (Me49, and local isolates BGD1, BGD14, and BGD26) was analyzed using ImageJ software. The analyzed parameters included diameter, circularity, packing density (PD), fractal dimension (FD), and lacunarity.
Results. Although cyst diameter varied widely, it was negatively correlated with PD. Circularity was remarkably close to 1, indicating a perfectly round shape of the cysts. PD and FD did not vary among cysts of different strains and age, nor among those derived from mice of different genetic background. Conversely, lacunarity, which is a measure of heterogeneity, was significantly lower for BGD1 strain vs. all other strains, and higher for Me49 vs. BGD14 and BGD26, but did not differ among Me49 cysts of different age, or those derived from genetically different mice. The results indicate a highly uniform structure and occupancy of the different *T. gondii* tissue cysts.
Conclusion. This study furthers the use of image analysis in describing the structural complexity of *T. gondii* cyst morphology, and presents the first application of fractal analysis for this purpose. The presented results show that use of a freely available software is a cost-effective approach to advance automated image scoring for *T. gondii* cysts.

Funding source: This study was supported by the Ministry of Education, Science and Technological Development of Serbia, through grant (contract № 451-03-68/2020-14/200015) to Institute for Medical Research, University of Belgrade, Serbia.

IN SILICO CHARACTERIZATION OF THE *Ixodes ricinus* AV422 SALIVARY PROTEIN IMMUNOGENICITY

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Background. *Ixodes ricinus* AV422 is a well-conserved salivary protein, secreted during the early stage of tick feeding. Immunogenicity of AV422 was demonstrated by serological testing, so it represents a good candidate for tick bite confirmation and new generation anti-tick vaccine design. To further validate AV422 immunogenic properties, a detailed *in silico* characterization of AV422 antigenic determinants (T and B cell epitopes prediction) was performed, together with modelling the predicted epitope:top-binding MHC II molecules interactions by molecular docking.

Material and Methods. AV422 immunogenicity was assessed by predicting T-cell epitopes in TepiTool (with allele MHC class II and Human host restriction parameters) and linear B-cell epitopes by available methods on IEDB server. For T-cell epitopes, cross-reactivity and conservancy was analyzed by blasting against the human proteome and the top-scoring homologues from other tick species, respectively. Globally-blind docking of the AV422 T-cell epitopes to the top-binding MHC II molecules was performed in MDockPeP, which samples flexible peptide conformers over the whole protein surface. Visualization of docked interactions was performed in BIOVIA Discovery Studio Visualizer.

Results. *In silico* analysis of AV422 sequence identified the most probable antigenic AV422 determinants in the regions between ~ 15 – 30 AA and ~ 180 – 205 AA of the mature protein sequence, which are highly conserved across different tick genera and display no cross-reactivity with human proteins. Docking analysis positioned predicted T-cell peptides exactly within the MHC II binding-grooves, in accordance with the common geometry of MHC II-epitope presentation.

Conclusion. *Ixodes ricinus* AV422 protein sequence *in silico* analysis enabled the prediction of highly-immunogenic, highly-conserved and non-toxic AV422 regions, prompting this salivary protein as a good candidate for tick bite confirmation and also in new generation anti-tick vaccine design.

Funding source: This work was supported by the Ministry of Education, Science, and Technological Development, Republic of Serbia (Project № 173006, Contract № 451-03-9/2021-14/200015, Contract № 451-03-9/2021-14/200178).

PALEOPARASITOLOGY
Organizers / Moderators: Matthieu Le Bailly & Gholamreza Mowlavi

INVITED LECTURES

NEW APPROACHES AND DATA SYNTHESIS IN PALEOPARASITOLOGY. 15 YEARS OF STUDIES IN THE NEOLITHIC PERIOD

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Paleoparasitological studies conducted for more than 15 years on Neolithic sites in humid contexts have provided a great deal of data, which contribute to a better understanding of the sanitary state and, more broadly, of the lifestyle of prehistoric European populations. Organic waste management, food and subsistence economy, or human/animal/environment interactions, are issues to which paleoparasitology brings elements of answers complementary to other archaeological and bioarchaeological studies.
The most recent studies have brought into play tools for improving diagnosis, such as paleogenetics, whose use is crucial for the future. Similarly, in the processing of data at the scale of a site, the use of spatialization shows a strong potential by allowing a better integration of paleoparasitological studies with archaeological problems.
On a larger scale, the compilation of data makes it possible to define distribution areas and to answer questions relating to the pathoecological status of certain parasites such as the fish tapeworm, genus *Diphyllobothrium*. Furthermore, variations in parasite frequencies or indices related to the relative importance of parasites, particularly in the Alpine arc, seem to be linked in part to economic choices, but also to regional climatic changes.

PARASITE INFECTION IN THE ROMAN PERIOD: CHANGE OVER TIME FROM PRE-ROMAN TO THE MEDIEVAL PERIODS

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The aim of this study is to characterize human parasite diversity across the Roman Empire, and to compare this with earlier and later time periods. This allows us to determine if there were changes in parasite species causing infections in the Roman period which may have been a result of the expansion of the Roman Empire and subsequent cultural and social changes. We will present new evidence from understudied regions and time periods including Neolithic and Roman period Turkey, Bronze Age Britain, as well as Roman period sites in the Mediterranean. This data is combined with previously published studies to begin to compare taxonomic diversity in the pre-Roman period, the Roman period, and the post-Roman period. Preserved parasite eggs and cysts were identified from archaeological sediments using microscopy and enzyme-linked immunosorbent assay (ELISA). Current data suggests a taxonomic shift in parasite presence from the pre-Roman to Roman period, characterized by a decrease in zoonotic parasites and an increase in parasites transmitted by faecal contamination of food and water, including roundworm and protozoa which can cause dysentery. The taxonomic diversity seen in the Roman period is very similar to that in the Medieval period. We will discuss factors that may have contributed to the establishment of the parasite diversity found in the Roman period including diet, urbanization, and human-animal interactions.

PALEOPARASITOLOGY AND NEW PRACTICAL PERSPECTIVES

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Paleoparasitology as an Interdisciplinary scientific approach may shed light on some hidden points of human life that might be more attractive than the merely retrieved parasites of ancient times. Since the various biotic and abiotic elements are involved in establishing the parasite's life cycles, the proven existence of a specific parasite in a period of distant past indicates the presence of the lifecycle's companions of that parasite at that time. Taking advantage of modern techniques in analyzing ancient genomes, will definitely increase our capabilities of scientific interpretation in describing diverse biological agents that participated in the parasites' evolutionary stages since their emergence on the earth. Meanwhile, through the wide access that we may have to plants genomes and those of other biological agents in coprolites, traditional and herbal anthelmintic therapies can be also discussed in ancient times. The extricated biotic elements that might have been involved in the lifecycle of certain parasites millenniums ago is another exclusive finding using hi-tech possibilities. The attractive topics agendas of the World Health Organization like "emerging and re-emerging infections" can also be discussed herein based on paleo parasitological evidence.

ORAL PRESENTATIONS

A COMBINED APPROACH TO STUDY PARASITES IN THE PAST: MICROSCOPY AND PALEOGENETICS

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Background. The study of parasites in the past has traditionally focused on the recovery of gastrointestinal worm eggs disseminated in past environments or archaeological structures by infested hosts. Contrary to other fields of paleoecology, the search for ancient DNA (aDNA) still remains under explored when it comes to parasites.
Material and Methods. We review here the past 20 years of development of molecular paleoparasitology and show its very recent advent in the last couple of years. We illustrate the state of the art by recent contributions of our group.
Results. We show how classical and molecular approaches complement each other, based on the opportunity to look for different kinds of remains in ancient environmental samples. Overlapping and complementary results plead for multi-proxy approaches in order to have a better comprehension of past parasitic diversity in ancient communities and environments from the past to the present.
Conclusion. As previously observed in other fields of paleoecology, the advent of paleogenetics in paleoparasitology tends to strongly complement classical proxies rather than replacing them. Meanwhile, farther than monitoring species presence/absence through time, major contributions of aDNA studies regarding molecular evolution and ancient paleogenomics still remain scarce.

THE STATUS OF PARASITIC INFECTION IN CHEHRABAD SALT MINE

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Background: Understanding of parasitic diseases in the past can help us to evaluate the status of health issue, the evolution of parasitic infections as well as the dominant diet and habits in ancient populations. The

appropriate preservation condition in Chehrabad archaeological salt mine, provided a unique opportunity to find the intact parasite eggs mostly in coprolites dated back to Sassanian and Achaemenid empires.
Material and Methods: Eighty paleofeces were selected during the excavation period of Chehrabad salt mine 2016. Origin and dating of the collected samples were determined by archeologisats in Bochum University. Five grams of each coprolite was rehydrated by Tri Sodium Phosphate (TSP) 0.5% W/V solution (5 times the volume of feces) and left for 10 days at room temperature. Liquid Glycerin-Gel solution was used to mount the picked up samples on slides. At least 50 microscopic slides were prepared and studied under the microscope for each. All retrieved parasites were photographed and measured using Olysia software.
Results: 21 Herbivores, 27 Donkeys, nine birds and some unknown faeces were found parasitized with Strongyles, Ascaris spp., *Paraascaris* sp., Tricocephala, *Taenia* spp., *Ascaris* spp., *Fasciola hepatica*, *Paraascaris* sp., *Anoplocephala* sp. And *Acantocephala* sp. Besides free living nematodes, Dictyocaulus, *Eimeria lukarti* and insects particles.
Conclusion: This is worth mentioning that appropriate preservation condition is of fundamental priorities to perform paleoparasitological investigations upon the coprolites of humans and animals in archaeological sites worldwide. The intact structural appearance of the eggs and larval stage of identified parasites in Chehrabad salt mine can be in favour of this fact.

FOODBORNE AND WATERBORNE PARASITES: CHANGING CLIMATE, CHANGING TRENDS, CHANGING PARASITES
Organizers / Moderators: Lucy Robertson & Joke van der Giessen

INVITED LECTURES

EFFECT OF CLIMATE AND ENVIRONMENTAL CHANGES ON THE DISTRIBUTION OF WBP/FBP

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Waterborne and foodborne parasite infections still affect public health across the world. Climate change is expected to influence infection rates, for example during extreme precipitation events when more parasites from manure applied to agricultural land are washed into the water used for recreation, or during droughts when more surface water irrigation can contaminate fresh produce. In this presentation, I will present the fate and transport of parasites, such as *Cryptosporidium*, from faeces and manure through the environment into the water and onto the food. Additionally, I will show the impact of socio-economic development and climate change on the fate and transport. My team uses models to simulate the loads of parasites to the water, the concentrations in the water and health risk due to exposure to contaminated water or fresh produce, for example after irrigation. These models use underlying understanding of processes and data on population, urbanisation, prevalence of infection, sanitation, waste water treatment, livestock numbers, agricultural management, hydrology and fate and transport processes. We use scenario analysis to assess potential future changes. These scenarios are built based on Shared Socio-economic Pathways and Representative Concentration Pathways from the intergovernmental panel on climate change and present a set of plausible futures for development of population, GDP, waste water treatment, temperature, precipitation and hydrology. The modelling and scenario analysis approach can be applied at different spatial and temporal scales and resolutions, from global to city scale, and I will provide several examples. Our approach is useful to better understand potential future changes and to improve understanding of possible interventions and adaptation strategies. Additionally, the approach is valuable for evaluating progress towards the Sustainable Development Goals.

ARE SOURCE ATTRIBUTION AND TRANSMISSION OF FOODBORNE PARASITES INFLUENCED BY CLIMATE CHANGE?

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Background. Attributing human cases of foodborne diseases, including those caused by foodborne parasites (FBPs), to putative sources and transmission pathways is crucial to identify targets for public health interventions. Beyond epidemiological methods like case-control studies and outbreak investigations, several microbiological approaches to source attribution are available and mainly applied to bacteria. These are often based on microbial subtyping data and quantitative microbial risk assessment (QMRA). Ideally, source attribution methods should also be able to capture trends and other changes in a pathogen's epidemiology. Methodologically, this is not straightforward, as source attribution methods tend to provide static outcomes.
Objectives. To provide an overview of source attribution methods applicable to FBPs, focusing on their ability to describe deviations from the usual epidemiological pattern in response to climate change, which may alter the living conditions for almost every species and affect FBPs in different ways.
Material and Methods. Both top–down and bottom–up source attribution approaches are applicable to FBPs. Top–down approaches assign human cases back to their sources of infection using epidemiological and/or microbiological data. Conversely, bottom–up approaches like comparative exposure assessment start from the contamination levels (prevalence and concentration) of a given pathogen in a source, and then

go upwards in the transmission chain incorporating factors concerning production and consumption. Other approaches are intervention studies and expert elicitations.
Results. There is no single method that satisfies all situations and needs for all FBPs in response to climate change, but combining different approaches or applying them comparatively seems to be the best strategy.
Conclusion. FBPs have varying sources and transmission routes and climate change can affect them in different ways. Methods to capture these changes should be considered based on the type, quality and quantity of data, the research question being addressed, and the (epidemiological and biological) characteristics of the pathogen.

RISK RANKING AND EVALUATION OF SURVEILLANCE SYSTEMS FOR FOODBORNE PARASITES IN EUROPE

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European Network for Foodborne Parasites in Europe

In 2012, foodborne parasites (FBP) were prioritized using multicriteria decision analyses, an approach often used to rank infectious and other diseases in a way that comparisons can be made. Of the twenty-four foodborne parasites of global concerns, *T. solium* ranked the highest followed by *Echinococcus multilocularis*, *E. granulosus* and *T. gondii*. All of which are zoonotic. One of the main recommendations was that the regional ranking could be very different from the global ranking and the same exercise should be carried out at the regional level. During a COST Action by the European network of foodborne parasites, the same methodology was repeated. *Echinococcus multilocular* was the highest ranked FBP followed by *T. gondii, E. granulosus* and *T. Spiralis*. Also here all were zoonotic and ranking could differ per region in Europe. The foodborne parasites of the top five list was subsequently used to give an overview of surveillance and reporting systems in Europe in the human and animal populations. Information on the surveillance systems was collected from 35 European countries and analysedaccording to the five different regions. Human surveillance is passive in most countries and regions in Europe and differs between countries and regions. Only for *T. spiralis*, being notifiable in 34 countries with active surveillance in susceptible animals under EU directive. It was concluded that although human and animal surveillance data are available for the five prioritised FBP, surveillance and reporting requirements between national experts and European bodies were not consistent. Recommendations for improved surveillance systems are discussed.

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CASE STUDY: UNUSUAL TOXOPLASMOSIS CASES IN SERBIA POTENTIALLY ASSOCIATED WITH IMPORTED MEAT

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Toxoplasmosis is the most common parasitic zoonosis worldwide, affecting one third of the global human population. Transmission occurs by ingestion of *Toxoplasma gondii* tissue cysts from undercooked meat and meat products, or oocysts from the environment with contaminated fresh produce or water. The WHO and FAO have recently declared toxoplasmosis as a foodborne infection of global concern, with a disease burden the greatest of all parasitic infections. Insight into the organism's population structure, which includes both clonal and atypical strains, is changing the view of toxoplasmosis as a generally innocuous infection adversely affecting specific categories including the fetus and immunosuppressed individuals, to a potentially serious disease depending on the genotype of the infecting strain. Indeed, atypical strains have been associated with more severe, often atypical ocular toxoplasmosis (OT), and even life-threatening disease, in both immunocompetent and immunosuppressed individuals. Moreover, cases of congenital toxoplasmosis in babies born to immunized mothers, re-infected with atypical strains, challenge the paradigm of congenital toxoplasmosis. In Serbia, like elsewhere in Europe, all typed strains

belong to type II and III, archetypes or, more often, variants. However, one atypical strain that led to a fatal outcome has been isolated from a teenage HSCT recipient who had never travelled outside the region. Attempts to explain the presence of this genotype in Serbia may require looking at the globalized world; movement of people but also of live animals and foods including meat (potentially contaminated with *T. gondii*) favors the spread of diverse strains out of the areas to which they are autochtonous. Another case probably associated with an atypical strain (but which unfortunately has not been isolated nor typed) was that of a young woman who, only weeks after a trip to the island of Mauritius, developed lymphoglandular toxoplasmosis simultaneously with bilateral OT which resulted in unilateral legal blindness.

INFLUENCE OF CLIMATE CHANGE ON THE OCCURRENCE OF CRYPTOSPORIDIOSIS OUTBREAKS IN EUROPE

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Species in the genus *Cryptosporidium* are a major cause of gastrointestinal infections in vertebrate hosts, including humans. The epidemiology of human cryptosporidiosis is complex, and involves direct (person-to-person and animal-to-person) and indirect (through water, food and fomites contaminated with infectious oocysts) transmission routes. *Cryptosporidium* is environmentally persistent and even few oocysts can cause infection. Disease incidence patterns show a distinct seasonality and strong associations with weather globally. Global climate change has the potential to severely affect human health, including diarrheal diseases, which are highly affected by environmental drivers such as water availability. In particular, ambient temperature, heavy rainfall, drought, and flooding are the meteorological conditions that are expected to increase with climate change. These effects are likely to be even stronger in countries with lower water and sanitation standards, where they are exacerbated by population growth. In Europe, many waterborne outbreaks have been reported, caused by the two major human pathogens *Cryptosporidium parvum* and *C. hominis*, and associated with drinking and recreational waters (mainly swimming pools), food consumption, animal contact, outdoor activities and person-to-person spread in the home and institutions. A comprehensive analysis of the impact of climate change on the occurrence of outbreaks of cryptosporidiosis in Europe is lacking. Nevertheless, the event that occurred in Halle, Germany, during 2013, showed that even in countries with high standards of sanitation, adverse climatic events (river flooding in the specific case) can cause epidemics of cryptosporidiosis.

EMERGENCE OF BILHARZIASIS IN CORSICA: WHERE ARE WE NOW?

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Summer 2013, an outbreak of urogenital Bilharziasis emerged in the south of Europe. More than 100 cases have been diagnosed in French hospitals, all of which having water contact in the Cavu river, in southern Corsica. Mathematical modeling has shown that more than 300 people were infected in the summer of 2013. After the river was closed in summer 2014, further independent events of contamination took place in the following years. Our first work consisted of a genetic characterization of the parasite, and we showed that the incriminated pathogen was a hybrid between a human parasite (*S. haematobium*) and an animal (*S. bovis*) parasite. Subsequently, several field studies and laboratory experiments were carried out in order to understand this epidemic. Despite our best efforts, the occurrence in summer 2019 of new case in another river (the Solenzara) shows that we have not been able to contain this epidemic and that many questions remain unanswered. The difficulty we have to contain the epidemic in a well-developed country shows that it will be particularly difficult to eradicate this disease.

TRANSMISSION OF *Echinococcus multilocularis* – NEW IDEAS EMERGING?

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Alveolar echinococcosis (AE), caused by *Echinococcus multilocularis*, belongs to the most serious parasitic zoonoses in the northern hemisphere. Although there appears to be a high degree of resistance against the development of AE in humans, the severity of disease and the unsatisfactory options for treatment and control are a matter of public health concern. Preventive measure should be a matter of priority, but data on the transmission pathways of this pathogen to humans are limited, mainly because the time lapse between the infection event and the onset of clinical symptoms may be longer than a decade and appears to vary drastically among individual patients. This effectively prevents any reliable source attribution and restricts preventive measures to basic recommendations on hygiene behaviour in order to reduce the contact with parasite eggs from the environment. To estimate the risk for human AE, numerous studies attempted to correlate human AE incidence with epidemiological parameters e.g. the number and species of infected definitive hosts, human contact with putative transmitting animals, human behaviour and attitude, the role of food as infection source, climatic conditions, and the genotype of the parasite. Here, an attempt is made to summarize the evidence for different routes for transmission and translocation of *E. multilocularis* in Europe and other regions of the northern hemisphere.

ORAL PRESENTATIONS

TRANSMISSION OF *Fasciola hepatica* FROM THE INTERMEDIATE HOST *Galba truncatula* TO THE DEFINITIVE HOST *Ovis aries* UNDER DROUGHT CONDITIONS IN A DANISH NATURE AREA

Leah LOURENÇO[1], Mita SENGUPTA[1,2], Nao TAKEUCHI-STORM[2], Stig THAMSBORG[2], Anna-Sofie STENSGAARD[1]
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Background. *Fasciola hepatica* is a snail-borne, parasitic trematode that causes fasciolosis in livestock and a range of wildlife species. In Denmark, recent studies have shown that the prevalence of fasciolosis has been rising in cattle. Additionally, forty years ago the dominant transmission pattern of *F. hepatica* was found to be the "summer infection" in snails.
Objectives. This study aimed to investigate the prevalence and seasonal transmission patterns of *F. hepatica* in both the snail and sheep hosts in a fasciolosis hotspot by 1) describing the infection status and population dynamics of the intermediate host (*Galba truncatula*), and 2) investigating the exposure of naïve lambs to *F. hepatica*.
Material and Methods. Samples from 22 lambs were collected every six weeks (June-December 2018) and analysed through sedimentation and ELISA. Snails were collected monthly at 7 sites in the area (April-December 2018), and snail shell morphology was used to identify *Galba* (n=321) and *Galba*-like snails (n=32). Snail infection status was done molecularly by targeting the liver fluke ITS-2 region on pooled snail DNA samples and COX-1 partial gene on individual snails of positive pools. To verify *G. truncatula* species identification, the Lymnaeid ITS-2 region was sequenced from the *Galba*-like snails and two snails from each site and time period.
Results. Prevalence of *F. hepatica* in *G. truncatula* was 0.9%. Although 90% of adult ewes had patent infections, only one lamb became infected. ELISA results indicate seroconversion occurred by August.
Conclusion. Seroconversion of the infected lamb may be a case of "winter infection." The low transmission was probably relayed to the dry, hot summer of 2018 and illustrates what the epidemiology of fasciolosis in Denmark may look like under future climate conditions.

FROM FEED TO FORK: CONTAMINATION OF LETTUCES BY EGGS OF *Echinococcus multilocularis* AND OTHER TAENIDAE SPECIES

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Background. Although few, scattered and heterogeneous data are available on the pathways of transmission of *Echinococcus multilocularis* (*Em*) to humans, food-borne is considered as one of the most important drivers. This study aims to produce data on the contamination of lettuce by *Em* and other *Taenidae* species.
Material and Methods. Specific PCR were settled for the analysis of pools of pellets obtained after sieving of 106 fresh lettuces collected in 2020 and 2021 from private kitchen gardens or local markets from French high endemic regions for *Em*.
Results. The limit of detection for *Em* was established at three eggs for 300g of lettuce. In 2019, two out of 35 pools of lettuce were positive for *Em* both from local markets, while six others were positive for *Hydatigera* sp., mainly from private kitchen gardens. Analyses of lettuces collected in summer 2021 will be available in September 2021.
Conclusion. Even if the viability of the eggs can't be proved, the detection of *Em* suggests that lettuce can be a potential source of infection as also highlighted by the detection of *Hydatigera* sp. A European multicentre study conducted in 13 countries was launched this summer inside MEME project to obtain data from different epidemiological settings.

Funding source: MEME project is supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement № 773830: One Health European Joint Programme.

FOODBORNE TREMATODE *Opisthorchis felineus* INFECTION: MECHANISTIC INSIGHTS INTO BILIARY NEOPLASIA FORMATION

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Backgroud. Opisthorchiasis caused by the foodborne trematode *Opisthorchis felineus* is a substantial public health problem in Eastern Europe and Russia, with 1.7 million persons infected worldwide. This disease is associated with hepatobiliary pathologies, including cholangiocarcinoma among chronically infected individuals. Molecular mechanisms of carcinogenesis by parasite infection are not well studied.
Material and Methods. To provide in-depth research and to gain insights into the mechanism by which *O. felineus* infection causes precancerous liver lesions we investigated (i) the profiles of chronic inflammation and fibrogenesis markers in the dynamics of opisthorchiasis by Western blot and immunohistochemistry; (ii) differential gene expression analysis of liver transcriptomes (Illumina HiSeq2500) of golden hamsters infected with *O. felineus* at 1 and 3 months postinfection was carried out as well as (iii) characterization of the parasitic extracellular vesicle (EVs) protein composition was performed.
Results. Principal components analysis revealed high clustering of samples by infection and by the time in infection. Gene Ontology annotation and functional enrichment analysis indicated that all of the differentially expressed genes were enriched in biological processes, molecular function and KEGG pathway of TGF-beta, MAPK, P53 signaling, cell cycle and cancer-associated pathways. EVs (50–150 nm in size) contained about 300 pathogen-specific proteins, many of them lack signal sequences, as well as canonical exosome marker proteins, such as Alix, syntenin, HSP70, and tetraspannins.
Conclusions. Our data provide knowledge about global changes in gene expression of *O. felineus*-infected host liver. These studies suggest that increased TGF-beta, MAPK, P53 signaling may contribute to the biliary

neoplasia formation associated with *O. felineus* infection. Further research must be undertaken to elucidate the exact role of exosome proteins in the initiation of pathological processes during the infection.

Funding source: This work was supported by the Russian Science Foundation [18-15-00098].

TROPISM OF *Toxoplasma gondii* IN THE TISSUES OF EXPERIMENTALLY INFECTED PIGS

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Background. *Toxoplasma gondii* is a food-borne zoonotic parasite capable of infecting essentially all warm-blooded animals, including humans. With a majority of infections in Europe estimated to be meat-borne, pork, as one of the most consumed meats in the Czech Republic, represents a potential risk for consumers. Therefore, we aimed to investigate and evaluate the anatomical distribution of *T. gondii* within the tissues of experimentally infected pigs and to identify the predilection sites for the different *T. gondii* isolates and the two infectious stages used.
Material and Methods. A selected set of samples, representing 4 commonly consumed meat cuts and organs were collected from each of the total of 22 pigs. Animals were divided into 4 groups, with each group inoculated orally with a different combination of *T. gondii* isolates (genotype II or III) and sources of infection (400 oocysts or 10 tissue cysts).
Results. In the four complete sets of samples selected for the testing, DNA of *T. gondii* was confirmed by qPCR in 27.2 % (6/22) hearts, 54.5 % (12/22) loins, 54.5 % (12/22) hams and 50.0 % (11/22) shoulder samples. Animals infected by oocysts proved to be positive for *T. gondii* by qPCR in 25 % (10/40) of cases, compared to 64.6 % (31/48) of those infected by tissue cysts. Infection by genotype II and III resulted in 22.9 % (11/48) and 75 % (30/40) *T. gondii* positives by qPCR respectively.
Conclusion. Results suggest an uneven concentration of cysts within the tissues of the tested animals, with an unexpectedly low prevalence in hearts. Differences between the infection with oocyst and tissue cyst stages, as well as between the two different isolates of *T. gondii* parasites used in this study, provide an interesting comparison of distribution patterns of *T. gondii* based on the source of infection.

Funding source: This work is a part of TOXOSOURCES project, funded by the European Union's Horizon 2020 Research and Innovation programme under grant agreement No. 773830: One Health European Joint Programme.

HOT CLINICAL TOPICS IN TOXOPLASMOSIS
Organizer / Moderator: Florence Robert-Gangneux

INVITED LECTURES

PROPHYLACTIC AND TREATMENT APPROACHES FOR TOXOPLASMOSIS IN IMMUNOCOMPROMISED PATIENTS (ICP): CHALLENGES AND GAPS

Jose Gilberto MONTOYA

Toxoplasmosis can be a life-threatening disease or lead to severe and long-term sequelae in immunocompromised patients (ICP). However, toxoplasmosis can be effectively prevented and treated with effective drug regimens. The greatest challenge of toxoplasmosis in ICP is not identifying those at risk by universal screening with Toxoplasma IgG and IgM. Every patient (and solid organ transplant donors) should be tested for Toxo IgG/IgM as soon as their status of immune deficiency becomes known or before they become immunocompromised. This strategy can successfully identify those at risk for developing toxoplasmosis and candidates for prophylaxis and treatment. Toxo IgG+ (positive) patients with primary or acquired immunodeficiencies, cancer, organ transplants, or taking immunosuppressive drugs are at risk of toxoplasmosis by reactivation which can be effectively prevented with drugs such as TMP/SMX or atovaquone, or, by strategies such as weekly Toxo PCR to further identify those at higher risk of reactivation. Toxo IgG – (negative) solid organ transplant recipients who receive an organ from a Toxo IgG + (positive) donor, are at high risk of developing life-threatening toxoplasmosis which can be prevented with drugs such as TMP/SMX or atovaquone. Treatment of toxoplasmosis in ICP is best achieved with pyrimethamine/sulfadiazine/folinic acid. Alternative regimens include pyrimethamine/clindamycin/folinic acid, TMP/SMX, atovaquone/sulfadiazine, or a combination of pyrimethamine with atovaquone, or clarithromycin, or dapsone, or azithromycin. Gaps remain in not having additional and reliable intravenous regimens in addition to TMP/SMZ and clindamycin since highly effective drugs such as pyrimethamine, sulfadiazine, and atovaquone are only available in oral formulations. Another gap is the lack of clinical trials in non-AIDS ICP. The vast majority of clinical trials in ICP were performed in AIDS patients between early 1980's and late 1990'S. Prophylactic and treatment recommendations for non-AIDS ICP are primarily extrapolations from studies in AIDS patients. In this presentation, a strategy to fill these gaps is proposed.

TOXOPLASMOSIS IN TRANSPLANT RECIPIENTS: NEW EPIDEMIOLOGICAL TRENDS, DIAGNOSIS AND PREVENTION

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Toxoplasmosis is a well-known life-threatening disease in immunocompromised patients. Recent epidemiological data show that there is a trend towards a higher incidence of toxoplasmosis in non-HIV patients than in HIV-infected patients, particularly in countries with a high activity in transplantation. Despite serological screening of organ donors and recipients, which is widely performed in European countries, prevention guidelines in transplant patients differ according to regulations and health care policies of countries. A recent review of prevention practices in Europe for hematopoietic stem cell transplant (HSC) or solid organ transplant (SOT) patients showed that special attention was given to allo-HSCT and heart transplant patients. By contrast, kidney or liver transplant were frequently disregarded, even in case of *Toxoplasma* mismatch, but fortunately indirect protection was warranted in most patients thanks to anti-*Pneumocystis* prophylaxis which was widely provided. However, gaps in prevention still exist when cotrimoxazole is not used for *Pneumocystis* pneumonia prevention. On the other hand, the paradigm of cotrimoxazole prophylaxis scheme in HSCT patients, with a starting date at one month post transplantation is being challenged, as some teams have reported a high rate of reactivation of toxoplasmosis during the first month, balancing the potential threat of toxicity. Due to the poor outcome of toxoplasmosis in transplant patients, early diagnosis and tight follow-

up of patients are important to improve survival. Diagnosis relies on imaging, serology and DNA detection, but result interpretation must be done with caution. Allo-HSCT patient monitoring using weekly blood PCR is useful to start preemptive treatment, but is also a matter of debate. This talk will provide a review on the latest data of the literature.

A PROSPECTIVE STUDY OF THE INCIDENCE OF *Toxoplasma* INFECTION AFTER HSCT AND HEART TRANSPLANTATION

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Background. Toxoplasmosis is an often neglected and misdiagnosed opportunistic infection in transplant recipients, which, whether graft-transmitted or reactivated, can not only compromise engraftment, but evolve into life-threatening disseminated infection.
Objectives. We conducted an eight-year-long prospective study on the diagnosis and monitoring of *Toxoplasma* infection (TI) in haematopoietic stem cell transplant (HSCT) recipients in a setting that withholds prophylaxis until engraftment, and in heart transplant (HT) recipients on continuous trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis. The objective was to determine the incidence of TI before it evolves into clinical, potentially fatal *Toxoplasma* disease (TD), in these two very different transplantation settings.
Material and Methods. Pre-transplantation serological and qPCR screening was followed by post-transplantation peripheral blood (PB)-based qPCR monitoring targeting the *Toxoplasma* 529 bp gene. In HSCT recipients qPCR was performed weekly while in HT recipients qPCR was performed monthly for two months post-HT and then yearly. TI was diagnosed based on a positive PCR result in at least one PB sample.
Results. TI was diagnosed in 21/104 (20.2%) HSCT recipients, predominantly after allogeneic (19/75) and rarely after autologous HSCT (2/29). Over 50% of TI cases were diagnosed during the first month post-HSCT, while awaiting engraftment without prophylaxis. On the other hand, TI was diagnosed in 3/35 (8.6%) HT recipients. Regardless of the TMP-SMX prophylaxis, qPCR became positive rather early post-HT (within a month post-HT in two and 40 days post-HSCT in third patient).
Conclusion. The presented results show that systematic PB-based qPCR monitoring is a valuable asset for the diagnosis of TI not only in HSCT but also in solid organ recipients, especially after HT. Frequency of qPCR monitoring should be adjusted according to the specificity of the transplantation setting, especially in terms of prophylaxis, in a manner allowing for prompt introduction of specific treatment in each case of TI.

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IF TOXOPLASMOSIS IS FOREVER COULD PREVENTION AND TREATMENT REDUCE THE RISK OF PSYCHIATRIC DISORDERS?

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Toxoplasma gondii is a ubiquitous intracellular parasite with a high tropism for the central nervous system (CNS), eye and testis, where it forms cysts establishing a chronic infection. Although parasite infection occurs apparently asymptomatic in immunocompetent individuals, several evidences showed that stable behavioral changes in both infected mice and humans are are induced by the parasite. Animal models of infection with a low virulence genotype showed the persistence of attraction to cat urine odor even after the clearing of the parasites from the brain, suggesting that the parasite may induce a chronic modification of neuronal environment.

In *Toxoplasma* exposed individuals, increased risk of epilepsy, psychiatric diseases, self-directed violence and impulsivity, and reduced psychomotor performances with high rates of road traffic accidents have been described.

Although the link between toxoplasmosis and schizophrenia has been the most extensively studied in last decades, more recently the association of *T. gondii* infection with bipolar disorders (BDs) and suicidal/ aggressive behaviors has yielded growing attention. Several mechanisms may be involved in the *Toxoplasma* induced brain disorders: i) neurotransmitter modifications, especially dopamine pathway; ii) induction of brain inflammation which plays a prominent role in psychosis and BDs, through the glia cell activation and the stimulation of inflammatory cytokine and chemokine production in the CNS; iii) increase of corticosteroid levels affecting the hypothalamus-pituitary-adrenal axis.

Several open questions still remain:

i) is seropositive population more susceptible to neuropsychiatric diseases and behavioural changes?

ii) does prenatal exposition to *Toxoplasma* facilitate the occurrence of psychiatric diseases such as autism spectrum disorders?

iii) are anti-*Toxoplasma* drugs useful in the treatment of neuropsychiatric diseases in seropositive patients?

iv) should also asymptomatic acute patients be treated with anti-*Toxoplasma* drugs?

It is a big challenge for future researchers to address these issues.

TOXOPLASMOSIS AND BEHAVIOURAL DISORDERS: CONTROVERSIAL ASSOCIATION

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Toxoplasmosis is a parasitic infection of global distribution due to the protozoan *Toxoplasma gondii,* capable of infecting all warm-blooded vertebrates including humans during its reproductive cycle. The parasite is found in humans in two forms: a rapidly propagating vegetative form capable of spreading throughout the body, and a resistant cystic form containing several hundred to thousands of parasites. After primo-infection and multiplication in organism, Toxoplasma can be present in muscles and brain where it remains quiescent throughout the life of the host with sometimes reactivations that can generate ocular and cerebral lesions.

People who have contracted the parasite therefore host cysts in their brains. Do these cysts cause neurological, behavioural disorders? The question is not settled, but numerous publications and some work are in favour of this hypothesis relating to a higher frequency of the disease in people with schizophrenia, bipolar diseases, obsessive-compulsive disorders than in the general population. Some publications even associate positive toxoplasmic serology with road accidents, suicide attempts, autism etc.

Toxoplasma gondii would manipulate the brain? Does epidemiological, clinical and psychological research provide any evidence on the reality of this association? There is currently no formal evidence of the impact of toxoplasmosis on human behaviour even some studies show that *Toxoplasma gondii* causes behaviour change in infected animals (rats, mice).

But, the question remains open.

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DIROFILARIOSIS IN EUROPE TODAY
Organizer / Moderator: Fernando Simón Martín

INVITED LECTURES

MAIN ASPECTS TO BE SOLVED IN THE MANAGEMENT OF CANINE AND FELINE DIROFILARIOSIS

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Despite the perception of a decrease in the prevalence of the parasite in endemic areas, the percentage of infected subjects in the Po Valley, Italy, continues to be between 20 and 40%.
Among the causes of the spread of the parasite, alien mosquito species must be considered. The urban environment also favours the infection because of the "heat islands": micro-environments in which temperatures even in cold seasons exceed 14 ° C
In cats due to the difficult diagnosis, epidemiological data are not available but theoretical data suggest that the prevalence in cats is about 10% of that found in dogs.
The tests for the detection of circulating antigens of *D. immitis* have a very high sensitivity but the increase in sensitivity has led to a reduction in specificity and false positives from cross reactions are documented. The indications are to perform a Knott test at the same time as the antigen test for to increase the positive predictive value of the tests and to highlight possible cross reactions.
In cats, the low sensitivity of antigen tests requires the contextual use of antibody tests, considering that even these tests have incomplete sensitivity. In case of positive antibody test, with negative antigen test, it is therefore necessary to perform echocardiography for a diagnosis of certainty.
In the dog, the therapies of choice are represented by Melarsomina (adulticidal medical therapy) and by the removal of adult worms by trans-jugular with a minimally invasive technique. Correct staging of the patient requires both thoracic radiographs and an echocardiography
The climatic variations do not allow to identify a seasonality of the parasite with an initial and an end phase of transmission risk. Prophylaxis for heartworm disease in both dogs and cats should be therefore performed 12 months a year.

CURRENT STATUS OF HUMAN DIROFILARIOSIS. A SCOPING REVIEW

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Background. Human dirofilariosis is a clinical entity caused by infection with nematode species of the genus *Dirofilaria*. The traditional picture depicts the disease as a sporadic event associated with the presence of a single immature worm causing a nodular lesion.
Objectives. We aimed to conduct a scoping review in the field of human dirofilariosis by analysing the information contained in clinical cases published throughout the 21st century.
Material and Methods. A systematic search based on PRISMA-ScR guidelines was conducted to identify publications reporting clinical cases on human dirofilariosis worldwide from 1 January 2000 to 31 December 2019. Data on different aspects of epidemiology, parasites characteristics and clinical process of the selected cases were extracted and analysed.
Results. Of the 1868 publications initially identified, 305 containing 576 case reports were included in the subsequent analyses. These showed that human dirofilariosis is a worldwide-spread disease currently caused by 5 *Dirofilaria* species [mainly *D. repens* (72.22%)]. Parasite maturation was described in 42.95% of cases and 6 microfilaremic infections were also reported. The predominant clinical manifestation was the presence of a worm encapsulated within a nodule, and parasites/nodules were found in 71 different anatomical locations, being the traditional nomenclature of human dirofilariosis unable to properly cover this complex situation. Delay in seeking medical assistance (patient perception) and the frequency of wrong clinical suspicions (doctor

knowledge), strongly influenced clinical management. The most frequent initial suspicion was a tumour not related to a parasitic origin and surgery was usually applied, regardless of the use of non-invasive techniques. Molecular approach was the most accurate technique to establish a species-level diagnosis.
Conclusion. Human dirofilariosis is not a sporadic clinical condition, but a global problem that physicians around the world are increasingly confronted with. In this context, a more up-to-date and objective picture of the various aspects of this disease has been constructed, which will improve and unify information and clinical management to deal with future cases of human dirofilariosis.

Dirofilaria immitis AND *D. repens*: CURRENT RISK OF SPREADING IN CENTRAL AND NORTHERN EUROPE

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Background. In the past decades the relevance of *Dirofilaria immitis* (causative agent of canine and feline cardiopulmonary dirofilariosis), and *D. repens* (subcutaneous dirofilariosis) is steadily increasing in Central and Northern Europe. On the one hand, these parasites are imported with dogs from endemic countries, on the other hand increasing temperatures benefit both vectors and parasites.
Material and Methods. A summary of published articles dealing with these parasites in Central and Northern Europe is given.
Results and Conclusion. It is obvious that the number of cases of *D. immitis* and *D. repens* is increasing in Central and Northern Europe. Various studies report imported (e.g. Austria, Northern Europe), but also autochthonous cases (e.g. Hungary, Poland). Housing conditions of dogs, vector species inventory, pet travel, climate change, and global change are important factors in the spread of these nematodes.

MOLECULAR RELATIONSHIPS BETWEEN *Dirofilaria* AND HOSTS. FROM SURVIVAL TO PATHOLOGY

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The evolutionary success of parasitism could be explained by the mechanisms evolved by these species to confront and evade the responses of their hosts, together with their capacity to adapt the hosts metabolic processes for their own benefit. An example of this type of manipulation is the interaction of many different parasites with the fibrinolytic system of their hosts, which comprises one of the main anticlotting mechanisms of haemostasis. Plasmin is the final enzyme of the fibrinolytic system and displays high, broad-spectrum proteolytic activity by degrading the fibrin of clots, among other different molecules. Due to its biological characteristics, *Dirofilaria immitis* supposes a paradigmatic example of this type of adaptations, since it is capable of surviving for long periods of time in the circulatory system of immunocompetent reservoirs, while it produces a vascular chronic parasitosis named cardiopulmonary dirofilariosis. A series of published studies have demonstrated that *D. immitis* activates the fibrinolytic system displacing the fibrinolytic balance towards the generation of plasmin. This would imply a survival mechanism by which *D. immitis* could control the formation of clots in its immediate intravascular habitat. However, later studies have linked the parasite-dependent over-production of plasmin with the long-term development of the pathogenic mechanisms that occur during cardiopulmonary dirofilariosis. These data demonstrate the dual role of plasmin in dirofilariosis: on the one hand, plasmin could favour the survival of both *D. immitis* and the host, while on the other, it could have a pathological effect at the vascular level. A deeper understanding of the way in which *D. immitis* uses the fibrinolytic system and other molecular pathways of its host could result in the definition of important mechanisms in the field of pathogenesis and virulence of parasites, potentially giving new insights into the development of drugs that could be applied not only to dirofilariosis, but also to other plasmin-induced pathologies.

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OTHER FILARIAE OF DOGS IN EUROPE

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Besides the well-known hearthworm *Dirofilaria immits* (Spirurida, Onchocercidae), in the last 15 years, other onchocercids infesting dogs in Europe have gained the interest of the scientific community. Indeed, many other filarioids may colonize subcutaneous tissues, muscular fasciae, and lymphatics (genera *Acanthocheilonema*, *Brugia*, *Cercopithifilaria* and *Dirofilaria*) as well as ocular tissues (*Onchocerca lupi*). The life cycle of most of those filarioids, either those vectored by mosquitoes, back flies or ticks, is little studied and, for most of them, remains largely unknown. For example, adults of *Cercopithifilaria* spp. are usually beneath cutaneous tissues and are transmitted by hard ticks, their microfilariae being always in the dermis. The first species known to infest dogs, namely *Cercopithifilaria grassii*, was described more than one century ago. Following the first report in a dog from Sicily (Italy), microfilariae of *Cercopithifilaria* sp. I (later on re-described as *Cercopithifilaria bainae*) have been morphologically and molecular characterized in many Countries of the Mediterranean region and also in US. The occurrence of this nematode overlapped the distribution of its vector (the brown dog tick, *Rhipicephalus sanguineus sensu lato*) with prevalence rates reaching up to 21.6% in dogs from Spain, Greece and southern Italy. Furthermore, morphological and molecular evidence indicate that at least another species of *Cercopithifilaria* sp. (i.e., *Cercopithifilaria* sp. II sensu Otranto et al., 2013) is present in the Mediterranean area. Another emerging filarioid nematode infesting the eyes of dogs has been reported in Hungary, Greece, Germany, Portugal, Romania and USA. This nematode was recently described as cause of infestation in human patients from Turkey, in Tunisia, Iran and the US. Undoubtedly, further research on the biology, clinical impact and immune response elicited by these parasites are advocated in order to gain more information on their actual impact on veterinary medicine and, for *O. lupi*, zoonotic potential.

ORAL PRESENTATIONS

TUMOR NECROSIS FACTOR ALPHA IN DOGS WITH HEARTWORM DISEASE

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Background. Canine heartworm disease (HWD) is clinically important, potential fatal disease caused by *Dirofilaria immitis.* The initial pathological changes, described as villous myointimal proliferation, occur in the pulmonary arteries where the parasite resides. The proinflammatory cytokine, tumor necrosis factor alpha (TNF-α), plays a key role in the cellular responses to inflammation and injury. Not only that TNF-α is produced by activated macrophages and lymphocytes, but also by endothelial and epithelial cells, smooth muscle cells and cardiac myocytes. The role of TNF-α in HWD pathogenesis, and thus its potential use in diagnosis or even in HWD therapy, has not yet been determined.
Material and methods. TNF-α was studied in 14 client-owned dogs with HWD. Clinical and parasitological examinations (modified Knott test for circulating microfilariae and SNAP Test IDEXX for circulating *D. immitis* antigen) were used for diagnosing *D. immitis* and HWD. All dogs were treated with an alternative therapy for HWD (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). Serum samples of all dogs at the moment of HWD diagnosis, during and at the end of therapy were frozen for further quantification of TNF-α by ELISA (Canine TNF-alpha ELISA kit, Thermo scientific).
Results. At the moment of HWD diagnosis TNF-α was detected in 9 dogs (7.21±12.44 pg/ml). There were no significant differences between TNF-α concentrations during therapy. Concentration of TNF-α was not related to the level of *D. immitis* antigen, nor were the changes of TNF-α concentrations in the dogs related to the changes of antigen level during therapy.

Conclusion. The inflammatory cytokine TNF-α is not necessarily detected in dogs with HWD. The alternative therapy for HWD has no influence on TNF-α concentration changes.

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OTHER ECTOPARASITES: FROM BIOLOGY TO CONTROL
Organizer / Moderator: Domenico Otranto

INVITED LECTURES

Bartonella spp. AND THEIR VECTORS: CO-EVOLUTION AND ZOONOTIC ASPECTS

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Bartonella are gram negative bacteria infecting erythocytes and endothelial cells of many mammalian species. Completion of the epidemiological cycle requires insect vectors. The most common one is *Bartonella henselae*, causing cat scratch disease (CSD). The oldest Bartonella species described at the end of the 19th century is *B. bacilliformis*, the agent of verruga peruana/Oroya fever, geographically limited to South America and vectored by sandflies. The second known species is *B. quintana* causing trench fever transmitted by body lice, first described in soldiers during WWI, which re-emerged at the end of the 20th century in lice-infested homeless people. It is also one of the two agents of bacillary angiomatosis (BA), a condition observed in AIDS patients in the 1990s. BA was also associated with AIDS patients who had exposure to cats or cat fleas, leading to the isolation and recognition of *B. henselae* as the agent of CSD. *B. henselae*, *B. clarridgeiae* and *B. koehlerae* in cats, are transmitted between cats by the cat flea, *Ctenocephalides felis*. For *Bartonella* species isolated from ruminants or bats, the vectors are likely biting flies. In sheep, the vector is *Melophagus ovinus* and in roedeer, *Lipoptena cervi*. In bats, several bat flies are likely vectors of a wide range of *Bartonella*. The role of ticks as true vectors is still debated, and they are not very efficient vectors. Bartonellae may have emerged from symbiotic bacteria of the gut fauna of insects which progressively adapted to a specific mammalian host, exemplified by *B. melophagi* which is specific to its ovine host and vector, and does not seem to infect cattle or goats living in close contact with infected sheep. Similarly, the two subspecies of *B. koehlerae* identified in mountain lions or bobcats are highly infeoded to these wild felids in a co-evolutionary adaptation.

VETERINARY PARASITOLOGY DISCOVERY IN ANIMAL HEALTH – OVERVIEW AND ISOXAZOLINE EXAMPLE

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The global market for animal health products and services is estimated to 80 billion Euros/year, with 19 billion being veterinary products with an annual growth rate superior to that of human medicine. The animal health market is driven primarily by three factors: Technically advanced and convenient treatments for companion animals; the growing demand of proteins for human population; and the development of chemoresistance in anti-infective and antiparasitic drugs. Parasiticides is the largest segment of the animal health market (i.e. 28%), followed by vaccines (26%), with € 7.1 billion sales.

Only a few new antiparasitics have been commercialized in recent years; these include the anthelmintic monepantel and derquantel and the insecticide isoxazolines. Most of the antiparasitic drugs used today were developed more than 20 years ago, and many drugs are generics. Animal health companies have focused largely on incremental innovations (i.e. new formulations, new combinations).

The antiparasitic field requires intense research for new actives and new formulations. Drug Discovery in Veterinary Parasitology is driven by the access to compound libraries in either pure chemical companies (e.g. Dupont, Nissan,…), crop science (e.g. Bayer, BASF,…), and rarely human health.

In general, once hits (i.e. promising molecules) show up and continue to produce favorable data, these hits can become lead molecules. On top of chemical optimization, parallel assessment of other parameters like formulation, IP, safety (including user and environmental safety) and of course efficacy are needed before entering in the development stage to generate the registration dossier.

The latest example is the discovery of the class of isoxazolines, which was first launched in veterinary medicine as insecticidal/acaricidal oral formulation in 2014 (i.e. afoxolaner, fluralaner), and now represents more than 60% of the anti-flea&tick products sold in veterinary clinics worldwide, including other isox molecules (sarolaner, lotilaner) and combinations to offer endectocide protection.

Acanthocheilonema reconditum AND ITS VECTORS. A LOOK INTO THE BIOLOGY OF AN UNUSUAL FILARIOID

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Among filarioids infesting dogs, *Acanthocheilonema reconditum is* regarded as the less significant species. Nonetheless, it has a global distribution and, in many geographical areas of the Old and New World, it is the sole or the most prevalent filarioid species of dogs. Despite this wide distribution, scant information is available on the biology and epidemiology of *A. reconditum*. There is evidence indicating that *A. reconditum* blood microfilariae are found in peripheral blood after a prepatent period of 67-101 days in experimentally infected dogs. However, no data is available on the patent period of the infestation in naturally infested animals or on the existence of periodicity of microfilaremia. Also, the similarity of the microfilariae of *A. reconditum* with those of *Dirofilaria immitis* can result in misidentification of the two species especially when specific diagnostic techniques such as antigenic test and/or PCR are not available. In the same manner, limited is the information on the intermediate hosts (i.e., fleas and lice) of *A. reconditum*, while the implication of ticks as the putative vector is still anecdotic.
In this presentation, the current knowledge on this unusual dog filarioid and its natural vectors will be reviewed and discussed along with some questions that remain unanswered and whose understanding is pivotal to address proper management in the exposed canine population.

ORAL PRESENTATIONS

DETECTION OF EMERGING TICK-BORNE DISEASE AGENTS IN THE FRENCH RIVIERA

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Background. Lyme borreliosis is a zoonotic tick-borne infection representing the most frequent vector-borne disease in the northern hemisphere. The mediterranean rim is generally described as unsuitable for the Western Europe vector, *Ixodes ricinus*. We conducted an epidemiological study to assess whether *I. ricinus* was present and study its infection status for tick-borne bacteria.
Material and Methods. Ticks originating from southeastern France were obtained from flagging sampling and removed from animals and tick-bitten patients. Species level identification used morphological keys and MALDI-TOF MS. Quantitative PCR and sequencing assays were used to detect and identify tick-associated bacteria (*Borrelia, Rickettsia,* Anaplasmataceae, *Bartonella, Coxiella burnetti*) in each specimen.
Results. A total of 1232 ticks were collected in several localities. Among these, 863 were identified as *I. ricinus* (70%). Bacterial screening allowed identification of Lyme group *Borrelia* among *I. ricinus* ticks originating from various regional areas. Other emerging tick-borne pathogens like *B. miyamotoi* and *Rickettsia* species were also detected.
Conclusion. The Alpes-Maritimes region, part of the French Riviera, harbours *I. ricinus* ticks infected with Lyme group *Borrelia* and several other tick-borne bacterial agents. Clinicians, travel medicine specialists and outdoor activity participants should be aware of the local Lyme borreliosis transmission risk.

DIAGNOSIS AND EPIDEMIOLOGY OF VISCERAL LEISHMANIASIS
Organizer / Moderator: Jean-Pierre Gangneux
Co-moderator: Guy Caljon

INVITED LECTURES

VISCERAL LEISHMANIASIS: WHAT'S NEW IN 2021

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Leishmaniasis is a neglected and lifethreatening disease, ranked by the World Health Organization (WHO) as the second most important human protozoan parasitic disease after malaria. Leishmaniasis is endemic in 98 countries, on every continent except Australia and Antarctica. The estimated incidence of visceral leishmaniasis (VL), the most severe clinical form of the disease, ranges from 0.1 to 0.5 million human cases. More than 95% of case reported to the WHO are from Brazil, China, Ethiopia, India, Kenya, Nepal, Somalia, and Sudan. Beside human visceral leishmaniasis, canine symptomatic and asymptomatic VL contributes to the spread of Leishmania and represents a Public Health concern, as the seroprevalence in dogs is estimated to be about 40% in endemic areas.
After inoculation by the sandfly vector, parasites infect macrophage cells and other phagocytic cells (neutrophils, dendritic cells) and diffuse to lymphoid organs, the bone marrow, the liver, and lymph nodes being the targeted tissues.
Diagnosis and management still differ amongst countries and a standardization is needed. We performed a European survey to describe the diagnostic practices and therapeutic strategies for the management of VL. Direct examination of the protozoa combined to quantitative PCR are the most used tools for the diagnosis. Regarding treatment, liposomal amphotericin B and antimonials remains the two main therapeutic strategies. However, discrepancies in (i) technical approaches of the diagnosis, as well as (ii) in treatment modalities, will be discussed during the session.

VISCERAL LEISHMANIASIS: DIAGNOSIS AND EPIDEMIOLOGY

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Visceral leishmaniasis (VL) is caused by *Leishmania* parasites transmitted by infected sand fly vectors. Since VL is an opportunistic infection, it affects persons with poor immunological profile (too young, too old, immunosuppressed in any way) which, if infected, will develop symptoms. Diagnosis and treatment are necessary since 95% of the cases will be fatal. The possibility of infection depends on the geographical spread of the vector and the parasite. Travelers to endemic countries are in danger of acquiring the disease while infected people leaving their endemic regions, for any reason, may transmit the parasite to new areas if the right vectors are present. The northward dispersion of sand fly vectors in Europe, which is favoured by global warming, is followed by VL cases. The common clinical symptoms of VL is a persistent, irregular, fever that does not improve with antibiotics and progressive pallor; if not treated will lead to hepatosplenomegaly, lymphadenopathy, anorexia, and weight loss. The laboratory diagnosis may be achieved by the detection of antileishmanial antibodies or/and parasite DNA, using various available test, which in combination with compatible clinical signs and the epidemiological profile of the patient would provide the diagnosis. It is important to know what parasite species/strains are present in an endemic region and their resistance to drugs so, isolating the parasite, when possible, will provide valuable information. It is also vital to know the sand fly vectors and the reservoir hosts present in the area for estimating the risk of spread of the parasite and for combating the disease.

L. martiniquensis AND *Mundinia* TRANSMISSION: WHAT'S NEW?

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Background. The genus *Mundinia* Shaw, Camargo & Teixeira 2016 is within the *Leishmania* Ross 1903 the one for which the transmission in natural conditions remains poorly documented despite the emergence of *L. martiniquensis* Desbois, Pratlong & Dedet 2014 leishmaniasis in South Eastern Asia.
Objectives. The main goal of this lecture is i) to provide a review about the transmission of the different species of *Mundinia*, focusing on *L. martiniquensis* and ii) to propose hypotheses on the transmission which remain to be demonstrated
Material and Methods. A review of the literature has been carried out, coupled to the personal author's field background.
Results. To date, the transmission under laboratory conditions exhibits that *Culicoides* Latreille have a better vectorial competence for *Mundinia* than Phlebotomine sandflies from New and Old Worlds. The data obtained from the field are mostly based on Leihmania DNA detection and consequently do not support the role of Phlebotomine sandflies in the parasite's transmission.
Conclusion. New field works have to be carried out in South Eastern Asia to demonstrate what is/are the vector(s) of *L. martiniquensis* in natural condition and the colonization of potential vectors from this area is of importance to understand the parasite's transmission.

ORAL PRESENTATIONS

DRUG RESISTANCE AND TREATMENT FAILURE IN VISCERAL LEISHMANIASIS: WHAT DO SAND FLY AND RODENT INFECTIONS TEACH US?

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Background. Visceral leishmaniasis (VL) is a disease caused by the parasites *Leishmania infantum* and *L. donovani* that can eventually be fatal when patients are left untreated. These parasites are transmitted by sand flies which requires a successful establishment in the insect gut and differentiation into infective stages. Upon transmission to the human host by an insect bite, parasites mainly replicate inside macrophages and further disperse to the liver, spleen and bone marrow as main target organs. Treatment of patients is currently based on a few drugs that all have various disadvantages such as the need for hospitalization, high cost, toxicity and/or the emergence of treatment failure.
Objectives. Therapeutic failure is known to have a multifactorial origin involving drug, host and parasite related parameters. As drug resistance or phenotypic adaptations of *Leishmania* leading to less effective treatment outcomes pose a significant threat to control programs in disease endemic countries, the risk of propagation of such traits needs to be carefully considered.
Material and Methods. The recent establishment of a *Lutzomyia longipalpis* sand fly colony in the new insectarium at LMPH, enabled us to study the impact of drug resistance on natural transmission by the insect vector and infection in the vertebrate host. Making use of engineered parasite strains, our studies have been able to study the impact of resistance on infection and unravel some of the underlying mechanisms of treatment failure.
Results/conclusion. The combined observations revealed the risk of rapid adaptation of parasites to monotherapy and identified specific tissue and cellular niches where treatment is less effective. Besides recommendations for rational drug use, these findings stimulate drug discovery efforts against parasites residing in a newly identified and highly permissive host cell.

DEVELOPMENT AND APPLICATION OF A MLST PANEL FOR THE IDENTIFICATION OF INFORMATIVE POLYMORPHISMS IN *Leishmania infantum* STRAINS IN THE MEDITERRANEAN REGION

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Background. Leishmaniasis is a zoonotic disease endemic in the Mediterranean region, where the causative agent of human and canine infection is *Leishmania infantum*. The spread of leishmaniasis is associated with population movements, ecology of phlebotomine vectors, and reservoir host. We used multilocus sequence typing (MLST) to explore the genetic variability of *L. infantum* strains in the Mediterranean region, including the borderline territory of Pantelleria island, and identify informative polymorphisms for rapid identification of genotypes through high-resolution melt (HRM)-based assays.
Material and Methods. A customized sequencing panel targeting 14 housekeeping genes was designed and MLST analysis was performed using the Ion Torrent S5 on 9 *L. infantum* strains/isolates: 5 canine isolates (3 from Pantelleria Island and 2 from central Italy), and 4 human isolates/strains from Tunisia, France, central and southern Italy. MLST results and in silico analysis of sequences available in Genbank allowed to select two informative polymorphisms on ME and GPI genes (390T/G and 1834A/G, respectively) used to develop two HRM-based assays for fast screening of 28 clinical samples.
Results. The MLST analysis identified a single *L. infantum* clonal complex regardless of the geographic origin or host (human or canine), except for the human isolate from central Italy that showed polymorphisms in 11 out of 14 housekeeping genes, and clustered independently in a molecular phylogenetic analysis. Successively, the screening through HRM-based assays of 28 clinical samples from central/south Italy and Pantelleria island allowed to identify 6 diploid sequence types (DSTs). Interestingly, the sequence type 390T/1834A was found only in Pantelleria island (prevalence 75%).
Conclusion. This study represents a description of the genetic variability of *L. infantum* through a first approach based on MLST and then by HRM analysis on selected polymorphisms. The HRM assays could be used as fast and cheap tools for epidemiological surveillance of *L. infantum*.

Funding source: Ricerca corrente IZS SI 01/17

MILTEFOSINE ENHANCES INFECTIVITY OF A MILTEFOSINE-RESISTANT *Leishmania infantum* STRAIN BY ATTENUATING THE ANTILEISHMANIAL IMMUNE RESPONSE

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Background. Miltefosine (MIL) is currently the only oral drug available to treat visceral leishmaniasis but its use as first-line monotherapy has been compromised by an increasing treatment failure. Despite the scarce number of resistant clinical isolates, MIL-resistance can easily be selected in a laboratory environment by mutations in a single aminophospholipid transporter gene. These mutations result in a reduced survival in the mammalian host, which can partially be restored by exposure to MIL, suggesting a kind of drug-dependency.
Material and Methods. To enable a combined study of the infection dynamics and underlying immunological events for differential *in vivo* survival, firefly luciferase (PpyRE9) / red fluorescent protein (DsRed) double-reporter strains were generated of MIL-resistant (MIL-R) and syngeneic MIL-sensitive (MIL-S) *Leishmania infantum*.
Results. Results in C57Bl/6 and BALB/c mice show that MIL-R parasites induce an increased innate immune response that is characterized by enhanced influx and infection of neutrophils, monocytes and dendritic cells in the liver and elevated serum IFN-γ levels, finally resulting in a less efficient establishment in liver macrophages. The elevated IFN-γ levels were shown to originate from an increased response of hepatic NK

and NKT cells to the MIL-R parasites. In addition, we demonstrated that MIL could increase the *in vivo* fitness of MIL-R parasites by lowering NK and NKT cell activation, leading to a reduced IFN-γ production.
Conclusion. In conclusion, we found that differential induction of innate immune responses in the liver was, partially, responsible for the attenuated phenotype of the MIL-R parasite and its peculiar feature of drug-dependency. The impact of MIL on hepatic NK and NKT activation and IFN-γ production following recognition of a MIL-R strain indicates that this mechanism may sustain infections with resistant parasites and contribute to treatment failure.

THE IMPACT OF DRUG RESISTANCE OF VISCERAL *Leishmania* SPECIES ON THE PARASITE- VECTOR-HOST INTERACTION

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Background. At the moment only few chemotherapeutics are approved for the treatment of visceral leishmaniasis and these are confronted with increasing treatment failure rates and the emergence of drug resistance.
Objectives. We aimed to predict the potential effects of miltefosine (MIL) and paromomycin (PMM) resistance on the parasite life cycle in the mammalian host and sand fly vector.
Material and Methods. To evaluate the impact of drug resistance, MIL and PMM resistant *L. donovani* and *L. infantum* strains were experimentally selected *in vitro*. The resulting parasites were phenotypically and genotypically characterized in comparison to the original wild-type population. Moreover, their adaptive behaviour in different sand fly species was studied in order to predict the effects on parasite transmission.
Results. Mutations in the *MT* transporter gene are sufficient for acquisition of MIL resistance and are linked to a clear reduction of parasite fitness in mice and sand flies. PMM resistance seems multifactorial and could not be associated to one specific genetic alteration. PMM resistant parasites develop normally in the insect vector and higher parasite burdens in the mammalian host suggest efficient transmission of this resistance trait.
Conclusion. The drug-dependent changes of parasite fitness indicate that not all drugs are at risk of an immediate spread of resistance, but that vigilant use is required.

SEROPREVALENCE OF LEISHMANIOSIS IN STRAY DOGS IN NORTH MACEDONIA

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Background. Canine leishmaniosis is a zoonotic protozoan disease caused by *Leishmania infantum* and vectored by phlebotomine sand flies. Canids (dogs, foxes, coyotes) have the main role in maintaining the disease in endemic regions as reservoirs and source of infection for the sand flies and humans. The disease is endemic in North Macedonia, hence the aim of this study was to assess the seroprevalence of canine leishmaniosis in stray dogs in 2019 and 2020.
Material and Methods. Serum samples were collected from January 2019 to December 2020. A total of 2654 stray dogs' sera from all the 8 regions of the country were tested for presence of anti-*Leishmania* antibodies with commercial IFAT Leishmaniasis test-kit (MegaFLUO® LEISH, MEGACOR Diagnostik, Austria). The test was performed following the manufacturer's procedure and using a 1:80 titre cut-off. Samples were considered positive at titres ≥ 1:160.
Results. Canine leishmaniosis was present in all regions and the seroprevalence varied from 1.9% in the Skopje region to 9.8 % in the Eastern region. The overall seroprevalence of canine leishmaniosis in this study was 4.8% (95% CI: 3.9-5.6).

Conclusion. The seropositive dogs have an important role in the epidemiology of leishmaniosis in North Macedonia. To reduce the possibility of human infection, it is necessary to detect infection in dogs in a timely manner and apply measures including treatment or elimination of positive dogs.

LONG-TERM HEMATOPOIETIC STEM CELLS AS SANCTUARY NICHE DURING TREATMENT FAILURE IN VISCERAL LEISHMANIASIS

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Background. The increasing frequency of treatment failure in visceral leishmaniasis (VL) has already resulted in discontinuation of various first-line drugs. Although most studies focus on associations with parasitic drug resistance, a knowledge gap remains about other factors determining cure versus relapse. For many pathogens it has been described that persistent infections can occur in many different tissues and cells throughout the host. Some of these niches give protection against active immunity and drug action.
Objectives. To identify the cellular niches responsible for *Leishmania* persistence and treatment failure.
Material and Methods. The present study used double bioluminescent/fluorescent *Leishmania infantum* and *L. donovani* reporter lines to study relapse at the tissue and cellular level, using bioluminescent imaging, flow cytometry and RT-qPCR as qualitative and quantitative techniques.
Results. In combination with observations made in golden Syrian hamsters subjected to 91 different treatments, the results provide evidence of parasites surviving in the bone marrow (BM), identifying this tissue as a sanctuary site from where the host can be recolonized. Long-term hematopoietic stem cells (LT-HSC; Lin- Sca1+ cKit+ CD48- CD150+) were found to become readily infected. Compared to other BM cells and macrophages, LT-HSCs constitute a hospitable cellular niche with low nitric oxide and reactive oxygen species levels and harbouring enormous parasite burdens. Moreover, we found that infected LT-HSCs are less sensitive to antileishmanial reference drugs in comparison to macrophages.
Conclusion. LT-HSCs are a protective cellular niche for persistent *Leishmania* parasites in the BM. Given the important clinical implications for the current field situation of increasing post-treatment relapse rates, this study represents an essential step towards unraveling *Leishmania* persistence and treatment failure.

Funding source: Fonds Wetenschappelijk Onderzoek (FWO), Flanders, Belgium

IDENTIFICATION OF ADIPOCYTES AS TARGET CELLS FOR *Leishmania infantum* PARASITES

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Background. *Leishmania infantum* is the causative agent of visceral leishmaniasis transmitted by the bite of female sand flies. The recommended drugs for treating leishmaniasis include Amphotericin B. But over the course of the years, several cases of relapses have been documented. These relapses call into question the efficiency of actual treatments and raises the question of potential persistence sites. Indeed, *Leishmania* has the ability to persist in humans for long periods of time and even after successful treatment. Several potential persistence sites have already been identified and named 'safe targets'.
Objectives. As adipose tissue has been proposed as a sanctuary of persistence for several pathogens, we investigated whether *L. infantum* could be found in this tissue.
Material and Methods. Experiments were approved by the ethics committee of the Nice School of Medicine, France (Protocol number: 2017-56). *In vitro* and *in vivo* experiments were performed with Recombinant *L. infantum* - expressing the Green Fluorescence Protein and *L. infantum* - expressing the Luciferase reporter,

respectively. Mouse and Human adipocytes were infected, and PCR, histology, microscopy and electron microscopy were used to follow the infection.

Results. *In vitro*, we demonstrated that *L. infantum* were able to infect mousse (Figure 1) and human adipocytes. Adoptive transfer experiments allowed us to demonstrate that *Leishmania* parasites were alive inside adipose tissue and had the capacity of infecting naive mice.

Conclusion. Altogether our results suggest adipocytes as a 'safe target' for *L. infantum* parasites. Treatment with poor access to the adipose tissue would be poorly effective at resolving the infection and would likely be followed by relapses with parasite exiting the adipocytes. In this context, it would be interesting to define new drugs against *L. infantum* with penetration into adipocytes to efficiently target parasites within these cells.

SEE TOXOPLASMOSIS
Organizers / Moderators: Branko Bobić & Barbara Šoba

INVITED LECTURES

Toxoplasma INFECTION IN SOUTHEAST EUROPE

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Background. In the Balkan region, research on *Toxoplasma gondii* infection has been conducted for the last sixty years.
Aim. The aim of this systematic review was to analyse the status of *T. gondii* infection in the Balkan region in the 2000-2019 period.
Source of data. A literature search was conducted to collect published articles, grey literature and official reports on *T. gondii* infection after the year 2000 in region. PubMed, Web of Science, and Google were searched, and search phrases were: toxoplasmosis OR *Toxoplasma* AND country name.
Results. From a total of 246 articles and 29 official reports identified by the search, after analysis, data were extracted from 34 full text articles and 29 official reports.
The dominant feature of Toxoplasmosis in the Balkans is a continuous decrease in the prevalence over time. In general, the prevalence of infection in the region is currently below 30%. Seasonality of infection, with significantly more cases of acute infection in the winter than in the summer, was observed in Slovenia in the west, Croatia, and Serbia in the east. Dominant risk factors for transmission appear to vary between countries in the region. Contact with cats was the infection risk factor In Slovenia, consumption of undercooked meat in Serbia and Albania, and soil contact in North Macedonia and Greece.
Conclusion. The existing results on toxoplasma infection in SEE countries, are not the result of systematic monitoring, except for Slovenia, but of individual studies that differ in the studies design, structure of the study population and the applied laboratory methods. Although this makes it difficult to draw general conclusions, the trend of declining prevalence of the infection over the observed 20 years is not questionable and is probably part of the changes in toxoplasmosis infection across Europe.

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HUMAN TOXOPLASMOSIS IN WESTERN ROMANIA

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Toxoplasma gondii is an obligate intracellular parasite that causes infection in most mammals worldwide. This protozoan infects approximately 30% of the global population. The most common sources of human infection are ingestion of food or water contaminated with oocysts shed by cats or by eating raw or undercooked meat containing tissue cysts and transplacental transmission. Acquired toxoplasmosis in immunocompetent hosts is frequently asymptomatic or may cause benign infection. However, this protozoan can severely affect immunocompromised patients and congenitally infected children. Given the severe complications that may be caused in these patients, it is important to survey the distribution of *T. gondii* infection. *Toxoplasma* prevalence may vary widely between countries, within a country and between different communities in one region. Recent studies performed in Western Romania suggest that *T. gondii* seroprevalence in this region may be among the highest in Europe. We present our experience regarding the seroprevalence of *T. gondii* in the general population and specific groups of population from Western Romania, including children, pregnant women or patients with psychiatric diseases. The risk factors that contributed to this high rate of *T. gondii* infection are also presented.

SLOVENIAN NATIONAL SCREENING PROGRAMME FOR PREVENTION OF CONGENITAL TOXOPLASMOSIS

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In Slovenia, compulsory screening of pregnant women for *Toxoplasma gondii* infection to prevent congenital toxoplasmosis was introduced in 1995. Susceptible pregnant women are screened for primary infection by serological tests for IgG and IgM antibodies against *T. gondii* three times during pregnancy, i.e., once in each trimester, with the first test as early as possible in pregnancy. Pregnant women with confirmed primary infection are administered therapy and followed up, as are their new-borns if congenital infection is confirmed. In the Laboratory of Parasitology at the Institute of Microbiology and Immunology, Ljubljana, approximately one quarter of all Slovenian pregnant women is screened yearly. In the period from 1996 to 1999, the seroprevalence of *T. gondii* in pregnant women who attended antenatal clinics in Ljubljana and its surroundings was 33.6% but it dropped to 24.8% in the period from 2000 to 2007. Primary *Toxoplasma* infection was detected in 0.62% and 0.73% of pregnant women screened for toxoplasmosis from 1996-1999 and 2000-2007, respectively. In the lecture, the Slovenian national screening programme for prevention of congenital toxoplasmosis and the results of a retrospective analysis of prevalence and incidence of toxoplasmosis in pregnant women in Central Slovenia from 2008 to 2019 will be presented.

GENOTYPES OF *Toxoplasma gondii* CIRCULATING IN SOUTH-EASTERN EUROPE, A REGION OF INTERCONTINENTAL STRAIN EXCHANGE

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Background. Four lineages of *Toxoplasma gondii* genotypes are in global circulation: I, II, III and Africa I. In Europe, genotypes of lineage II are dominant, with the archetype, ToxoDB#1, being most frequently detected. The abundance of lineage III genotypes varies geographically, while lineage I are exceedingly rare, yet present in several regions of the continent. Africa I lineage has thus far been detected solely in Turkey and France.
Objectives. Data on the *T. gondii* population structure in South-Eastern Europe (SEE) are scarce, yet necessary to appreciate the diversity of the species in Europe. To help fill this gap, we identified the genotypes of *T. gondii* detected in and isolated from various species of intermediate hosts in Serbia
Material and Methods. The population structure was determined based on 50+ strains from humans, domestic animals (*O. aries, S. scrofa, G. gallus, E. caballus*) and wildlife (*V. vulpes, C. aureus, C. lupus, C. liva*). Genotyping was performed by MnPCR-RFLP on a minimum of 4 loci.
Results. Lineage II genotypes comprise 63% of the structure, with ToxoDB#1 (36.8%) being most common in the domestic environment, while lineage III genotypes represent 19% and ToxoDB#3 (8.7%) is distributed evenly. Lineage I and Africa I were not detected. While 45.4% of the total genotype population consists of just ToxoDB#1 and ToxoDB#3, of note is that we detected one atypical genotype, variant genotypes in 62.9% of the population in wildlife and 26.6% in domestic animals, including two lineage III variants with intercontinental distribution**.**
Conclusion. Based on the findings obtained on this considerable *T. gondii* strain collection, SEE is a region of underappreciated *T. gondii* genetic diversity.

Funding source: This work was supported by grants (project № III 41019 and contract № 451-03-68/2020-14/200015) from the Serbian Ministry of Education, Science and Technological Development.

ORAL PRESENTATIONS

POSTNATAL OCULAR TOXOPLASMOSIS IN IMMUNOCOMPETENT PATIENTS IN SEE: A CASE SERIES AND REVIEW OF THE LITERATURE

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Background. Ocular toxoplasmosis (OT) is the most common cause of infectious posterior uveitis worldwide. It can be acquired congenitally or postnatally. Despite estimations that postnatal OT is more prevalent, only several cases of proven postnatal OT have been reported in non-epidemic settings.
Objectives. We report a series of four cases of conclusively proven postnatal OT in immunocompetent patients in Serbia. These cases prompted us to review the data on OT published in South-East Europe since 2000.
Material and Methods. Postnatal OT was diagnosed based on clinical diagnosis supported by longitudinal detection of *Toxoplasma gondii*-specific IgG, IgM, and IgA antibodies in the serum, as well as by direct detection of the parasite (bioassay) and/or its DNA (real-time PCR) in aqueous humor.
Results. Three cases involved adults in whom OT developed during primary *T. gondii* infection, as part of the clinical presentation in two and as the sole manifestation in one patient. The fourth patient was a case of inactive OT in a 14-year-old boy, where postnatal infection was confirmed by exclusion of maternal infection. The causative parasite strain was genotyped in only one case, and it belonged to genotype II, the dominant type in Europe. One patient acquired the infection in Africa, suggesting an atypical strain. Review of the literature has shown a total of 13 studies on OT published in the region, but with no specific data on postnatal origin, and subsequently no clinical or diagnostic information.
Conclusion. The distinction between prenatal and postnatal OT requires extensive laboratory investigation yet is still only possible in particular clinical situations. Although with no immediate clinical relevance at present, awareness of postnatal origin, along with genotyping of the infecting parasite strain, may become important as treatment options tailored to particular genotypes become available, all the more so if atypical strains are involved.

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TRICHINELLOSIS AND TRICHINELLA INFECTION IN SEE: CURRENT STATUS
Organizer / Moderator: Vasile Cozma

INVITED LECTURES

TRICHINELLOSIS AND *Trichinella* INFECTION IN CROATIA: CURRENT STATUS

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Trichinellosis was an important public health issue in Croatia in the mid-1990s. The peak of human cases was in 1998 with 575 registered cases, though over the past 10 years, from zero to 37 human cases have been registered. The epidemic curve of *Trichinella* infection in the domestic swine population is similar to the human curve, with a peak of 4478 positive carcasses in 2000, followed by a continual decline in the number of positive swine carcasses to 14 or fewer positives in the last six years. The exception was in 2018, when 194 positive swine carcasses were registered. *Trichinella* infection has been proven in 31% of tested wolf samples (1996-2007), in 30.7% of golden jackals (2009-2020), and in 12.5% of badgers, 2,7% of bears, 2,4% of foxes, and 0,15% of wild boars (2010-2020). *Trichinella* infection has never been confirmed in solipeds (horses and donkeys). The most widespread species are *T. britovi* in wild animals and *T. spiralis* in domestic swine, though both species are also found outside their natural circles. The first report of *T. pseudospiralis* dates back to 2008 in a domestic pig, though it was later confirmed in populations of wild boars and foxes. Trichinellosis will remain an expected disease in Croatia in the future. *Trichinella* spp. have many hosts among wild animals, and should accordingly be listed in future monitoring plans. Special attention should be focused on education to ensure the safe, proper epidemiological disposal of carcasses of both domestic and wild animals. Also, hunters are required to submit a mandatory sample of possibly infected game meat to the authorized veterinary service for *Trichinella* testing.

TRICHINOSIS IN ROMANIA: UPDATES REGARDING *Trichinella* spp. INFECTIONS IN WILD ANIMAL SPECIES

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Background. Nematodes of the genus *Trichinella* are zoonotic parasites and are widespread in Romania. *Trichinella spiralis* is widespread in domestic animals such as pigs and often can be found in wild animals when a domestic life cycle spillover occurs. *Trichinella britovi* is encountered in wild omnivores and wild canids.
Objectives: The main objective of this study was to assess the evolution of these two *Trichinella* species in wild animals in Romania over the past 20 years.
Material and Methods. A literature review of original studies concerning *Trichinella spiralis* and *Trichinella britovi* in wildlife in Romania was conducted and corroborated with results of our original research concerning the topic.
Results. In Romania, the direct detection of *Trichinella* species in muscle samples from wild animals was accomplished by trichinoscopy and artificial digestion. The identification of the parasite species was performed with the help of molecular biology (PCR). Afterwards, the detection of anti-*Trichinella* antibodies was done with serological methods such as ELISA and Western blot. Lately, new approaches started to surface regarding this parasite in Romania, by using experimental devices (microfluidic) for the detection and numbering of the

larvae. This extensive parasitological research has shown that, in Romania, European minks could be infected with *T. spiralis*, while wolves, European wild cats, Eurasian lynx, golden jackals, stone marten, and European badgers could have *T. britovi* infection. Both species of *Trichinella* were identified in foxes, bears, wild boars, and Ermines, but mixed infections were found only in European polecats. Anti-*Trichinella* antibodies were found in wild boars from Bihor County thanks to serological methods (ELISA, Western blot). Recently, an experimental microfluidic device showed promising results in identifying and counting *Trichinella* larvae in golden jackals, but has proven lower efficiency than artificial digestion.

Conclusion. The results indicate that in Romania, *Trichinella* spp. are still present in wildlife, and new approaches start to appear regarding their detection.

Funding source: This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement № 816172.

SERBIAN *Trichinella* STORY

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Trichinellosis used to be one of the most important foodborne zoonotic diseases in Serbia. Activities performed by Serbian National Reference Laboratory for trichinellosis concerning specific antibody detection in human and animals and an establishment of PT panels, alongside EU harmonized measures applied at national level, contributed to the better control of *Trichinella* infection and trichinellosis in Serbia. Epidemiological data from the last 2 decades indicate that the number of human cases as well as the prevalence of infection in animals has decreased significantly in last 10 years period. The number of confirmed cases dropped from 2257 in 2001-2010 to 716 in 2011-2020 period. Moreover, in 2018-2020 only a small number of single cassis with or without any *Trichinella* outbreaks appeared. The average prevalence of infection in domestic swine was 0.01% in 2011-2020 (reaching 0,003% in 2018-2020 period, when the prevalence was lowest than ever before) compared to 0.1% in 2001-2010. The prevalence of *Trichinella* infection in wild boars has been monitored for all 25 districts in Serbia from 2015 (with average prevalence 0.83% in 2015-2018 period). Over the last 10 years period the consumption of untested pork containing *Trichinella spiralis* larvae was the most frequent source of trichinellosis in Serbia. Cases generally occurred in small family outbreaks. Meat and meat products may be source of *Trichinella* infection when backyard pigs are raised without any compliance with hygienic rules and animals are not veterinary tested. The first confirmed *T. britovi* outbreak appeared in 2016 after consumption of uninspected wild boar meat (Cajetina, 111 people). It should be emphasized that: 1. Hunters and meat consumers in Serbia should be better educated about the risk associated with consumption of untested meat, 2. Control of *Trichinella* testing QA system in veterinary subjects and regular participation in PTs are needed to achieve safe food for consumers.

Funding source: Ministry of Education Science and Technological Development of Serbia contract № 451-03-9/2021-14/200019

SEE DIROFILARIOSIS & OTHER EMERGING VECTOR-BORNE ZOONOSES
Organizer: Suzana Otašević

INVITED LECTURES

DIROFILARIOSIS & THELAZIOSIS: EMERGING VECTOR-BORNE ZOONOSES ON THE TERRITORY OF THE CENTRAL BALKANS

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Emerging vector-borne zoonosis caused by *Dirofilaria* spp. and spreading of oriental eye-worm, *Thelazia (T.) callipaeda* in Europe have garnered increasing attention of veterinaries, physicians and entomologists.
In Serbia, after many years of negligence, results of first systematic surveys in XXI century demonstrated that North part of Serbia (NS) (Vojvodina Province) was endemic for dirofilarial infection in dogs with domination of *D. repens* species. Additionaly, 28 human cases as well as very high seroprevalence in humans were reported. This was followed by detection of biological vectors and dirofilariosis in different carnivore hosts. Recently, epidemiological scenario has changed. Thus the first molecular investigation of *Dirofilaria* spp. showed that Central Balkan (Serbia and North Macedonia) is endemic for dirofilariosis and the most prevalent was *D. immitis* (NS: D. *immitis,* 7.05%; *D. repens* 0.81%; North Macedonia: *D. immitis* 8.75%; South Serbia-overall prevalence 3,02%). In 2020, analysis of human sera with a non-commercial ELISA and confirmed by Western blott testing showed the overall seroprevalence of dirofilariosis was 3.77%. Notably, all diagnosed human dirofilariosis cases were due *D. repens* (10 new cases-3 ocular, 1 submucosal and 6 subcutaneous with one microfilaremic patient).
At the same time *T. callipaeda* (Spirurida, Thelaziidae), has been spreading through the Balkan Peninsula. The first thelaziosis cases in Serbia were reported in 2014 in dogs and cats. This was followed by the evidence of *T. callipaeda* infection in foxes and one human case of thelasiosis. Interestingly, the same patient had thelaziosis again in 2020.
Results of these investigations showed the need for better strategy for vector-borne zoonosis control and prevention of spreading through Balkan Peninsula as well as in Europe.

Dirofilaria INFECTIONS: THE ONE HEALTH APPROACH

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Dirofilaria immitis and *Dirofilaria repens* are nematode parasites of dogs and other carnivores. Depending on the species, they inhabit the pulmonary arteries (*D. immitis*, canine heartworm) or subcutaneous tissues (*D. repens*) and have an emerging zoonotic significance. Indeed, it has been evidenced that in enzootic areas, humans are at risk of infection. Both parasites have an indirect life cycle, are transmitted by the bites of infected mosquitos (intermediate hosts) and occur mainly in tropical and temperate regions. *Dirofilaria immitis* has a worldwide distribution while *D. repens* is present in the Old World. Accordingly, human cases are exclusively due to *D. immitis* in the Americas, while they are mainly related to *D. repens* in the Old World. Heartworm disease is one of the most significant and severe parasitic diseases of dogs, with potentially fatal outcome. The clinical implications include right heart failure, pulmonary hypertension, and pulmonary arterial embolism. In humans, *D. immitis* usually causes nodules in the pulmonary parenchyma. Infection may remain subclinical or cause coughing and painful breathing. *Dirofilaria repens* infection in dogs often remains asymptomatic, but may also manifest with subcutaneous nodules, localised alopecia, and cutaneous inflammation. In humans, *D. repens* infections are associated with subcutaneous and ocular lesions. The climate change and the movements of dogs due to adoption, commerce, and vacation, influence the transmission and distribution

dynamics of *Dirofilaria* spp. and favour their expansion in new areas that were considered non-endemic until recently. Because of i) the clinical impact of *Dirofilaria* infections in dogs and other animals, ii) the zoonotic potential of the parasites, iii) the complexity, risks, and cost of heartworm treatment in dogs, and iv) the documented trend of distribution expansion of these parasites, infection preventive measures are imperative. Furthermore, constant surveillance of the epizootiological/epidemiological status, healthcare professionals' education, and public awareness campaigns are necessary.

EPIDEMIOLOGY OF DIROFILARIOSIS IN HUNGARY – PAST AND PRESENT

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Dirofilariosis is an endemic parasitosis in the Mediterranean area, and it is an emerging zoonosis in Hungary. Climate changing, migration of host animals, humans, and tourism also may facilitate the spread of these infections. Since 1879 – when the first human case was described from Hungary – 149 cases were reported until 2020 from our country. All of them were caused by *Dirofilaria repens*. Most frequent form of this infection was subcutaneous dirofilariosis, followed by ocular localization. However, there were some reports about worms eliminated also from other body sites (spermatic cord, ligamentum gastrosplenicum, lymph node, abdominal cavity and lung granuloma). The territorial distribution of these human infections can be connected to the Danube and the Tisza rivers, as well as to their catchment areas. Based on the available epidemiological data, it can be concluded that most of these cases were autochthonous infections.

FISH PARASITOLOGY
Organizer / Moderator: Ivona Mladineo
Co-moderators: Serena Cavallero & Jerko Hrabar

INVITED LECTURES

Anisakis´s "OMICS": TRANSCRIPTOMICS, PROTEOMICS AND METAGENOMICS REVELATIONS

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Transcriptomics, Proteomics and Metagenomics analysis were focussed on *A. simplex*, *A. pegreffii* and their hybrids L3 larvae. **Transcriptomes** were generated using Illumina RNA-Seq, and assembled de novo. The transcript sets were functionally annotated and globally compared (differentially expressed). The allergome of the two species and their hybrids has also been characterized. We detected 938 sequences expressing 121 different allergens belonging to 74 allergen families from 509 non-redundant food allergens (from fungi, plants and animals). The **total proteomes** of these taxa were compared by quantitative proteomics (iTRAQ approach) by means of two independent experiments considering four biological replicates of *A. simplex* and two each for *A. pegreffii* and hybrids. A total of 1811 and 1976 proteins have been respectively identified in the experiments. Results of pairwise Log2 ratio comparisons among them were statistically treated and supported in order to convert them into discrete character states. This comparison selected thirty seven proteins as discriminant taxonomic biomarkers among *A. simplex*, *A. pegreffii* and their hybrid genotype. The proposed methodology (proteomics and statistical) solidly characterizes a set of proteins that are susceptible to be used in character compatibility phylogeny approach (Perfect Phylogeny) and also to take advantage of the new targeted proteomics. Proteomics approaches allows to describe for the first time as antigenic and potentially new allergens in *Anisakis*. **Metagenomics** analysis of 113 L3 individuals obtained from fish captured along the FAO 27 fishing area allows consider a total of 2,689,113 16S rRNA gene sequences. Bacteria were characterized at least through 1803 representative operational taxonomic units (OTUs) sequences. Fourteen phyla, 31 classes, 52 orders, 129 families and 187 genera were unambiguously identified. We have found as part of microbiome an average of 123 OTUs per L3 individual and its general relationship structure has been defined.

A miRNA CATALOGUE FROM THIRD-STAGE LARVAE AND EXOSOMES OF *Anisakis pegreffii*

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Background. 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).
Objectives 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-RNAs were isolated from L3 and EX of *A. pegreffii* from *Merluccius merluccius* and sequenced using Illumina (single-end, 50bp). Raw reads were mapped to *Anisakis simplex* genome

and to a collection of *A. pegreffii* hairpins and mature predicted by a double approach (using miRNAs from *Ascaris suum* and miRDeep* software). Mapping reads provided *A. pegreffii* miRNAs catalogue. Differential expression analysis was performed by EdgeR. Selected miRNAs was validated by Stem&Loop PCR.
Results. A total of 101 million and 71 million reads were obtained for *A. pegreffii* L3 and EX, respectively. With a list of 206 predicted miRNAs from the two approaches, 156 reads showed a match in the samples (76%) representing the final catalogue. Forty miRNAs (25.6%) were found significantly differentially expressed.
Conclusion. The most abundant miRNAs in the L3 sample showed identical seed regions with other parasitic helminths suggesting a possible conserved function across evolutionary distant taxa. Among miRNAs found enriched in exosomes, some showed targets related to host immunity and inflammation.

UNWANTED GUEST – A RAT MODEL FOR STUDYING EARLY IMMUNE RESPONSE TO AN UNUSUAL HUMAN PATHOGEN, *Anisakis pegreffii*

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Background: Anisakiasis, a zoonotic disease caused by *Anisakis* spp. larvae, represents a rising public health problem. Presentation of the disease is variable with unspecific gastrointestinal and/or allergic symptoms, accounting for the high number of misdiagnosed cases.
Objectives: To better understand the onset of anisakiasis in Sprague-Dawley rats experimentally infected with *Anisakis pegreffii*, we aimed at characterising early cellular and molecular (6-72 h p.i.) immune response in affected tissues and general regulatory mechanisms.
Materials and Methods: Two experiments were performed on 35 and 10 male rats, respectively, and each animal was intubated by the gastric probe with 10 live *A. pegreffii* larvae or 1.5 mL of saline (external control). At predefined time points, animals were sacrificed and tissues with visible lesions were processed for i) Illumina NextSeq 500 paired-end sequencing, ii) target genes and microRNA expression analysis, iii) histopathological evaluation (HE, IHC, TEM), and iv) global DNA methylation quantification.
Results: In total, there were 1372 differentially expressed (DE) genes in the *Anisakis*-infected rat stomach tissues and 1633 DE genes in the muscle tissues. *Il6*, *Il1b*, and *Ccl3* showed particularly strong expression in the stomach and visceral adipose tissues. In total, three microRNAs were differentially expressed. Histopathology revealed severe inflammatory/haemorrhagic lesions in stomach tissues, dominated by neutrophils and macrophages. Different numbers of $CD3^+$, $CD4^+$, $CD68^+$, $iNOS^+$ and $S100A8/A9^+$ cells were found in stomach, intestine, and muscle tissues. TEM revealed the presence of eosinophils in the inflammatory infiltrate with a lack of mast cells and diffuse areas of tissue necrosis, particularly in muscle tissues. No changes in global DNA methylation were observed between infected and non-infected tissues.
Conclusions: *Anisakis* infection induces strong immune responses in infected rats with marked induction of specific proinflammatory cytokines and miRNA expression, which seems to favour the activation of the interleukin 17 signalling pathway and the development of the T helper-17 response.

Anisakis AND ANISAKIASIS - AN OLD PATHOGEN AND AN EMERGING DISEASE

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Anisakiasis, a diseases caused by species of genus *Anisakis* infecting humans, has recently been ranked as fifth in the European risk ranking and the second of 24 foodborne parasitoses with the highest “increasing illness potential”. It manifests in four specific forms; gastric, intestinal, ectopic and gastro-allergic, although a high number of asymptomatic *Anisakis*-sensitised individuals have been observed in countries with high per capita fish consumption. Being frequently misdiagnosed with underreporting of cases, anisakiasis data result in biased interpretation and speculative incidence. In most cases anisakiasis expresses only as mild, almost inapparent gastrointestinal perturbation, but the rise in hypersensitised subjects might lead to rise

in allergy cases, which become detected also through component-resolved diagnosis or epidemiological studies. However, anti-*Anisakis* IgE antibodies in sensitised patients are detectable in serum during many years, sometimes boosted by continuous ingestion of *Anisakis* allergens with food, which impedes even the approximate determination of time of primo-infection. Previous studies reported a wide range of anti-*Anisakis* IgE seroprevalence in asymptomatic population and population with allergic symptomatology, but the great variation among study design, anamnesis, group size and most importantly, serodiagnostic tools used to detect the sensitisation, hampers a clear resolution of the situation. However, proper evaluation of serological status within countries is in particular important when it comes to assessment of populations exposed to professional risk, such as fishermen, fishmongers and fish-processing workers. Contact and air borne exposures have been reported in occupational settings of fishery and aquaculture workers, cooks, fishmongers and anglers, resulting in patients exhibiting symptoms of rhino-conjunctivitis, asthma, anaphylaxis and dermatitis, frequently leading to increased incapacity and absenteeism from work. While recently a step forward was made for standardised detection of the pathogen in fish products, much more needs to be done in standardisation of *Anisakis* serodiagnostics.

NEGLIGIBLE RISK OF ZOONOTIC ANISAKID NEMATODES IN FARMED FISH FROM EUROPEAN MARICULTURE

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Background. The diffusion of new eating habits leading to an increasing demand for raw or undercooked fish products, raises concerns about the transmission risk to humans of zoonotic fish parasites. Among these, anisakid larvae have been widely reported in wild fish, while the risk of their presence in farmed fish still need to be fully investigated.

This has led to the current European Union (EU) Regulation No 1276/2011 amending Annex III of Regulation (EC) No 853/2004 and mandating a freezing treatment of such products, except the farmed Atlantic salmon (*Salmo salar*) for which the risk is already considered negligible according to EFSA Opinion (2010).

Objectives. To assess the zoonotic Anisakidae parasite risk in European farmed marine fish other than Atlantic salmon.

Material and Methods. From 2016 to 2018 a parasitological survey was carried out on 6,549 farmed fish including 2,753 gilthead seabream (*Sparus aurata*), 2,761 European seabass (*Dicentrarchus labrax*) and 1,035 turbot (*Scophthalmus maximus*) from 14 farms in Italy, Spain and Greece. Furthermore, 200 rainbow trout (*Oncorhynchus mykiss*) sea-caged in Denmark, as well as 352 seabream and 290 seabass imported in Italy and Spain from other countries were examined. Fish were subjected to visual inspection and candling. Fresh visceral organs/fillet samples were artificially digested or UV pressed and visually examined for zoonotic anisakid larvae.

Results. No zoonotic parasites were found in any of the fish investigated. One European seabass from an Italian farm, showed the presence of a non-zoonotic raphidascarid fourth stage larva encysted on the liver, identified as *Hysterothylacium fabri*.

Conclusion. The risk linked to zoonotic Anisakidae in the examined fish species from European mariculture appears negligible. This study laid the groundwork for considerations to amend the current EU regulation and for planning surveillance activities in EU fish farming systems, as it appears feasible and reliable for the industry.

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ORAL PRESENTATIONS

LENGTH AND DEPTH ARE MAJOR DRIVERS OF *Anisakis* LEVELS IN A ZOOPLANKTON-FEEDING FISH

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Background. Identifying major drivers of parasite infestation levels in fish is a crucial prerequisite to assess potential hazard for human health. It is also needed to address how the actual integration of parasites in marine food webs and environment.

Objectives. By coupling two trophic tracers with an analysis of parasite prevalence, this study aimed at comparing the relative effect of size and environment on the intensity of *Anisakis* sp. observed in tissues of jack mackerel *Trachurus trachurus*, a species of major importance in the English Channel ecosystem and fisheries.

Material and Methods. Fish were collected during the 2014 CAMANOC ecosystemic survey. For each fish, diet was inferred by coupling stable isotopes and stomach content analyses. *Anisakis* infsetation levels were estimated in five tissues or organs.

Results. Size and size-driven dietary variation is the major driver of parasite number. Small mackerel displayed low numbers of parasites, as they consume copepods, a zooplankton species which do not host *Anisakis* larvae. On the contrary, the higher number of *Anisakis* in large fish is directly driven by their consumption of euphausiids, intermediate hosts of *Anisakis*. This number is however largely variable, and is controlled by depth. In shallow stations, mackerel can access benthic (and less parasited) preys. As *Anisakis* is a parasite of pelagic pathway, its intensity in fish is higher in deep stations, as fish diet is exclusively based on pelagic preys.

Conclusion. Results of the present study illustrated that levels of parasitism in fish are under the control of both biotic and environmental drivers. These results confirm the interest of considering parasitism under the integrated "One health" approach, as their level and consequently potential effects on fish and associated ecosystemic services (*e.g.* fisheries) or on human health are completely driven by ecosystemic functioning, like food web structures.

Funding source: This work was funded by Ifremer Scientific Directorate (project RETROGRAD), by the French government and the Hauts de France Region (CPER MARCO, project RECOMANCHE), and by the SFR Campus de la Mer (projects PARATROPH and PATROFLU).

A FINE-SCALE ANALYSIS REVEALS MICROGEOGRAPHIC HOTSPOTS MAXIMIZING INFECTION RATE BETWEEN A PARASITE AND ITS FISH HOST

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Background: For parasites, finding their hosts in vast and heterogeneous environments is a task that can be complex. Some parasite species rely on elaborate strategies to increase encounter rate with their hosts (e.g., behavioral modification of host), but others do not. For these parasites, a key issue is to reveal the processes that enable them to successfully find their hosts and complete their life cycles.

Objective: Here, we tested the hypothesis that infectious larvae of the freshwater ectoparasite *Tracheliastes polycolpus* are not homogeneously distributed along the river and preferentially occur in very specific microhabitats that maximize encounter rate, and hence infection rate, with their host fish.
Material and Methods: To do this, we combined an *in-situ* experiment (caging) with an empirical survey carried out on the same sites to identify potential "hotspots" of infection at the microgeographic scale and their environmental characteristics.
Results: Experimental and empirical results demonstrated that infections were not evenly distributed among microhabitats, and that infections were spatially aggregated in hotspots at a very fine spatial grain. We further found that certain combinations of environmental variables were consistently and non-linearly associated with higher infection rate for both caged and wild-caught fish. Microhabitats characterized by very low or high stream velocities, associated with medium and very small substrate respectively and a deep water column were strongly and repeatedly associated with higher infection rates. These microhabitats could concentrate parasites and/or promote physical contact with the hosts.
Conclusion: We conclude that the characteristics of some microhabitats could facilitate contact between hosts and parasites and explain how some parasites manage to find their hosts in complex environments.

Funding source: This work was supported by the Agence Nationale de la Recherche (project INCLIMPAR, grant № ANR-11-JSV7-0010). BiodivERsA (project PROBIS) and the Institut Universitaire de France. EMB was financed by a PhD grant from the French Ministry for Education and Sciences. This work was carried out within the framework of the "Laboratoire d'Excellence" (LABEX) TULIP (ANR-10-LABX-41).

INVESTIGATING THE ROLE OF PARASITE PLASTICITY IN PARASITE HOST SHIFT: EVIDENCES FROM A TRANSCRIPTOMIC APPROACH

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Background: The ability of parasites to use and adapt to new host species strongly affects their ecological and evolutionary dynamics, as well as their pathogenic effects on host communities. Although host shift has been reported in many parasite species, little is known about the processes permitting parasite adaptation to alternative host species.
Objective: Here, we tested whether plasticity could be a mechanism favoring host shift in parasites.
Material and Methods: Focusing on an emerging parasite (*Tracheliastes polycolpus*) that infect freshwater fish species, we used a transcriptomic approach to compare patterns of gene expression between parasites infecting their main host species to those of parasites infecting two alternative host species.
Results: We found 120 protein-coding genes that were differentially expressed (DEGs) between parasites infecting different host species. Most DEGs were found between parasites using the main host species compared to parasites using the two alternative host species, whereas only a few DEGs were identified when comparing parasites from the two alternative host species (7 DEGs). The main biological processes associated with the exploitation of different host species were related to cellular machinery, energetic metabolism, muscle activity and oxidative stress. Furthermore, we found no evidence for selection associated with host specificity among 55 645 identified SNPs, which rather suggests that adaptation to alternative host species results from plasticity.
Conclusion: This study provides unique empirical evidence that plasticity is a key mechanism for parasites to use and adapt to alternative host species, which probably facilitates invasion in this particular parasite species.

Funding source: This work was supported by the Agence Nationale de la Recherche (project INCLIMPAR, grant № ANR-11-JSV7-0010). BiodivERsA (project PROBIS) and the Institut Universitaire de France. EMB was financed by a PhD grant from the French Ministry for Education and Sciences. This work was carried out within the framework of the "Laboratoire d'Excellence" (LABEX) TULIP (ANR-10-LABX-41).

Cryptocotyle (METACERCARIAE) PARASITIC COMMUNITIES FROM SEVEN COMMERCIAL FISH SPECIES SAMPLED IN THE ENGLISH CHANNEL AND THE NORTH SEA

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Background. Marine fish are often speckled with "black spots" caused by host response to larval trematode infection. Moreover, the presence of parasites may lead to important economic loss in fishery and aquaculture sector and may have impacts on public health. As many other zoonotic trematodes, *Cryptocotyle* (Lühe, 1899) are present in marine fish species. So far, its impact on human health is still unknown and few publications exist dealing with its recovery, identification and distribution among commercially important fish.
Objectives. The present study reports for the first time, *Cryptocotyle* metacercariae communities of marine commercial fish species in the English Channel and the North Sea.
Material and Methods. An epidemiological study was performed on seven commercial fish species: herring (*Clupea harengus*), sprat (*Sprattus sprattus*), whiting (*Merlangius merlangus*), pout (*Trisopterus luscus*), dab (*Limanda limanda*), flounder (*Platichthys flesus*) and plaice (*Pleuronectes platessa*). The samples were collected during the sea campaign of International Bottom Trawl Survey in the Eastern English Channel and the North Sea in January 2019 and 2020. *Cryptocotyle* infection was estimated by counting visible black spots or lesions due to this parasite on the fish skin. Metacercariae were isolated and characterised from a morphological point of view with microscopic observations and from a molecular perspective with Sanger sequencing of fragments of mtDNA *cox1* gene and of rDNA *ITS* region.
Results. The prevalence, intensity and abundancy show differences between fish species and fishing areas. Whiting and pout were the most infected species for all sampling areas. Morphological data were compared to available literature data to identify the isolated metacercariae. Phylogenetic trees including reference sequences were built to confirm morphological and molecular identifications.
Conclusion. This survey constitutes the first description of *C. lingua* and *C. concava* metacercariae in the English Channel and North Sea ecosystems.

Funding source: French Agency for Food, Environmental and Occupational Health & Safety and Hauts-de-France Regional Council.

TELL ME WHAT YOU EAT, I WILL TELL YOU WHAT YOU ARE! A STUDY OF THE HYPERPARASITE *Cyclocotyla bellones* (MONOGENEA, PLATYHELMINTHES) USING INTEGRATIVE TAXONOMY

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Cyclocotyla bellones Otto, 1823 is a monogenean characterized by an outstanding way of life. It is a hyperparasite that attaches itself to cymothoid isopods, themselves parasites of the buccal cavity of fishes. *Cyclocotyla bellones* was found on *Ceratothoa parallela* (Otto, 1828), an isopod parasite of *Boops boops* off the Algerian coast. We used integrative taxonomy combining a morphological description of *Cy. bellones* with a molecular analysis of COI mtDNA sequences. We provided, for the first time, molecular barcoding of a hyperparasitic monogenean, the parasitic crustacean host, and the fish host. We also investigated the diet of the monogenean and its morphology, compared it to a close non-hyperparasite monogenean, and addressed the question "is the monogenean really a hyperparasite (does it feed on the isopod?)?" The walls of the oesophagus and of the intestine of *Cy. bellones* were lined by dark pigment resembling those observed in hematophagous polyopisthocotyleans, derived from ingested host blood. We suggest that *Cy. bellones* would cumulate blood meals from the fish and egest them at intervals via the mouth; the indigestible haematin appear thus as black pigment. *Cyclocotyla bellones* should be considered a symbiont of the crustacean, as it uses the latter merely as an attachment substrate while feeding on fish blood. The body shape of various

diclidophorids from fish gills or parasitic isopods was compared. Our results showed that the anterior stem is observed only in diclidophorids dwelling on parasitic isopods and never in those infesting gills. We conclude that the anterior stem of the body of *Cy. bellones* is an anatomical adaptation for nutrition of the monogenean on the host fish. *Cyclocotyla bellones* is thus a hyperparasite in terms of location (it dwells on a parasite), but not in terms of nutrition (it does not feed on a parasite but on the primary host).

Funding source: ISYEB, DeepBlue Project; European Maritime and Fisheries Fund (EMFF); Ocean Space; TBA21 Academy; TBA21.

A ONE HEALTH APPROACH TO MANAGE PARASITIC INFECTIONS
Organizers / Moderators: Frank Katzer & Rachel Chalmers

INVITED LECTURES

ZOONOTIC TRANSMISSION OF INTESTINAL HELMINTHS IN THE PHILIPPINES

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Background. Intestinal helminths are highly prevalent infections of humans and livestock worldwide with significant impacts on public health and food production. Many intestinal helminths are transmitted through environmental contamination with parasite eggs by human and animal defaecation. In other instances, infection occurs through ingestion of infected meat. However, there is a poor understanding of the role played by animals in transmission of intestinal helminths to humans.
Objectives. The ZooTRIP project aims to assess the contribution of zoonotic transmission and environmental reservoirs to the human intestinal helminth infection burden in the Philippines, and determine effective strategies for helminth control and elimination.
Material and Methods. A cross-sectional household-based study was undertaken in eight municipalities in Region XIII, Mindanao Island. Household members (10-60 years) were requested to provide faecal samples and to complete a questionnaire gathering demographic, socio-economic and environmental information. Faecal samples were collected from companion animals (dogs and cats) and livestock (cattle, pigs and water buffalo), as well as environmental samples. Parasitological and molecular approaches are being used to diagnose intestinal parasites. Data will be integrated into mathematical models of parasite transmission.
Results. Analysis of initial data from 663 human participants revealed a soil-transmitted helminth prevalence of 22.8% and *Schistosoma japonicum* prevalence of 8.3%. Examination of faecal samples from 91 dogs, 136 pigs, 146 water buffalo and 18 cattle revealed 16 species of zoonotic helminths, with overall prevalence of 52.8%. Hookworms and *Toxocara* spp. were most prevalent in companion animals while *Fasciola* spp. and strongyles were most common in livestock. Molecular analysis is ongoing to confirm helminth species present and determine the extent of zoonotic transmission.
Conclusion. The project outcomes will provide an evidence-base for enhanced control and elimination strategies for intestinal helminths considering zoonotic and environmental reservoirs.

Funding source: Newton Fund, Medical Research Council, Department of Science and Technology - Philippine Council for Health Research and Development.

NEW APPROACHES TO INVESTIGATING ZOONOTIC *Cryptosporidium* OUTBREAKS

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Background. *Cryptosporidium parvum* is the major cause of zoonotically-acquired human cryptosporidiosis. Outbreaks are linked to animal contact, drinking or recreational waters, and food. It is necessary to identify sources of contamination and routes of transmission by epidemiological investigations, environmental inspections, and microbiological analysis. Although sequencing a hypervariable region of the gp60 gene is commonly used to further characterise *C. parvum*, multilocus analyses are more discriminatory and rapid

characterisation of isolates using multiple loci variable-number tandem repeat analysis (MLVA) may be more informative.
Objectives. To improve environmental inspections, triggers for visiting and sampling processes at premises linked to human cases. To investigate the application of a seven-marker MLVA panel for outbreak investigations, historical outbreaks, background cases and suspected sources were re-analysed using for typability, discriminatory power, and epidemiological concordance. To test the laboratory workflow, reporting, interpretation and communication of results in real time, the MLVA scheme was piloted during an outbreak investigation in 2021.
Material and Methods. Triggers and processes for sampling animals were reviewed and a lower threshold discussed with relevant agencies. PCRs were developed for seven markers identified on different chromosomes, and multiplexed as four-plex and three-plex reactions, and applied to a panel of 260 related and unrelated *C. parvum* isolates. Amplicons were sized by capillary electrophoresis to identify the number of repeat units calibrated against sequenced reference standards. A MLVA profile was compiled by listing the calculated number of repeats for each marker in chromosomal order: cgd1_470_1429 (GRH); cgd4_2350_796; cgd5_10_310 (MSF); cgd5_4490_2941; cgd6_4290_9811 (MSC6-5); cgd8_NC_ 4440_505; cgd8_4840_6355 (MM19). *C. parvum*-positive stools from patients who drank milk from an on-farm vending machine and from a calf at the premises were analysed.
Results. A protocol for first response sampling was agreed between agencies.
The MLVA typability of 260 samples was 89%, discriminatory power for unrelated samples was 0.99, and epidemiological concordance of 60 samples in five historical outbreaks demonstrated. Patient and calf samples from the farm were indistinguishable at seven loci.
Conclusion. Stronger evidence can be obtained in outbreak investigations by improved environmental and microbiological approaches.

PUBLIC HEALTH IMPACT OF FOODBORNE PARASITE IN A ONE HEALTH APPROACH

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Various ranking methods were used to rank foodborne parasites in Europe. Toxoplasma gondii has the highest disease burden using Disability adjusted life years. To estimate the relative contribution of the two main transmission routes, we developed quantitative risk assessment (QMRA) models with the aims to get more insight in the relative contribution of different pathways and risks for human infections. The QMRA results showed that raw meat consumption was important in the Netherlands and the relative contribution was strongly influenced by preparation and consumption habits and can differ from country to country. In the Netherlands, fresh meat is generally heated properly and two popular ready-to-eat raw meat products were most important and add up to 90% of the total of predicted meat borne infections. This has guided further research towards the effects of meat processing and may lead to intervention measures targeted at these specific products, either by freezing of the meat used for production or by adjusting other processing parameters (e.g. salt concentration) to ensure *T. gondii* inactivation. To compare the meat products with the highest risk that means which gives the most human infections between countries, it is important to use food consumption data collected using a similar method. A project aiming to study meat borne source attribution just started under the European Joint Programme One Health. The described QMRA model will be assessed with food consumption data from ten European countries to obtain better insight of control options in Europe. In conclusion, we have shown that QMRA models for foodborne parasites can be used to give more insight what control options are effective.

SQUEEZING *Giardia* UNDER THE ONE HEALTH UMBRELLA

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Our understanding of how different intestinal parasites fit into the One Health paradigm has swung backwards and forwards, along with our development of knowledge on those parasites, our examination of their morphology, and the development of methods that allow us to dissect transmission routes in greater detail. *Giardia duodenalis* has been particularly enigmatic, and, in this presentation, the intention is to summarise how our understanding has developed, and where we stand now. Different mammalian host species, all known to be susceptible to *Giardia* infection, will be used as examples; from domestic animals - such as cattle and dogs - to wild animals - such as raccoons, foxes, and (incriminated/wrongly incriminated) beavers - via non-domesticated animals frequently kept as pets / fur animals - such as chinchillas. In addition, other intestinal protozoa (*Cryptosporidium* spp., *Entamoeba* spp.) will be used for contrast and comparison, and also for discussing whether we are yet able to reach any firm conclusions on zoonotic potential based on comparisons of relatively short sequences at different target genes, or whether WGS is the only approach for reaching resolution on this question. Based on past history, this is hardly likely to be the last word on a subject that is relevant for both human and animal health, and also for understanding the evolutionary history of this parasite.

ORAL PRESENTATIONS

PILOT SURVEY OF CYSTIC ECHINOCOCCOSIS IN MASAAI LIVESTOCK-KEEPING COMMUNITIES OF NORTHERN TANZANIA

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Background. Maasai communities of Northern Tanzania are suffering the consequences of a very high prevalence of cerebral coenurosis (*Taenia multiceps*/*Coenurus cerebralis*), causing high mortality in small ruminants. Given the close similarity between the life cycle of *T.multiceps* and *E.granulosus sensu lato* (causing cystic echinococcosis, CE, in humans), this unusual epidemic raises concerns about an increased risk of CE in people.
Objectives. To estimate the prevalence of human abdominal CE and the prevalence and genotypes of *E.granulosus* s.l. circulating in livestock in Maasai communities of Northern Tanzania.
Material and Methods. Human CE was diagnosed by abdominal ultrasound on volunteers ≥7 years old in 5 communities of Longido and Ngorongoro districts. Infection in ruminants was evaluated through inspection in local abattoirs. 1-3 cysts/animal were collected and DNA extracted from 1 cyst/animal, prioritizing hepatic cysts. Molecular identification was performed using PCR targeting the COX1 gene, followed by RFLP and Multiplex PCR. The COX1 PCR product of non-*E.granulosus* s.l. samples was sequenced.
Results. Ultrasound was performed on 823 voluteers (n=352 in Longido, n=471 in Ngorongoro). 6 hepatic CE cases, 3 of which with active cysts, were diagnosed in Ngorongoro (1.3% prevalence for this district). Of the 696 ruminants inspected, 34.2% had parasitic cysts. Molecular identification, achieved for 139 cysts, identified *T.hydatigena* in 48.2% and *E.granulosus* s.l. in 51.8%: *E.granulosus sensu stricto* (G1-G3) in 87.5% cases; *E.ortleppi* (G5) in 9.7%, and *E.canadensis* (G6-10) in 1 cyst. The 3 human cysts removed surgically were G1-G3.

Conclusion. Human CE was detected only in Ngorongoro, despite a high prevalence of cysts in livestock in both districts. Several *E.granulosus* s.l. genotypes are circulating in Maasai livestock communities of Northern Tanzania. Understanding specific risk factors for CE in Ngorongoro is needed. Interventions targeting transmission routes common to these parasites would have dual benefits for preventing human CE and livestock diseases.

Funding source: This study was funded by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Research Grant 2019. Molecular analysis was supported by funding from the European Union's Horizon 2020 Research and Innovation programme grant agreement number 773830: One Health European Joint Programme (MEME project).

14th INTERNATIONAL SYMPOSIUM OF GEOSPATIAL HEALTH – GnosisGIS
Organizer / Moderator: Laura Rinaldi & Robert Bergquist
Co-moderator: Sherif Amer

INVITED LECTURES

COMPARING SPATIO-TEMPORAL DISTRIBUTION OF THE MOST COMMON HUMAN PARASITIC INFECTIONS IN IRAN: A SYSTEMIC QUANTITATIVE LITERATURE REVIEW

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Background: This study analyses the spatio-temporal trend of the prevalence of the four most prevalent parasitic diseases in Iran over two periods, 2007 to 2012 (period 1) and 2013 to 2018 (period 2), indicating high-risk and low-risk areas.
Material and Methods: Out of 19,126 articles, we selected 220 articles for data extraction and calculated the pooled prevalence for cutaneous leishmaniasis, human toxoplasmosis, giardiasis and blastocystosis for all 31 provinces in the country. Anselin local Moran's *I* was used to identify clusters and outliers in the prevalence rates.
Results: The mean prevalence of cutaneous leishmaniasis patients was found at 35.12 per 100,000 in period 1 but fell to 19.12 per 100,000 in period 2. The mean prevalence of acute and of chronic toxoplasmosis reached 2.36% and 32.5%, respectively, in period 1, which changed to 2.28% and 31.14% in period 2. The total prevalence of giardiasis declined from 9.8% in period 1 to 4.8% in period 2, while the mean prevalence of blastocystosis declined from 8.9% in period 1 to 6.76% in period 2. There was only one High-High cluster in period 1 (giardiasis), while there were two in period 2, one for blastocystosis and one for chronic toxoplasmosis.
Conclusion: The total prevalence of blastocystosis, giardiasis and cutaneous leishmaniasis in Iran has continually declined since 2007. In contrast, the prevalence of toxoplasmosis in pregnant Iranian women has not been changed. Iran's Midwest has more parasitic infections compared to the Mideast, which may be explained by the existence of vast deserts and consequently dry and hot climate in that part of the country.

PREDICTIVE TOOLS FOR ASSESSING THE INFECTION RISK BY RUMEN FLUKES IN SMALL RUMINANTS OF SOUTHERN ITALY

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Background. During the past decade the rumen fluke, *Calicophoron daubneyi*, has established as a prominent parasite of livestock within numerous European countries (Jones et al., 2017 Vet Parasitol. 240: 68-74). Climate and environmental changes have boosted the geographical expansion of this snail-borne trematoda, hence favoring the host-parasite contact during the grazing activities of ruminants. Geospatial health represents the ideal approach for a thorough infection risk detection and consequent spatio-temporal monitoring activities.
Objectives. The aim of the study was to assess the spatial distribution of *C. daubneyi* infection in sheep and goats in southern Italy and to develop a predictive model of the geographical distribution of rumen flukes in small ruminants.
Material 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 by the FLOTAC technique utilizing a zinc sulphate flotation solution (specific gravity = 1.35) (Cringoli et al., 2017 Nat Protoc. 12(9):1723-1732). A Hot-Spot analysis was developed to recognize any spatial patterns of infection in the study area.

Then, a univariate statistical analysis revealed soil texture, land use, elevation, aspect, slope and the presence of natural watercourses as potential predictors of rumen fluke infection. Two models explaining the presence of *C. daubneyi* were created using a stepwise the logistic regression and the random forest machine learning technique.

Results. The results showed 7.9% of sheep farms and 2.7% goat farms infected by *C. daubneyi*. Spatial patterns with highest prevalence were identified in the western part of the region. The soil texture and the presence of natural watercourses were found to be variables significantly ($P<0.05$) associated to the *C. daubneyi* distribution in study area.

Conclusion. The study confirms the importance of geospatial technology in supporting parasite control strategies and demonstrates that a combined use of different geostatistical techniques can improve the prediction of the *C. daubneyi* infection risk in small ruminants.

CHANGING PATTERNS OF SNAIL-BORNE DISEASES

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Snail-borne parasitic diseases, such as schistosomiasis and fascioliasis pose risks to human and animal health and cause major socioeconomic problems in countries all over the world, but most notably in sub-tropical and tropical countries. Many different species of parasites and snail host are involved in the transmission of snail-borne diseases, and the effects of climate and land-use change will vary with each the snail-parasite species' specific ecologies and the spatio-temporal scale of investigation. This makes it difficult to predict the exact effects and disease transmission and distribution. In recent years, there has been a considerable growth in the attention given to the impacts of climate change on several snail-borne diseases; most notably schistosomiasis and fascioliasis in the scientific literature. Yet, little consensus about the exact impact and the direction of outcomes have emerged, highlighting the complexity of the system we try to model. Studies from the northern and southern range margins for many snail-borne diseases indicate an increase in transmission as the most likely outcome of warming, whereas contractions or status quo scenarios emerged from the central, tropical parts of the disease distribution. A comparison between the current geographical distributions and the thermo-physiological limitations of the parasite and intermediate host snail species may offer additional insights.

Funding source: The Knud Højgaard Foundation

ORAL PRESENTATIONS

SPATIAL AND TEMPORAL ANALYSIS OF SARS-CoV-2 CONCENTRATION IN WASTEWATER IN THE NETHERLANDS

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Background. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been a public health emergency of global concern since its outbreak in 2019/2020. In order to monitor the spatial and temporal patterns of SARS-CoV-2 transmission, public health authorities and universities have established a variety of dashboards worldwide. These dashboards usually track multiple epidemiological indicators such as the number of confirmed COVID-19 cases, hospital admissions, and mortality. In The Netherlands, wastewater surveillance of SARS-CoV-2 is used as additional indicator serving as an early warning for (re-)emergence of SARS-CoV-2 circulation in communities.

Objectives. Our research assesses the added value of the SARS-CoV-2 wastewater surveillance data in relation to the standard epidemiological indicator data collected in the Netherlands across space and time.
Material and Methods. Publicly available secondary data on epidemiological indicators and wastewater SARS-CoV-2 concentrations were compiled into a unified spatial database involving data fusion and spatial (dis) aggregation. Subsequent analysis consisted of three stages. In stage 1, the spatio-temporal patterns of SARS-CoV-2 transmission were visualised through time series mapping at multiple geographic scales. In stage 2, the statistical association between all epidemiological indicators was assessed based on bivariate correlation analysis. In stage 3, the feasibility of using SARS-CoV-2 wastewater data - as a spatially disaggregate early warning indicator of transmission intensity - was determined based on cross-correlation analysis.
Results. Overall, SARS-CoV-2 concentrations in wastewater have a medium to strong correlation with the weekly number of confirmed COVID-19 cases. Considerable variations, however, are apparent across municipalities and the (much larger) safety regions. Cross-correlation results indicate the absence of a time lag between SARS-CoV-2 concentrations and confirmed cases in most municipalities in the Netherlands. This indicates that SARS-CoV-2 wastewater concentration data - although with merit - is probably not very suited as an early warning indicator.
Conclusion. Our novel spatio-temporal approach shows that SARS-CoV-2 wastewater concentration data has added value as a complementary epidemiological indicator. We recommend further testing of our methodology.

A SPATIAL ANALYSIS OF COVID-19 IN AFRICAN COUNTRIES: EVALUATING THE EFFECTS OF COVID-19 VULNERABILITY RISK FACTORS

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Background: The ongoing highly contagious coronavirus disease 2019 (COVID-19) pandemic, which started in Wuhan, China, in December 2019, has now become a global public health problem. The pandemic is exerting great strain on the already fragile economies, health systems, and education systems of all countries in the African continent. However, there are inter-country variations in the levels of COVD -19 risk and impacts due to the varied economic, health systems, and social strengths and vulnerabilities. We aimed to assess how spatial vulnerability risk factors and neighbourliness between countries could generate potential insights into COVID 19 disparities across Africa.
Methods: Using publicly available data from the COVID-19 data repository of Our World in Data, effects of the risk factors were compared between two time periods, namely first wave (January-September 2020) and second wave (October 2020 to May 2021) using spatial regression models. Results: As of 31st May 2021, there was a total of 4 748 948 confirmed COVID-19, with an average, median and range per country of 101 041, 26 963 and 2 191 to 1 665 617, respectively. We found differential effects in risk depending on the Wave of COVID 19.
Conclusions: Our findings provide evidence that could guide countries on how to prepare for and respond to the initial and subsequent stages of an infectious disease pandemic like COVID 19. Particularly where a future novel coronavirus is most likely to occur.

COVID-19 PANDEMIC RELATED WORRIES COMPARED TO EVERYDAY LIFE: EVIDENCE FROM A CROSS-SECTIONAL NATIONAL SURVEY OF AUSTRALIAN FAMILIES

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Background. The harm caused by the change of life behaviour to interrupt the spread of COVID-19 may have a prolonged lifelong impact on families' well-being. Earlier research revealed that a child's resilience

to distress varies by individuals and links to their parent and cultural identity. Prompt detections of the vulnerable population may aid in mitigating potential damages.

Objectives This paper is part of broader research work that aimed to understand the impact of COVID-19 on Australian parents and their primary-school-age children level of worry and its association to modifying children's outdoor active mobility behaviour.

Material and Methods. We carried online national survey sampling parents (n=332) and their primary school-aged children of grades (4-6) across five Australian states. Parents filled the socio-demographic, history of being worried and distressed, perception of neighbourhood safety, and other pandemic-related life stressors of economic stress, health, and pandemic-related media exposure. Children supported by their parents have reported their active behaviour two separate weeks before and during the pandemic. Finally, children independently have assessed their level of worry, amount of screen time and exposure to pandemic-related news during the COVID-19 outbreak. Spatial and statistical analyses were undertaken, and dissemination of findings was through ArcGIS StoryMaps.

Results. Ethnic background, history of daily distress, and safety perception were associated with a higher level of parents' worry during the pandemic. However, excessive daily exposures to media news largely by parents may have primarily contributed to heightened children's anxiety.

Conclusion. Limiting parents' exposure time towards unneeded news may reduce children anxiety in crises. Furthermore, model prediction of the vulnerable population may aid in the preparedness of adequate and timely physiological first aid in higher-demand areas to promote healthy recovery for parents, children, and communities in current or future crises.

Funding source: The PhD candidate (RZ) is receipient of the RTP Australian Scholarship.

CYCLING TO GET MY VACCINATION: HOW ACCESSIBLE ARE THE COVID-19 VACCINATION CENTERS REALLY IN THE NETHERLANDS BY BICYCLE?

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Background. Ensuring that populations can easily access COVID-19 testing and vaccinations is important for minimizing future risks of infections. Many factors can affect access. Of these physical accessibility and the ease in which people can get to a center is key.

Objectives. In this study we examine accessibility to COVID-19 vaccination centers in the Netherlands. Since the vaccine became available in the Netherlands, we concentrated on examining how accessible vaccination centers were by bicycle, a common and popular mode of transportation.

Material and Methods. Our study fully relies on publicly available, secondary data which were compiled into a unified spatial database. The addresses of all COVID-19 vaccination centers were obtained from the regional public health services and geocoded. Transportation network data were obtained from OpenStreetMap. High resolution population data at neighborhood level were retrieved from PDOK, an online platform providing high quality geodata. Multiple network based- accessibility measures were used to identify variations in accessibility by bicycle (e-bike and regular). Population data were used to assess disparities in accessibility.

Results. First results indicate that the current distribution of vaccination locations provides adequate accessibility to people living in densely populated regions of the Netherlands but that travel times can become considerable in more peripheral, less dense regions in Northern and Eastern parts of the country where the population is relatively older and of lower socio-economic status.

Conclusion. Our study shows how a computationally simple but sound GIS-based approach can provide accurate and timely information both to the general public and to public health officials. It also highlights that open data can increasingly be used to critically assess infrastructure needs and areas where health services/facilities are lacking. Finally, our approach can be extended to support decision-making on up/down scaling of the number of vaccination (and testing) locations in a region-specific manner.

COVID-19 IN THE RUSSIAN FEDERATION: REGIONAL DIFFERENCES AND PUBLIC HEALTH RESPONSE

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Our study presents a medical-geographical analysis of the SARS CoV-2 pandemic development in the Russian Federation during its first wave in 2020. In general, the initial course of pandemic in Russia was characterized by a basic reproductive ratio (R_0) of 2.41 (2.22 – 2.60), which is relatively low as compared to most affected countries. A suggested population immunity threshold was therefore estimated as 58% (55% - 62%). The observed cumulative number of COVID-19 cases varied from 5 to 195 per 10,000 population across the 85 Russian regions, while reported number of deaths ranged from 0 to 3.63 per 10,000. A spatial analysis was conducted to reveal relationships between the main epidemic indicators and a number of socio-demographic factors, including the quality of healthcare, transportation network connectivity, population density etc. Spatial regression models demonstrated that the onset of the epidemics by the regions of Russia was determined by their proximity to major international airports and connectivity of transportation network, while morbidity and mortality rates show a pronounced relationship with the population density, urban population proportion and proportion of the population over working age. No patterns were detected that would allow assuming an influence of climate factors onto the disease course in different regions, though further investigations are being conducted to reveal environmental factors potentially related to the intensity of epidemics at the local level.

Funding source: The spatial analysis was funded by the Russian Science Foundation (Grant 17-77-20070 "Assessment and Forecast of the Bioclimatic Comfort of Russian Cities under Climate Change in the 21st Century"). The GIS mapping was supported by Russian Foundation for Basic Research (Grant 18-05-60037 "Medical-Geographic modeling the space-time changes of environmental and socially significant diseases under the conditions of changing climate and economic development of the Russian Arctic")

E.V.E.: AN INTEGRATED SYSTEM FOR THE MANAGEMENT OF ENVIRONMENTAL DATA, TO SUPPORT VETERINARY EPIDEMIOLOGY

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Background. Climate and environment may have a marked effect on the onset and transmission of infectious diseases, and in particular of Vector-borne Diseases (VBD). In fact, environmental factors affect both the population dynamics of vectors, and the vector-pathogen interface. Therefore, accounting for climatic/environmental variables at different spatial and historical/temporal scales can improve the organisation of VBD response measures and research.
Objectives. The heterogeneity of remote sensing repositories and ground sensors requires data to undergo a strict harmonisation process. The developed system allows acquiring remote- and ground-sensed data, which are pre-processed and stored, permitting a quick and easy access to ready-to-use information.
Material and Methods. The system is composed of several modules implemented using R scripts (R x64 3.5) and other open-source third-party software (e.g. Modis Reprojection Tool, Grass). Input data are acquired from freely available online sources (e.g. MODIS, Copernicus program), resampled to a common origin and spatial resolution. The resulting data are then stored in a dedicated server (for raster format) and in an Oracle (Oracle 11g) Relational Database Management System (for table format and indexes). Outputs are available in multiple format. A web application was also implemented through PHP5 as an in-house data catalogue.
Results. The system, named E.V.E (Environmental data for Veterinary Epidemiology), is currently in use at the IZSVe. At present, available data include Land Surface Temperature, Vegetation indexes, Rainfall, Sea Surface Temperature, Primary net production. The stored information are continuously updated, according to availability of the online data-sources; the historical data start from 2010.

Conclusion. E.V.E. have already provided data for extensive researches on Avian Influenza and West Nile Virus. The system allows obtaining historical series to support studies on infectious diseases of animals at different timescales, up to almost real-time. Thanks to the modular solution adopted, further data and elaboration can be easily integrated without re-engineering the system.

CHIKUNGUNYA BEYOND THE TROPICS: A THREAT FOR EUROPE?

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Background. Chikungunya is a mosquito-borne viral disease caused by the chikungunya virus (CHIKV). First isolated in Tansania in the 1950s, during the past two decades CHIKV has seen a dramatic spread across the globe. While it has most commonly occurred in the tropical regions, repeated outbreaks in France and Italy are proof that temperate climates are not excempt from chikmungunya transmission. As *Aedes albopictus*, a competent vector for CHIKV, occurs in large parts of southern Europe, further outbreaks can be expected in the future. However, spatio-temporal risk assessment for CHIKV so far has mostly focused on global patterns and tropical regions – potentially underestimating the potential for chikungunya in temperate areas around the world.
Material and Methods. Based on long-term climate data, we build an ecological niche model (ENM) for chikungunya, that is specifically aimed at estimating the potential for CHIKV transmission outside the tropics and compare it with a previous global approach. Additionally, we employ a probabilistic epidemiological disease transmission model (EM) based on weather station data to capture the temporal variability of the CHIKV transmission potential in European cities.
Results. The new ENM for non-tropical regions reflects the spatial distribution of real-life CHIKV outbreaks in Europe considerably better then previous approaches. Large areas along the Mediterranean and Atlantic coasts are predicted to be climatically suitable for CHIKV transmission. The EM suggests a potential transmission season of up to 93 days in high-risk areas.
Conclusion. The spatial extent of potential CHIKV transmission in Europe is likely to be much larger than previously anticipated, and may further increase due to climate change.

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SPATIAL PATTERNS OF WEST NILE VIRUS DISTRIBUTION IN THE ENDEMIC AREA WITH FOCUS ON THE URBAN ENVIRONMENT

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Background. Southern Russia remains the area most affected by West Nile virus (WNV). Territories of the lower reaches of the Volga and Don Rivers are thought to be long-existing WNV foci. We aimed to identify the spatial determinants of WNV transmission occurrence in the endemic area with focus on the urban environment.
Material and Methods. To identify spatial patterns, the data for virus isolation from mosquitos, ticks, birds, and mammals in the environment during 1999-2016, as well as possible places of infection according to epidemiological investigations of human cases, were used. Population density was included as an offset to

correct for possible bias. The environmental suitability was modelled using an ecological niche modelling approach with MaxEnt software.

Results. Analysis of places of virus isolation in the environment showed significant contributions from surface temperature, distance to water bodies, and altitude. When indicators of the location and mobility of the population were included, the relative impact of factors changed, in which the roads density became most important. However, distance to water bodies remains significant contribution. When the places of possible places of human infection were added to the model, the percentage of leading factors changed slightly.

Conclusion. The urban environment could significantly increase the epidemic hazard of the territory. Quite favourable conditions for the circulation of the virus are created here, which is due to both the involvement of new hosts and vectors and the formation of favourable environmental conditions for their existence. The private building sector with low-storey houses and garden plots located in the city suburban area provides a connection between urban and rural transmission cycles. Road system components such as culverts, storm drains and roadside ditches easily become perfect larval habitat. If case detection is insufficient and diagnosis is not timely in such territories, adverse consequences may develop.

Funding source: This research was funded by the Russian Science Foundation (Grant 17-77-20070 "Assessment and Forecast of the Bioclimatic Comfort of Russian Cities under Climate Change in the 21st Century")

EVALUATING SPATIAL RISKS OF EMERGING ANIMAL DISEASES INTRODUCTION IN THE REPUBLIC OF KAZAKHSTAN: THE CASE OF AFRICAN SWINE FEVER AND PESTE DES PETITS RUMINANTS

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Background. The Republic of Kazakhstan (RK) is a land-locked country in Central Asia. Livestock breeding is one of the main industries of the RK. Historically, the RK has been free from African swine fever (ASF) and Peste des petits ruminants (PPR). Here, we used a variety of spatial analysis methods, to identify those regions within the RK that are most susceptible to the introduction and subsequent spread of PPR and ASF.

Material and Methods. To evaluate the susceptibility of RK districts to an ASF introduction, we applied a *conjoint analysis* that elicitated the opinion of a group of experts in relation to the extent at which relevant epidemiological factors influence the risk for ASF introduction into disease-free regions.

With regard to PPR, we developed a *spatial regression* model trained using PPR outbreak data from China, 2014-2020, considering a number of socio-economic and environmental factors. The model was then transferred to the RK to reveal those districts in which conditions may be most appropriate for PPR outbreaks.

Results. Share of pigs in backyard holdings, density of pig population and wild boar, and a common border with ASF-infected countries were the most influencing risk factors for ASF**.** Two clusters of districts at highest risk for ASF were identified in northern and south-eastern RK, respectively.

Susceptible population density, transportation network and landscape were the most influencing factors for PPR outbreaks. Three clusters of districts at highest risk for PPR were identified in eastern, western, and southern RK, respectively.

Conclusion. Results suggest that risk is heterogeneously distributed for both diseases in RK. Results here will help to define a national strategy to prevent the introduction of ASF and PPR in the RK from infected neighboring countries.

Funding source: This work was conducted as a part of scientific research by the Agro-Industrial Complex under the Scientific-Technical program "Scientific basics of the veterinary wellbeing and food safety", budgetary program #267, Research Project BR06249242.

SEE VECTORS AND VECTOR-BORNE PATHOGENS
Organizer / Moderator: Snežana Tomanović

INVITED LECTURES

GLOBAL WARMING AND INFECTIOUS DISEASES: THE NEED TO CATCH THEM BEFORE THEY CATCH US

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It is thought that 75% of emerging diseases are related to zoonotic diseases therefore, any change in the ecology that can affect wildlife can also affect human health. Zoonotic pathogens and parasites are maintained in animal reservoirs and regularly or sporadically spill over to cause disease in humans, sometimes leading to human-to-human or vector-borne epidemics (eg, SARS-CoV, Ebola) but more commonly to endemic or sporadic disease (eg, leptospirosis, helminthiases, Lyme disease, hantavirus diseases). Pathogens and their respective vectors/reservoirs depend, to a large extent, on local microclimates, the so-called climatic shell (temperature, rainfall, rising sea levels, wind, sunshine, etc). Climate change will bring major changes to the epidemiology of infectious diseases. Microbes can adapt to higher temperatures and global warming may select for microbes with higher heat tolerance that will reveal new infectious diseases. Human activity [migrations, vector control practices), deforestation, land use change, the development of intensive crops, water storage, the expansion of cities in suburban areas] may, also, affect transmission. The overall influence of climate change on disease is complex. The current pandemic has underlined the potential for infectious disease to cause massive destruction. Increasing understanding of how climate change can affect the dynamics and distribution of infectious disease seems more relevant than ever before. The information gathered daily is enormous and to understand the linkage among multiple factors several models are currently used. Experimental models contain a multitude of meteorological data and have been developed to describe the “bioclimatic envelope” for various vector-borne pathogens. The combination of climatic models with the field study which uses spatial analysis methods seems unavoidable together with the introduction of complex mathematical models and Artificial Neural Networks. We need to continue to invest in surveillance, epidemiology, antimicrobial therapeutics, basic research into mechanisms of microbial pathogenesis and the development of algorithms using sophisticated mathematics.

IMPACTS OF CLIMATE CHANGE ON WEST NILE DISEASE AND VECTORS IN SERBIA (1985 – 2100)

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West Nile virus (WNV) is endemic in the Republic of Serbia since 2011. Integrated WNV surveillance, based on the unofficial collaboration among human, animal and environmental health institutions (One Health approach), was implemented, from 2005 until 2018, in the Vojvodina Province, Serbia (VPS). We present the development of this cooperation between the VPS actors and the organisation of WNV surveillance in Serbia. We evaluated the integrated WNV surveillance system in VPS and discussed the most significant results on WNV circulation in VPS and mosquito control efforts in France, Greece, Italy and Serbia. The spatial analysis of the effects of climate change (CC) on the most important vector species in Serbia (*Culex pipiens, Ixodes ricinus, Aedes albopictus, Anopheles hyrcanus,* and *Phlebotomus papatasi)* was carried out using the Multi Criteria Decision Analysis (MCDA) model developed within the framework of the draft National Climate

Change Adaptation Plan. Climate projections from the EURO-CORDEX ensemble of regional climate models (RCM) were used to investigate the MCDA risk for the: (i) short-term (2011 – 2040); (ii) medium-term (2041 – 2070); and long-term (2071 – 2100) scenarios.
The results showed that most of Serbia would become significantly more suitable for all the investigated vector species with the number of high-risk districts increasing from 1 to 23 (median of 9) by the end of the century. Strong correlations were observed between the vector index values and the incidence of human West Nile neuroinvasive disease (WNND) cases recorded at the district level. The occurrence of WNV detections in *Cx. pipiens* was significantly correlated to overwintering temperature averages and seasonal relative humidity at the sampling sites. Model projections indicate a twofold increase in the incidence of WNV positive *Cx*. *pipiens* for a rise of 0.5 ˚C in overwintering $T_{October–April}$ temperatures. The inter-institutional and interdisciplinary integration provided highly satisfactory results in terms of area specificity (the capacity to indicate the spatial distribution of the risk for human cases of WNND) and sensitivity to detect virus circulation even at the enzootic level.
For the successful implementation of vector control, it is crucial to know how to use already available tools. Using a useful tool in the wrong way could yield unsatisfactory results. Also, it seems that a lot of money is spent on mosquito control without really knowing the consequences, emphasising the need to include quality assessment in the system.

SHOULD WE BE SCARED FROM A TICK BITE (IN SOUTHEASTERN EUROPE)?

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It could be assumed that humans have been exposed to ticks since the early age of humankind. The oldest samples of ticks in amber are dated as far as 90 million years ago, and the earliest data on the impact of ticks on human health originate from Egyptian papyrus scroll from approx. 1550 B.C. Understandably, fear and disgust toward ticks are developed in humans as protective emotional responses.
Feeding exclusively on the blood of vertebrate animals and occasionally humans, ticks can cause a direct effect on their hosts, but more important is their role as vectors of diseases. Despite the worldwide distribution, ticks are considered as vectors of local importance since the epidemiology of tick-borne diseases is strongly correlated with the ecology of ticks as vectors and their vertebrate hosts. Furthermore, social, demographic, and environmental factors influence transmission dynamics of tick-borne diseases in a great manner. With over 40 viral, bacterial and protozoan pathogens, global risk maps of tick-borne diseases follow the patchwork pattern, urging for locally tailored prevention, control, diagnostic and therapeutic approaches. People in Southeastern Europe are more or less frequently bitten by over 10 hard tick species, which together vector nearly 20 different pathogens. Distribution of major vector species, such as *Ixodes ricinus*, *Dermacentor marginatus*, *Dermacentor reticulatus*, *Rhipicephalus sanguineus*, *Haemaphysalis concinna*, *Hyalomma marginatum* are overlapping in this region, and the area is characterized by favourable conditions for the establishment of natural foci of several tick-borne diseases (Lyme borreliosis, Crimean-Congo hemorrhagic fever, Tick-borne encephalitis, Mediterranean spotted fever, Ricketsiosis, Tularemia). With the dynamic interplay between vectors, pathogens, and hosts in the region, humans are exposed to ticks bearing single or multiple infections with relatively high prevalences. As part of nature, ticks are not to be scared of but taken with caution.

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ORAL PRESENTATIONS

THE OCCURRENCE OF *Hyalomma* spp. IN GERMANY – RESULTS OF A CITIZEN SCIENCE STUDY

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Background. *Hyalomma* spp. are mainly distributed in Southern and Eastern Europe, Africa and parts of Asia. While *H. rufipes* occurs especially in the arid regions of Sub-Saharan Africa and the Arabian Peninsula, *H. marginatum* is also endemic in the more humid regions of Southern and Eastern Europe. Findings outside of these areas are presumably due to the spread by migratory birds. In contrast to the most common tick species in Germany, *Ixodes ricinus*, which is an ambushing tick species, *Hyalomma* ticks are actively hunting their hosts

Objectives The occurrence of questing *Hyalomma* ticks in Germany was monitored by a citizen science approach.

Material and Methods. In late summer 2018, an unusually high number of *Hyalomma* ticks was detected in Germany. Afterwards, a press release was published. In February 2019, a website was launched and interested citizens were asked to send in ticks of the genus *Hyalomma*. From the 27th of February, several press releases were published promoting this website and presenting our latest data.

Results. In the study presented here, we report the occurrence of 210 specimen of the genus *Hyalomma* in two years in Germany. In 2018, 35 *Hyalomma* specimens were reported to us, of which 17 were only available as pictures and were identified as *Hyalomma* spp., 18 specimens were sent in and identified to species level. In 2019, we received 112 *Hyalomma* spp. in total, of which 95 originated from Germany. In Addition, 14 specimens of *Hyalomma* spp. were recorded in Germany based on pictures in 2019. In 2020 we received a total of 63 *Hyalomma* spp. of which 56 originated from Germany.

Conclusion. These results show that exotic tick species get imported to Germany, presumably by migratory birds and can develop into host-seeking adult ticks. They can be considered as a potential threat for humans and livestock in Germany. Additionally, citizen science projects can be used to collect important information about imported tick species in Germany.

SMALL RODENTS AS HOSTS OF TICK-BORNE PATHOGENS IN SERBIA

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Background. Tick-borne pathogens (TBPs), the agents of tick-borne diseases (TBDs), such as Lyme borreliosis, babesisosis, Q fever, hepatozoonosis, rickettsiosis, anaplasmosis, tularemia, relapsing fever, are mainly maintained in natural foci through the transmission cycles of competent tick vectors and vertebrate reservoirs. Number of small mammal species have been identified as reservoirs or hosts of TBPs.

Objectives. The aim of the present study was to explore the role of small mammals in enzootic cycles of TBPs in Serbia.

Material and Methods. A total of 179 small mammals (90 females and 89 males), identified as *Apodemus agrarius*, *Apodemus flavicollis*, *Microtus arvalis* and *Crocidura* sp., were collected from five localities in Belgrade region (Avala, Kosmaj, Košutnjak, Titov gaj, Ada Ciganlija), one locality (Veliko Gradište) in Northestern Serbia

and one from Western Serbia (Milošev Do). After necropsy, tissue samples (liver, spleen, kidney, and bladder) were collected from each animal and pulled. All samples were tested for the presence of TBPs belonging to genera *Borrelia*, *Rickettsia*, *Coxiella*, *Hepatozoon*, *Babesia*, *Francisella*, and family *Anaplasmataceae*, by conventional PCR and sequence analyses.

Results. In 179 samples, the DNA of following TBPs was detected in small rodent species - *Rickettsia helvetica* (1.1%), *Rickettsia monacensis* (0.6%), *Coxiella burnetii* (1.7%), *Borrelia afzelii* (0.6%), *Hepatozoon canis* (0.6%), *Babesia microti* (0.6%), and *Candidatus* Neoehrlichia mikurensis (0.6%). Further, coinfection with *Bo. afzelii* and *Ba. microti* was found in one sample (0.6%). Samples collected from Belgrade localities were positive to all tested TBPs (except to *Francisella*) while two samples from locality in Northestern Serbia were positive to *C. burnetii*.

Conclusion. The study shows the presence of number of TBPs - *Bo. afzelii*, *Ba*. *microti*, *R. helvetica*, *R. monacensis*, *C. burnetii*, *H. canis*, and *Ca.* N. mikurensis, in tissues of small rodents, suggesting potential role of these animals in temporarily maintaining and spreading of these bacterial and protozoan pathogens in Serbia.

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KNOWLEDGE, ATTITUDE AND PRACTICES OF GENERAL POPULATION, PROFESSIONALLY TICK- EXPOSED PERSONS AND HEALTH CARE WORKERS FOR TICK-BORNE ENCEPHALITIS AND TICK-BORNE DISEASES IN SERBIA

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Background. Tick-borne diseases (TBDs) represent a significant burdain to public health and economy in Europe. Amongst TBDs, tick-borne encephalitis (TBE), caused by a flavivirus, might lead to the lethal outcome in humans and animals.

Objectives. The aim of this study was to establish the level of knowledge, attitude, and practices (KAP) between general population (GP), professionally tick-exposed persons (PTEP), and health care workers (HCW) in Serbia for TBE and TBDs.

Material and Methods. To get insight into KAP for TBE and TBDs in Serbia, three different groups of people (PTEP, HCW and GP) were subjected to an anonymous, voluntary, on-line questionnaire. It consisted of 68 questions divided in four groups: general questions, knowledge, attitude, and practices. The statistical analysis was performed in JASP 0.14.1.0 and Microsoft Excell software packages.

Results. Total of 663 questionaries were collected in the period from February to March 2021, from which 642 were further analysed. Expectedly, the results showed significant difference in knowledge for TBE and TBDs between GP and PTEP and HCW ($p<.001$). Interestingly, the perception of risk to tick exposure and TBDs was high in all three groups (42.38% [33.60-51.16] of GP, 44.87% [35.81-53.93] of PTEP and 46.23% [37.96-54.49] of HCW), while the parameters of fear towards ticks and TBDs remained low and without statistical differences between tested groups (13.74% [7.95-19.53] of GP, 12.57% [7.29-19.86] of PTEP and 13.48% [7.45-19.51] of HCW).

Conclusion. Problem of TBE and TBDs in Serbia is still neglected due to low annual number of severe clinical cases in human population. Continuous education of persons at risk, HCW and general population towards prevention of tick bite and transmission of TBDs is needed.

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SEE ONE HEALTH
Organizer / Moderator: Ivana Klun

INVITED LECTURES

Cryptosporidium AND *Giardia* IN THE EASTERN PART OF EUROPE: THE ONE HEALTH PERSPECTIVE

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Background. One Health is an approach where multiple sectors communicate and work together to achieve better public health outcomes, since it is critical to addressing health threats in the animal, human and environment interface.
Objectives. This presentation reviews the current knowledge and understanding of *Cryptosporidium* spp. and *Giardia* spp. in humans, animals and the environment in countries in the eastern part of Europe.
Material and Methods. Published scientific papers and conference proceedings from the international and local literature, official national health service reports, national databases and doctoral theses in local languages were reviewed to provide an extensive overview on the epidemiology, diagnostics and research on these pathogens, as well as analyse knowledge gaps and areas for further research.
Results. *Cryptosporidium* spp. and *Giardia* spp. were found to be common in eastern Europe, but the results from different countries are difficult to compare because of variations in reporting practices and detection methodologies used.
Conclusion. Upgrading and making the diagnosis/detection procedures more uniform is recommended throughout the region. Public health authorities should actively work towards increasing reporting and standardising reporting practices as these prerequisites for the reported data to be valid and therefore necessary for appropriate control plans.

IS SERBIA READY FOR THE ONE HEALTH APPROACH IN THE FIELD OF AMR CONTROL?

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Antimicrobial resistance (AMR) is a complex, multidimensional problem that can harm human and animal health. Hence, it is of utmost importance to include integrated and holistic multisectoral One Health approach in tackling AMR and in particular the need for better integration of aquatic ecosystem issue into current approaches. Although first Serbian National Programme with Action Plan to control AMR 2018-2022 has elements of intersectoral collaboration and coordination, water ecosystem was neglected.
In terms of the density of their occurrence and the physicochemical water quality parameters, The Republic of Serbia is among the wealthiest regions on the European continent, with almost 1,200 registered water sources. However, the development of the wastewater system is significantly behind the development of water supply system. Serbia has 44 wastewater treatment plants (WWTPs) for municipal wastewaters, with only six of them being operational. In order to abide by the EU environmental standards, 320 wastewater treatment facilities should be built in Serbia. Of upmost importance is the fact that 92% of the wastewater is released directly into recipients (i.e. rivers) without adequate treatment.
A pilot study called "Wastewater and river waters are reservoirs of clinically relevant carbapenemase-producing *Enterobacteriaceae*" was conducted in 2017. The aim of the study was to evaluate the presence of carbapenem-resistant enterobacteria (carbapenems are last-resort antibiotics) in wastewater and river water (the Sava and the Danube) in Belgrade. This study showed the presence of carbapenem-resistant *Enterobacteriaceae* in water systems in Belgrade and highlighted the potential role of aquatic environments as reservoirs of clinically relevant antibiotic-resistant bacteria.

Therefore, we need to raise awareness of abundance and ample scope of the AMR issue in water sector in Serbia, emphasising importance of inclusion of preventive activities and mitigating measures in future AMR Action Plan.

LEISHMANIASIS AND A ONE HEALTH APPROACH

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Leishmaniasis are vector borne zoonotic diseases spread by the bite of sand flies infected with pathogens Leishmania sp. Since it is a disease that can affect humans and animals, and also needs a vector for transmission, Leishmaniasis is a perfect example for how to apply One Health approach. One Health implies collaboration of medics, veterinarians, biologists and other experts in joining forces to control and understand the disease. Over 200 million people live in Asia, Africa, South and Central America, and southern Europe in the areas where Leishmaniasis is common, but the disease is also found in other European countries. For the conformation of disease presence in a region, several factors are needed: The presence of Phlebotominae as vectors, the presence of the pathogen Leishmania infantum (for the European region) in vectors, the presence of seropositive dogs with clinical symptoms and clinical cases of human leishmaniasis. Leishmaniasis was considered to be a Mediterranean disease, but during the last few years, the presence of L.infantum has been confirmed in vectors or dogs in Serbia, Hungary, Romania, Bulgaria, Austria, Germany and Slovenia. The occurrence of leishmaniasis is in relation with environmental / climate changes and socioeconomic risk factors, so that is why the One Health approach is important for control and surveillance of the disease.

Toxoplasma gondii INFECTION AND THE ONE HEALTH APPROACH IN SERBIA

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Research on *Toxoplasma gondii* infection in Serbia started more than 60 years ago, and was continued over the decades, to evolve into a complete One Health approach at present. In the early '60s, studies focused on the isolation of viable *T. gondii* from various animal species, including fowl, waterfowl, and pigs. Early serological investigations in animals (cattle, sheep, pigs, horses, dogs, cats, mice, and rats) were also performed, but except for pigs, on samples of limited size. In the following years, extensive research of *T. gondii* infection in humans, including seroprevalence studies in women of generative age began, and continues to this day. Analysis of the epidemiological risk factors showed that the major one for human infection was the consumption of undercooked meat and/or meat products. This finding, which was obtained consistently throughout the decades, prompted the systematic research of infection prevalence in food animals (cattle, sheep, pigs, and horses). Given the demonstrated significance of pork as a source of human infection in our milieu, studies in pigs have been periodically repeated, allowing for a temporal perspective. The most recent focus involves understanding environmental contamination and first studies on the presence of *T. gondii* oocysts in various water sources have been initiated, using an innovative methodology. The integrative approach to infection control in dealing with *T. gondii* infection in one centre in Serbia presents a unique real-life example of the application of the One Health concept.

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POSTER PRESENTATIONS

CLINICAL AND TROPICAL PARASITOLOGY

CLINTP1

ZOONOTIC INFECTIOUS DISEASES IN TRANSPLANTED IMMUNOCOMPROMISED PATIENTS

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Background. Immunocompromised patients, like transplant recipients, are a particularly vulnerable group being at higher risk of developing several infectious diseases. Among them, zoonotic diseases, such as visceral leishmaniasis, bartonellosis, Q fever and leptospirosis are a growing concern in immunosuppressed patients as they are more susceptible to develop severe symptoms of the diseases.

Objectives. The study aimed at the detection of *Leishmania infantum*, *Bartonella* spp., *Leptospira* spp. and *Coxiella burnetii* DNA in immunocompromised hosts through molecular methods.

Material and Methods. The study included fifty-eight transplanted subjects with suspected zoonotic infections, hospitalized at ISMETT from 2016 to 2021. Genomic DNA extraction was carried out from EDTA blood or tissue biopsy, using commercial kits. On the basis of clinical suspicion, samples were analyzed to search for DNA of *Leishmania infantum* by a Taqman Real Time PCR targeting the kinetoplast DNA, *Bartonella* spp. by both a PCR (16S-23S rRNA intergenic transcribed spacer) and a SYBR Green RT-PCR (16S-23S rRNA intergenic region), *C. burnetii* by a PCR (*htpB*) and a TaqMan RT-PCR (*IS1111*), *Leptospira* spp. by a multiplex TaqMan RT-PCR (*16S rDNA*, *lipL32*).

Results. Overall, out of the 55 transplanted patients subjected to analysis for different zoonotic agents following clinical suspicion, 10 (18.2 %) were positive for one of the examined pathogens. In detail, five patients resulted positive to *Leishmania infantum*, four patients were positive to *Bartonella* spp., one to *C.burnetii*. The only patient with clinical suspicion of leptospirosis resulted negative for the pathogen.

Table 1. Examined immunocompromised patients for each pathogen and relative results

	Leishmania infantum	*Bartonella* spp.	*Coxiella burnetii*	*Leptospira* spp.
Examined pathogens	42	12	3	1
Positive results	5	4	1	0

Conclusion. Our results suggest that a correlation can be found between immunosuppression and susceptibility to infectious zoonotic diseases and that immunosuppression due to a transplant may predispose patients to these infectious agents. Diagnosis of zoonotic diseases should be thus considered in the differential diagnosis of transplant recipients and may be useful in the management of patients in post-transplant phase.

CLINTP2

NOSOCOMIAL AND COMMUNITY ACQUIRED MYIASIS

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Background. Myiasis (B87 (2021 ICD-10-CM)) is the invasion of living tissues of humans and other vertebrates by dipterous larvae (maggots). Though most prevalent in tropic countries, there is a rise in literature data that testify the occurrence of myasis in temperate countries as well.The first case that we present is a nosocomial

myiasis in an unconscious patient on mechanical ventilation, treated in a specialized part of the hospital ward for 4 months. Fast moving maggots were detected and removed from his left axilla. The second case was a community acquired myasis in a patient treated for COVID-19 in OTPs. Although the COVID-19 symptoms resolved, the stuffy nose, irritation sensation, anosmia, sinusoidal headache and sticky thick white discharge remained for 2 months. The patient discharged 4 alive maggots from the nose.
Material and Methods. The collected maggots were analyzed by macroscopic and microscopic examination. Photographs were taken using Olympus SZX9 and Carl Zeiss Stemi 508 Stereo Microscope with an integrated high-resolution digital camera. Maggots from nosocomial myiasis were cleared in order to visualize the morphological details of the cephalon-pharyngeal skeleton and the integument. The species identification was performed by using well established identification keys.
Results. The maggots from the patient with nosocomial myiasis were identified as *Sarcophaga argyrostoma* (Diptera: Sarcophagidae) second instar larvae (length: 6-8mm). Unfortunately, we failed to perform more detailed examination of the larvae discharged from the nose of the post COVID-19 patient. However, MRI ruled out further infiltration of the larvae into the nose, sinus, orbit, face or brain.
Conclusion. Infestation by dipterous larvae reveals a broad range of symptoms depending on the anatomical location and maggots' burden. Its outcomes banks on host health status and medical staff awareness and experience. Collaborative approach is essential for its proper identification, managing and prevention.

CLINTP3

EFFICACY OF THE CRUDE EXTRACT OF *Holarrhena pubescens,* AGAINST COMMON TAPEWORM INFECTION OF FOWL

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Background: Indigenous Rajbanshi and Koch communities of Cooch Behar district of West Bengal, India use plant materials as curative for various infections/diseases of veterinary significance. Cestodes constitute one of the most important groups of poultry helminths, both in terms of number of species as well as pathology. Globally, traditional medicinal systems have taken advantage of the various useful natural products in controlling or eradicating various types of helminth diseases, of both humans and animals with lesser or no side effects.
Objective: The current communication focuses on depicting the anthelmintic efficacy of ethanolic extract of the stem bark of *Holarrhena pubescens* through ultra structural and histochemical studies against a model tapeworm infecting country fowl.
Material and Methods: Live parasites (*Raillietina spp.*) were collected in 0.9% phosphate buffer saline (PBS) from the intestine of domestic fowl slaughtered in local market. The parasites were treated with various dosages of ethanolic plant extract and reference drug Praziquental in PBS for efficacy testing and further studies. Ultrastructural studies (SEM) and histochemical localization of some tegumental enzymes like Acid Phosphatase, Alkaline Phosphatase, Adenosine triphosphatase, 5´- Nucleotidase were performed.
Results: The results of efficacy were based on 1mg/ml, 2mg/ml, 5mg/ml, 10 mg/ml, 15mg/ml and 20mg/ml doses of plant extract used to treat the parasites. Significant ultrastructural changes in the tegumental architecture of the worms treated with the most efficacious dose were noted, compared to their controls. Histochemical studies indicated a marked reduction in the activities of the enzymes.
Conclusion: From the ultrastructural and histochemical studies, it can be concluded that the ethanolic extract from stem bark of *Holarrhena pubescens* seems to be anthelmintic in nature.

CLINTP4

EVALUATION OF COMMERCIAL CONCENTRATION METHODS FOR MICROSCOPIC DIAGNOSIS OF PROTOZOA AND HELMINTHS IN STOOL SAMPLES

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Background: The diagnosis of intestinal parasitic infections still relies largely on microscopic examination of stools and requires quality reagents and expertise. The ParaFlo® assays (Eurobio Ingen), "Bailenger" and "Diphasic Concentration" (DC) products, are ready-to-use concentration methods for detection of ova and parasites. The use of such marketed techniques could alleviate the reagent traceability overload due to in-house methods, but needs evaluation.

Material/methods: Ninety-three stools were analyzed with the ParaFlo® concentration methods together with routine microscopic examination (direct wet mount and in-house Thebault's or Bailenger's and/or Merthiolate-Iodin-Formalin (MIF) concentration methods). Results obtained with the ParaFlo® Bailenger assay were compared to those obtained with in-house methods using McNemar χ^2 test.

Results: 79 were analyzed with ParaFlo® Bailenger and in-house concentration (Bailenger cohort), and 65 were analyzed with ParaFlo® DC and in-house concentration (MIF cohort). Concordance with in-house methods was 71% for ParaFlo® Bailenger and 78% for ParaFlo® DC, with Cohen's κ = 0.43 and 0.53 respectively (moderate agreement). Considering all parasites, the ParaFlo® Bailenger and the in-house Bailenger/Thebault detected 38/78 (49%) and 47/78 (60%) positive samples, respectively (p=0.004). The ParaFlo® DC and the MIF detected 20/55 (36%) and 21/55 (38%) positive samples, respectively (ns). In-house methods showed better performances than ParaFlo® Bailenger for protozoa detection: 49% (39/79) and 29% (23/79) of positive samples, respectively, but the ParaFlo® Bailenger was also able to detect helminths in 12 (15%) samples, while in-house techniques detected only 8 (10%). Protozoa cysts showed important morphological changes with ParaFlo® Bailenger, preventing identification in 3 samples (scored negative). The ParaFlo® DC and the in-house MIF detected helminthes in 31% (17/55) and 29% (16/55) of samples, respectively (ns). Agreement was strong for helminth detection (κ =0.75 and 0.70 in Bailenger and MIF cohorts, respectively).

Conclusions: In-house methods showed better performances than ParaFlo® concentration methods for protozoa detection. However, ParaFlo® assays showed equivalent results for helminths detection. This suggests that these marketed concentration methods could be used in routine associated to reliable techniques for protozoa detection, such as multiplex PCR.

Funding source: the ParaFlo® kits were kindley provided by Eurobio, but results were independently analyzed.

CLINTP7

ACCIDENTAL FINDING OF THE HORSEHAIR WORM (*Gordius* sp.) IN HUMANS: NO REASON TO WORRY

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Background. Horsehair worms (Nematomorpha) are parasitic animals morphologically similar to nematodes. They can be confused with bowel and urogenital parasites and sent to the laboratories for diagnosis of human parasitic infections.

Objectives. The aim of the paper is to refer to possible errors in doctors' offices, where patients with certain complaints bring the samples claiming that they have excreted them from their bodies and asking for a confirmation that these are the cause of their complaints, i.e. diseases.

Material and Methods. An adult worm was brought to the parasitology laboratory, found by a patient in the receptacle after urination. After macroscopic and microscopic properties were established, histological analysis of the worm was performed. The tissue of the medium part of the worm body was routinelly

processed to paraffin blocks. Five µm thick serial sections were cut from paraffin blocks by using microtome, and were subsequently stained with hematoxylin and eosin.
Results. The sample was 12 cm long and 0.3 cm wide along the entire length. The body was dark brown, cylindrical and with uniform diameter along the entire length. Individual parts of the worm were observed under a light microscope. The histological analysis revealed the presence of the cuticle, muscular layer, parenchyma and ventral nerve cord, which corresponded to the microscopic structure of *Gordius* sp.
Conclusion. The knowledge of parasite biology abrogates any pathogenic potential for people and public health relevance. In ambiguous cases, complete parasite identification is necessary, requiring electron microscopy and phylogenetic analysis, i.e. a multidisciplinary approach to diagnosis.

CLINTP8

SERO-EPIDEMIOLOGICAL SURVEY OF *Echinococcus granulosus* INFECTION IN PATIENTS WITH CYSTIC LIVER DISEASE FROM CROATIA

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Background: Cystic liver disease (CLD), characterized by solitary or multiple cysts in the liver, represents a widespread diagnosis. There is a wide range of possible causes; however, CLD of infective origin is usually caused by parasitic species of the genus *Echinococcus*. The aim of this sero-epidemiological study was to determine the seroprevalence of *Echinococcus (E.) granulosus* infection in patients with CLD from Croatia.
Material and Methods: During three consecutive years, a total of 540 serum samples from Croatian patients with hepatic cysts previously detected by imaging methods were screened for the presence of *E. granulosus* IgG antibodies with the use of semiquantitative enzyme-linked immunosorbent assay (ELISA). Western blot technique was used as confirmatory test for the CE diagnosis. Statistical significance was set at $p<0.05$.
Results: The overall *E. granulosus* seroprevalence rate in patients with CLD was 3.9%. There was no statistically significant difference in seroprevalence rates between male and female patients ($p=0.541$). The seroprevalence rate in participants residing in rural regions was 5%, compared to 3% of participants residing in urban regions ($p= 0.291$). There was a significant difference in seropositivity among different age groups ($p=0.002$). More specifically, the highest seroprevalence rate was detected in the youngest age group (i.e., up to 18 years), both in men and women (20% and 13%, respectively).
Conclusion: This study indicates that CE still represents a salient public health issue in Croatia, particularly in younger individuals; hence, effective prevention measures have to be implemented to reduce the infection burden, which may otherwise lead to various symptoms and complications in the infected individuals. As the initial phase of primary *Echinococcus* infection is always asymptomatic, small cysts can remain undetected for many years. Furthermore, echinococcal cysts must be differentiated from many other conditions (even various malignancies) by using imaging techniques in combination with serological assays.

CLINTP9

SEROLOGICAL DIAGNOSIS OF CYSTIC ECHINOCOCCOSIS – A NINE-YEAR SURVEY FROM SOUTH EASTERN SERBIA

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Background. Cystic echinococcosis (CE) is a parasitic disease caused by the larval stage of the *Echinococcus granulosus* complex and it is the cause of diseases in humans, domestic and wild animals. The diagnosis of human CE is still problematic since it is insufficiently or incidentally detected.
Objectives. The aim of the study was to establish the significance of confirmatory immunoblot test in the

diagnosis of CE compared to serological screening tests, as well as to establish demographic characteristics of the examinees.
Material and Methods. In the period from January 2011 to the end of 2019, in the region of South Eastern Serbia, 1817 individuals with suspected CE were examined in the cross-sectional study. The following commercial screening tests were used for the detection of specific antibodies: Hydatidosis IgG ELISA and Indirect hemagglutination assay (IHA). The commercial confirmatory Western Blot (WB) test was used as well. The results of the examination were statistically processed.
Results. In order to determine the agreement between the utilized diagnostic methods we calculated kappa value and prevalence-adjusted and bias-adjusted kappa (PABAK). Although the female gender was more frequent in our study, the only significant difference in EC positivity between the genders was found for the results obtained from ELISA test, where the females were more frequently diagnosed as positive than males. Calculated kappa and PABAK between ELISA and WB were 0.437 and 0.434, while between IHA and WB these were found to be 0.721 and 0.732, respectively. These values, along with the probability value ($p<0.001$), indicated a significant moderate and substantial agreement between the tests.
Conclusion. The studied region is endemic for echinococcosis. In a coordinated health surveillance system, control, and implementation of measures to prevent echinococcosis, serological diagnosis is a necessary tool, together with screening and confirmatory tests.

CLINTP10

CONSERVATIVE TREATMENT OF HEPATIC AND PULMONARY HYDATIDOSIS WITH A COMBINATION OF ALBENDAZOLE AND PRAZIQUANTEL

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Background. Conservative treatment of hydatidosis (cystic echinococcosis) in certain clinical indications with benzimidazole derivatives (mebendazole, albendazole) improved the clinical prognosis and the therapeutic success exceeded 60%. WHO documents mention a possible effective combination of albendazole and praziquantel.
Material and Methods. In order to increase the therapeutic efficacy of albendazole (15 mg/kg body weight, in courses of one month and intervals without treatment of 15 days), simultaneously the isoquinoline derivative praziquantel was taken by the patients. It was prescribed in a dose of 40 mg/kg, once every week. The therapy lasted from 2 to 6 one-month treatment courses.
Results. In observations of 20 treated patients (14 patients with liver and 6 patients with pulmonary cystic echinococcosis, only adults) in the last 5 years, imaging gave the following results - degeneration of echinococcal cysts in 17 (85%) of patients. Only 3 patients (2 patients with liver and 1 patient with pulmonary hydatidosis) did not respond to conservative treatment and were referred for surgery. No side effects were observed.
Conclusion. The combination of albendazole and praziquantel is an opportunity to improve the therapeutic efficacy of the conservative treatment of cystic echinococcosis in endemic countries such as Bulgaria.

CLINTP12

EOSINOPHILIC MYOCARDITIS CAUSED BY *Toxocara canis* LARVAE INFECTION: A CASE REPORT

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Background. Toxocariasis caused by *Toxocara* spp larvae, is a worldwide occurring zoonosis. Humans become infected by accidental ingestion of embryonated *Toxocara* eggs contaminating the soil or vegetables, or larvae found in domestic/wild paratenic hosts tissues. Typical target organs are liver and lungs, causing symptoms classically described as visceral larva migrans, however eye, CNS and heart could be affected. The disease is mostly asymptomatic and common in children. Myocarditis presents to 10-15% of the cases and can be caused by direct migration of the parasite larvae to the myocardium and/or by the body's hypersensitivity reaction due to myocardial infiltration by eosinophils.
Case report: A 54-year-old woman submitted at the hospital with a history of fatigue, apraxia, gait instability and involuntary movements of the upper limbs for a week as well as diplopia and blurred vision for two days. Clinical and neurological examination revealed disorientation in space and time, upper limb dysmetria, gait instability and bradyphrenia. Laboratory tests on admission revealed increased eosinophils count (3500 cells/mm3) and troponin (cTnI) levels (10ng/mL - upper normal limit of 0,04ng/mL) and the electrocardiogram showed nonspecific electrocardiographic changes. MRI of the brain revealed multiple abnormal foci in the T2 sequence in the cortex, deep white matter, and both hemispheres of the cerebellum, possibly attributed to multiple emboli. Cerebrospinal fluid and ocular examination didn't reveal signs of active infection. Cardiac MRI confirmed the inflammation of the myocardium (eosinophilic myocarditis) and the presence of a thrombus in the apex of the left ventricle. Parasitological examination using ELISA technique confirmed the presence of *T. canis* specific antibodies (titre 1/2400). Based on the above the patient treated with prednisolone, albendazole and acenocoumarol which lead to clinical improvement.
Conclusion. Toxocariasis is an important, potentially fatal yet neglected zoonosis. *Toxocara* infection should be considered as a possible cause of myocarditis especially when presented with eosinophilia.

CLINICAL HOT TOPICS IN TOXOPLASMOSIS

CLTOXO1

THE IMPACT OF *Toxoplasma gondii* INFECTION ON COGNITIVE DEFICITS, OTHER SYMPTOMS AND DIGITAL NEUROSCIENCE-INFORMED COGNITIVE TRAINING IN PATIENTS WITH SCHIZOPHRENIA

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Background: Studies indicate that digital cognitive training can remediate cognitive impairments in schizophrenia. However, it is not clear which factors contribute to deficits and response to cognitive remediation in this population. *Toxoplasma gondii (T. gondii)* is a neuroinvasive protozoan parasite that has been linked to poorer cognitive performance, with a higher prevalence in schizophrenia subjects.
Material and Methods: In this study, we investigate whether seropositivity to *T. gondii* leads to poorer cognitive performance and more severe symptoms in 62 patients with schizophrenia, and whether it affects their adherence and response to 40 hours of digital cognitive training.
Results: At baseline, seropositive subjects (n=25) presented lower global cognition when compared to the seronegative group (n=37) ($F=3.78$, $p=0.05$). Specifically, seropositive subjects showed worse performance in verbal memory ($F=4.48$, $p=0.03$) and social cognition ($F=5.71$, $p=0.02$). After cognitive training, the TOXO-positive group showed higher adherence to intervention ($X^2=9.31$, $p=0.01$), larger improvements in attention ($\beta=0.64$, $p=0.02$), social cognition ($\beta=0.40$, $p=0.03$) and associations between IgG titres and total symptoms measured by PANSS scale ($r=0.40$, $p=0.04$).
Conclusion: In conclusion, previous *T. gondii* infection was associated with worse baseline cognition, higher adherence and larger responses to treatment, and IgG titres were positively correlated with schizophrenia symptoms. On the other hand, we must be aware that *T. gondii* infection is closely linked to poor socioeconomic status, which can broadly impact cognition and overall schizophrenia symptoms.

CLTOXO2

A SYSTEMATIC REVIEW AND META-ANALYSIS OF *Toxoplasma gondii* SEROPREVALENCE AMONG TUBERCULOSIS PATIENTS

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Background. *Toxoplasma gondii* and *Mycobacterium tuberculosis* are intracellular pathogens that are able to cause chronic infections as well as severe disease in humans.
Material and Methods. We conducted a systematic review and meta-analysis to estimate pooled *T. gondii* seroprevalence among tuberculosis patients.
Results. From altogether 1389 documents identified from three international databases, eight papers were included in the systematic review and meta-analysis. Overall, few studies have been conducted on *T. gondii* among tuberculosis patients, and geographical data gaps were clear. The pooled *T. gondii* seroprevalence (proportion anti- *T. gondii* IgG antibody positive) among tuberculosis patients was 35.9% (95% confidence interval 19.3–56.7%). In the case-control studies, the pooled *T. gondii* seroprevalence (proportion anti- *T.*

gondii IgG antibody positive) was 29.5% among tuberculosis patients and 17.2% among controls, and the odds ratio by random effects model was 1.63 (95% confidence interval 1.28–2.08).
Conclusion. The results suggest an association between *T. gondii* seropositivity (anti- *T. gondii* IgG antibody positivity) and being a tuberculosis patient, but this should be interpreted with caution because the approach did not account for the timeline of the infections and the disease. The results showed that *T. gondii* seropositivity (anti- *T. gondii* IgG antibody positivity) was relatively common among tuberculosis patients.

CLTOXO3

NEW ASPECTS IN THE IMMUNOLOGICAL AND MOLECULAR DIAGNOSIS IN CONGENITAL TOXOPLASMOSIS, IN ORDER TO IMPROVE THE TREATMENT SCHEME

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Background. Toxoplasmosis is one of the most prevalent parasitic disease caused by *Toxoplasma gondii* an obligate intracellular protozoan. The life cycle involves a definitive host (*Felidae)* and a wide range of intermediate hosts. Sexual and asexual reproduction involve both hosts. Human patients enter accidentally in the life cycle of the parasite.
Material and Methods. 1789 biological materials (serum, CSF, amniotic fluid, aquous humour) were collected from patients with suspected toxoplasmosis. As methods: screening (ELISA) - 1623, confirmation (Western Blot WB) - 80 and PCR(RT-PCR) - 86. 40 pregnant patients were monitored, 26/40 followed up the level of antibodies in the newborn, up to 1 year old.
Results. 1115/1623 samples analyzed by the screening method were tested for acute phase antibodies (IgA, IgE and IgM) and 508/1623 for chronic phase antibodies (IgG). 40 patients recorded a recent infection, the avidity test was performed: 6/40 (15%) recent infections and 34/40 (85%) late infections. All acute infections were confirmed by WB.RT-PCR confirmed an infection in 5 cases. Monitoring the antibodies in pregnant patients (quarterly and in dynamics), the results showed 95 / 320(29.68%) positive -IgA, 37/639 (5.79%) - IgE, 27/116 (23.27%) - IgM and 392/508(77.16%) - IgG. Of the mother-newborn pairs, all children presented specific IgG-type antibodies at birth, which are transmitted passively maternal-fetal and have a titer that decreases in dynamics until the age of 1 year.
Conclusion. In the case of the diagnosis of toxoplasmosis, the laboratory methods have of major importance in monitoring the treatment and in preventing the risk of congenital transmission. Early detection of acute-phase antibodies (IgA, IgE, IgM anti-*Toxoplasma gondii*) in every quarter, together with molecular diagnostics allows the clinician an early diagnosis orientation and prompt establishment of an appropriate treatment, which leads to an increase of the quality life of the patient.

CLTOXO5

Toxoplasma gondii IN DOLPHINS STRANDED ALONG SICILIAN COASTS

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Background. *Toxoplasma gondii*, a protozoan pathogen causing zoonosis, represents a serious threat for aquatic mammals including dolphins, as it causes severe brain lesions in dolphins leading them to stranding and death.
Objectives. This study was aimed to investigate *T. gondii* DNA presence in organs collected from dolphins stranded along Sicilian coasts.
Material and Methods. From the beginning of 2021, nine dolphins were analysed, found stranded along the coast of Sicily (Table 1). Five animals were *Stenenella coeruleoalba*, 2 *Delphinus delphis* and 2 *Tursiops*

truncatus. Different organs were collected, including brain, spleen, liver, lung, lymph node, muscle and heart, for a total of 45 examined organs. One gram of tissue sample, diluted in 9 mL of saline solution, was homogenized in the Stomacher. Genomic DNA was extracted from the homogenate using a commercial kit. *T. gondii* DNA was amplified by both a nested PCR targeting the *B1* gene and a TaqMan Real Time PCR targeting the 529 bp repeat element. Positive PCR products were sequenced.

Results. Two *S. coeruleoalba* dolphins resulted positive for *T. gondii* DNA (Table 1). The first animal was found in the Western coast of Sicily and all the examined organs (brain, lymph nodes, spleen, hearth, liver and muscle) were positive. The second dolphin was found in the Eastern Sicilian coast and had the spleen, liver and muscle positive. Sequencing of the amplicons from both the dolphins confirmed positivity to *T. gondii*.

Table 1. Stranded dolphins examined in this study

Dolphin n.	Specie	Place of finding	Examined Organs	*T. gondii*
1	S. *coeruleoalba*	Santa Flavia (PA)	Brain, hearth, lymph nodes, spleen, muscle, lung	-
2	*D. delphis*	Campobello di Mazzara (TP)	Brain, liver, lymph nodes, spleen	-
3	S. *coeruleoalba*	Alcamo (TP)	Brain, hearth, liver, lymph nodes, spleen, muscle	-
4	S. *coeruleoalba*	Alcamo (TP)	Brain, Lymph nodes, Spleen, Hearth, Liver, Muscle	+
5	S. *coeruleoalba*	Messina	Brain, Lymph nodes, Spleen, Hearth, Liver, Muscle	+
6	*T. truncates*	Palermo	Brain, Muscle, Lung, Lymph nodes	-
7	S. *coeruleoalba*	Palermo	Brain, Lymph nodes, Lung, Spleen	-
8	*D. delphis*	Priolo	Brain, Hearth, Muscle	-
9	*T. truncates*	Castelvetrano	Lymph nodes, Spleen, Muscle, Hearth, Liver, Brain	-

Conclusion. The study reports *T. gondii* presence in dolphins stranded in a short period in a small Mediterranean area. Pathogen DNA was detected in different organs including those of choice for this pathogen. Positive animals belonged to cetacean species living in the open sea, making interesting better understanding the transmission routes of *T. gondii* for such animals in this area.

COMBATTING ANTHELMINTIC RESISTANCE IN RUMINANTS – COST ACTION 16230 (COMBAR)

CMBR1

PREVALENCE OF STRONGYLE GASTROINTESTINAL NEMATODES IN SHEEP IN THE CZECH REPUBLIC

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Background. In sheep, strongyle gastrointestinal nematodes are an important cause of parasitic gastroenteritis, a disease of great socioeconomic importance worldwide. Typically, grazing animals are simultaneously infected with several species. These multi-infectious cases contribute to final clinical effect and severity of disease. The high nematode burden together with increasing anthelmintic resistance leads us to focus on integrated control with justified and species-dependent use of drugs based on rapid and accurate diagnostics.
Material and Methods. In 2019, approximately 600 individual faecal sample were collected rectally on 34 farms in the Czech Republic from 10% animals in each flock. The samples (5 g) were tested by McMaster technique and RT-PCR.
Results. A total of five genera were confirmed in the faeces: *Haemonchus*, *Teladorsagia*, *Trichostrongylus*, *Nematodirus* and *Chabertia*. Mixed infection of several species was found in most sheep. Preliminary data indicate that the highest percentage of samples were positive for *Trichostrongylus*, followed by *Teladorsagia* and *Haemonchus* species.
Conclusion. Samples were analysed by coproscopy and compared with real-time multiplex PCR, which proved to be a sensitive and reliable approach to identify strongyle eggs in faeces. Our results suggest that *Trichostrongylus*, *Teladorsagia* and *Haemonchus* species are the main causative agents of disease in sheep in the studied area.

Funding source: The work was supported by an INTER-COST project by the Czech Republic Ministry of Education, Youth and Sports (LTC19018) and the COST Action COMBAR CA16230.

CMBR2

GASTROINTESTINAL PARASITES IN SMALL RUMINANTS IN SOUTH-WESTERN SPAIN: QUESTIONNAIRE AND SURVEY

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Background. Gastrointestinal parasitic infections (GIN) are one of the main problems in the livestock production of small ruminants, and there are scarce data in our country.
Objectives. The present study was focused on: 1. A survey of farmers on management systems, veterinary advice and anthelmintic treatment and rotation. 2. The coprological study of gastrointestinal parasites and the relationship between the parasite burden and the type of production as well as the answers obtained in the survey.
Material and Methods. A questionnaire was supplied to 198 farmers, asking about different items: type of production; physiological status; veterinary advice; control measures; antiparasitic treatment; drugs and frequency used; rotation of anthelmintic drugs; perception of disease.
In the same farms, faecal samples were obtained from three rearing animals and three breeding animals, and two pools were made from them. Three grams per pool were analysed by McMaster method.

Fisher's exact test was applied to study the association between survey items and parasite burden.
Results. Parasites were identified as *Eimeria* spp., *Moniezia* spp., *Dicrocoelium dendriticum* and *Trichuris* spp. According to the type of production, a significant association was observed in meat-producing sheep in relation with the presence of Strongylida (P = 0.04) and *Moniezia* spp. (P = 0.006). The replacement animals (92.86 %) presented higher percentage than breeding animals (7.14 %).
Respect to the questionnaire, only the frequency of deworming influenced the prevalence of Strongylida, and there was an association between treatment (more than twice a year) and the lowest percentage of animals having no parasites.
Conclusion. The prevalence was influenced by different factors: type of small ruminant, type of production and physiological status. The type of infection depends on the ruminant specie, production, and physiological status. The most frequent infections were the single infection with *Eimeria* spp. and double infection with *Eimeria* spp. and Strongylida.

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CMBR3

HIGH PREVALENCE OF BENZIMIDAZOLE RESISTANCE ASSOCIATED β-TUBULIN POLYMORPHISMS IN *Haemonchus* spp. AND *Trichostrongylys* spp. FROM MERINO SHEEP IN PORTUGAL

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Background. Gastrointestinal nematodes (GIN) are one of the most important health constraints in sheep production worldwide. Intensive anthelmintic treatment has contributed to the spread of resistance to all classes of anthelmintics. Resistance to benzimidazoles (BZ) correlates particularly with several polymorphisms in the isotype 1 β-tubulin gene.
Objectives The study aimed to assess BZ resistance in GIN of sheep in Portugal by analysing the frequency of polymorphisms at codons F167Y and F200Y in the isotype 1 β-tubulin gene.
Materials and Methods. Faecal samples were collected between September 2019 and July 2020 from 250 Merino sheep aged 2-14 months on 22 farms in the Alentejo region, Portugal. Time to last deworming was > 2 months for all animals. Data on last anthelmintic used and deworming frequency were recorded. Coprocultures were set for all farms. GIN eggs were purified from individual samples on a sugar gradient and subsequently combined into 50 pools of 5 animals each (1-4 pools/farm). Allele frequencies for β-tubulin codons F167Y and F200Y were measured by pyrosequencing.
Results. Genus-specific PCR amplification of the β-tubulin gene confirmed *Haemonchus* on 95.5% and *Trichostrongylus* on 86.4% of farms. *Trichostrongylus* was the dominant genus in coprocultures. The frequency of resistance-associated polymorphisms at codons 167 and 200 was 7-47% and 34-85% for *Haemonchus*, respectively, and 1-10% and 81-100% for *Trichostrongylus*, respectively. Deworming was performed once a year on 36.4% and twice on 66.6% of the farms. BZs were the last used anthelmintic on 66.7% of the farms.
Conclusion. The study provides first molecular evidence of BZ resistance in GIN from sheep in Portugal. Similar to previous reports for trichostrongyloids in Europe, resistance-associated alleles were more frequent at codon 200 compared with codon 167. The high prevalence of BZ resistance identified urges the implementation of available integrated parasite control strategies and further research on non-chemical options.

Funding source: This study is based upon work from COST Action COMBAR CA16230, supported by COST (European Cooperation in Science and Technology). The project was funded by FCT - PTDC/CVT-CVT-28798/2017.

CMBR4

SEGREGATION OF RESISTANT AND SUSCEPTIBLE CANARIA SHEEP AND CANARIA HAIR BREED LAMBS TO GASTROINTESTINAL NEMATODES AT WEANED

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Background. Gastrointestinal nematodes (GIN) are one of the main constraints in small ruminant production worldwide. They have been controlled by anthelmintics but increasing drug resistance makes a priority identify alternative/complementary control methods. One of these options is breeding for diseases resistance. However, there are some concerns on the productive performance of resistant animals. Two local breeds of sheep are mainly exploited at the Canary Islands: The Canaria sheep (CS) and the Canaria Hair Breed (CHB) sheep. They are exposed to several gastrointestinal nematode species in local mountains. Adult CHB sheep are more resistant to GIN than CS. However, lambs younger of 6 months are considered poor immune competent against GIN.

Objectives. (1) Compare within/between breeds FEC in naturally GIN-infected lambs of CS and CHB at weaned (2) Repeatability of individual FEC (3) Correlate FEC with individual weight.

Material and Methods. 24 CS and 26 CHB lambs born in a local farm in Valleseco village (Gran Canaria, Spain) were selected. FEC were performed weekly at weaned for 9 weeks. All lambs were weighted at weaned and on week 9.

Results. There were no differences between breeds in FEC. Great individual differences in FEC were observed within breeds, with high repeatability. Animals were segregated as resistant/susceptible, attending to their FEC in both breeds. No significant correlation between FEC and weight were observed.

Conclusions: (1) No differences in resistance/susceptibility in CS and CHB lambs <6 months of age. (2) Lambs could be segregated as resistant/susceptible within breed and their status is maintained at least for 9 weeks. (3) Resistance in these two breeds was not associated with weight, suggesting that selection of lambs of these breeds at weaned may not compromise productivity. (4) Considering all these data, breeding for GIN-resistance is a promising option at the Canary Islands.

Funding source: Agencia Canaria de Investigación, Innovación y Sociedad de la Información (ACIISI) of The Canary Government (PROID2017010109).

CRYPTOSPORIDIUM

CRYPTO1

***Cryptosporidium parvum* INFECTION AND COLON CANCER. RESULTS OF A MICROARRAY APPROACH**

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Background. Accumulative experimental and clinical evidences link *Cryptosporidium parvum* infection and digestive adenocarcinoma.
Objective. This study aimed to identify the gene expression profile and significant pathways involved in *C. parvum*-induced neoplasia.
Material and Methods. 30 SCID mice were divided into 4 groups: 1) uninfected and 2) infected at 45 days post-infection (PI), 3) uninfected and 4) infected at 93 days PI. Histopathology and Agilent SurePrint G3 Mouse microarray analysis were performed. Ingenuity Pathway Analysis (IPA) and gProfiler allowed pathway analyses. Microarray data was validated by RT-qPCR.
Results. The gene expression profile was significantly altered with 92 and 755 genes upregulated at 45 and 93 days PI, respectively, and 39 and 303 downregulated at 45 and 93 days PI, respectively (absolute logFC > 2.0) IPA analysis for Group 4 identified 27/173 genes of the tumor microenvironment pathway (z score >2). The software also annotated 166 genes from the dataset corresponding to 14 different functions associated with cancer (z score >2). Molecular gene network built using highly regulated genes such as Defa1 and IDO-1 predicted the role of Paneth cells, which are specialized intestinal epithelial cells present in the crypts of small intestine to be involved in parasite infection propagation and tumour progression. Either at 45 or 93 days PI, genes were significantly enriched in the biological process of the immunological response, and the cellular component indicated that these genes were predominantly located in the extracellular region, membrane and cell surface. As for molecular function, these genes were enriched in GTP binding at 45 days PI, and signalling receptor and integrin binding at 93 days PI. The most significant KEGG pathways were associated with cytokine-cytokine and Extracellular Matrix receptors interaction.
Conclusion. In the current study, we identified for the first time the alteration in the gene expression profile of the *C. parvum*-induced neoplasia.

CRYPTO2

FIRST CHARACTERIZATION OF THE ROLE OF EPIGENETICS IN THE DYNAMICS OF *Cryptosporidium parvum* INFECTION

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Background. Epigenetic mechanisms are known to be targeted by pathogens to hijack cellular host functions during infection
Objectives. Our aim was thus to explore the role of epigenetics and in particular of histone lysine methylation in *Cryptosporidium parvum* and in the host.
Material and Methods. *In silico* analysis was performed to identify epigenetic regulators. Phylogenetic analysis allowed prediction of substrate specificities of identified lysine methyltransferases (KMTs). Gene expression profile of the identified KMTs was performed by RT-qPCR. Immunofluorescence analysis using antibodies recognizing specific methylated-lysine modifications in the parasite as well as in the host was performed.
Results. 11 putative KMTs were identified. Subsequent alignment of the SET-domain sequences of these KMTs classified the predicted *C. parvum* KMTs into 5 subfamilies: CP SET1, CP_SET2, CP_SET8, CP_KMTox and CP_AKMT. CP_SET1, CP_SET2 and CP_SET8 are predicted as histone lysine methyltransferases (HKMTs) while CP_KMTox and CP_AKMT have been identified as KMTs and exclusively found in Apicomplexa. No evidence of histones lysine demethylases was observed. Phylogenetic analysis confirmed the classification of the 5 subfamilies of *C. parvum* KMTs and their associated putative substrate specificities. Site specific methylation at lysine 4 (K4) and K36 of histone H3 and K20 of histone H4 in sporozoite stage of *C. parvum* confirmed substrate specificities of the HKMTs. Gene expression profile of these putative KMTs during different stages of the parasite development was compared. HKMTs (CP_SET1, CP_SET2) were shown to be highly expressed during the trophozoite stage. Consistently, the specific histone lysine marks displayed dynamic changes during the parasite development. Furthermore, we showed that the infection induces global downregulation of the histone lysine methyl marks in the host cell.
Conclusion. This study highlights the importance of epigenetic mechanisms in gene regulation of virulence factors of *Cryptosporidium* and the potential of this parasite to exploit host epigenetic regulation to its advantage.

CRYPTO3

HOW TO DETECT *Cryptosporidium* spp. OOCYSTS IN FOOD BY MOLECULAR METHODS: AN EXTENSIVE LITERATURE REVIEW

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Background. Parasites associated with ready-to-eat (RTE), bagged salad leaves and other fresh produce are of particular concern for both public health and the food industry. The lack of validated detection methods hampers our knowledge and progression towards improved food safety in this field.
Objectives. The aim of the EFSA-funded IMPACT project is to increase the European-level capacity for risk assessment of foodborne protozoa using *Cryptosporidium* and RTE salad leaves as a model. One of the project objectives is to develop an SOP for molecular detection of *Cryptosporidium* oocysts in leafy greens by real-time PCR. To achieve this objective, a literature review of molecular methods for the detection of *Cryptosporidium* spp. oocysts was conducted.
Material and Methods. We searched PubMed and Scopus databases for all published data on the topic and followed the PRISMA guidelines. The following search phrases were used: i) cryptosporidi* AND pcr AND detection and ii) cryptosporidi* AND pcr AND genotyping. The databases were searched for papers in English published up to 15th August 2019. Selected full texts were assessed for both general attributes as well as method-quality attributes.
Results. Out of 899 screened papers, a total of 65 relevant papers were identified encompassing four main groups of sample types that have been used for spiking and PCR method development: faeces (31 studies), environmental samples (23), food (4), and other matrices (15). Only a few of the methods described were fully validated and thus fulfilled the IMPACT requirements.

Conclusion. The results of our review highlighted the lack of sensitive and robust molecular methods on which the IMPACT SOP could be based. Most published molecular methods were found inappropriate due either to cross-reactivity of the PCR primers or lack of applicability to all relevant *Cryptosporidium* species.

Funding source: Partnering Grant Project Grant Agreement № GP/EFSA/ENCO/2018/03 – GA03 IMPACT: Standardising molecular detection methods to IMprove risk assessment capacity for foodborne protozoan Parasites, using *Cryptosporidium* in ready-to-eat salad as a model.

CRYPTO4

MOLECULAR EPIDEMIOLOGY OF CRYPTOSPORIDIOSIS ON CATTLE FARMS IN THE CANARY ISLANDS

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Cryptosporidiosis is a disease caused by different species of the genus *Cryptosporidium*, a protozoan parasite capable of causing large economic losses in ruminant livestock farms, as well as diarrhoea in humans. The objective of this study has been to determine the prevalence and identity of *Cryptosporidium* species and GP60 subtypes in cattle from farms in the Canary Islands. Fifteen cattle farms located in different municipalities of the island of Gran Canaria were sampled, and faeces from 8 calves less than 30 days old and 8 adult cows were collected from each farm. In addition, potential risk factors linked to infections were identified by means of a questionnaire for veterinarians and livestock farmers. *Cryptosporidium* oocysts were microscopically identified using a Kinyoun staining of the sediment concentrated from faecal specimens. The presence of the parasite was also investigated using molecular tools and results were correlated with microscopical analyses. Total DNA was extracted from faecal sediments using the QIAamp Fast DNA Stool Mini Kit® after three cycles of freezing in liquid nitrogen/thawing at 100ºC. *Cryptosporidium* species and subtypes were identified using PCR protocols and sequence analyses of the small-subunit ribosomal RNA (SSU rRNA) and GP60 gene loci, respectively. The results of the questionnaire study show that most cattle farmers are not aware of the presence of cryptosporidiosis in their farms, in spite of the high prevalence recorded (> 50% of the farms were positive). Positive sample were more frequently found in calves compared to cows and a significant correlation was observed between faecal staining and PCR results. The present study provides the first description of *Cryptosporidium* in cattle farms in the Canary Islands. Risk factors linked to cryptosporidiosis and zoonotic potential *Cryptosporidium* species found in calves will be discussed.

DIROFILARIA

DIF1

RARE ZOONOTIC DISEASES CAUSED BY NEMATODES *Gongylonema pulchrum* AND *Dirofilaria repens* IN CLINICAL PRACTICE

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Background. Some zoonotic parasites such as zoonotic nematode species that infect humans represent rare and surprising clinical findings.

Material and Methods. We present clinical cases of rare helminthic diseases gongylonematosis and dirofilariasis, that are not unfamiliar to the population of Bulgaria.

Results. The nematode *Gongylonema pulchrum* was isolated from the oral mucosa of a young woman, who pulled it out of her mouth. It looked like a thin but mobile worm. In the last 3 decades two similar cases were registered. In one of them peritonsillar abscess caused by the worm was found. In the same period 24 patients with the disease caused by zooparasite *Dirofilaria repens* were diagnosed. Clinically, the invasion was manifested by subcutaneous nodules in various parts of the body. The cases were sporadic, as the infected people sought medical help from various specialists, most often surgeons and ophthalmologists when the eyes were affected. The exact diagnosis was made by examination of the parasites in the laboratory of parasitology at the Medical University.

Conclusion. The presence of infected dogs (more than 10% are infected with *Dirofilaria repens*), which are most often the source of infection, abundant mosquito fauna that occurs seasonally in our country, and global warming all favour the occurrence of local cases of gongylonematosis and dirofilariasis in humans.

DIF2

CANINE EXPOSURE TO *Dirofilaria* sp. NEMATODES

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Dirofilaria repens and Dirofilaria immitis are nematode worms belonging to Dirofilaria genus which are mainly parasites of carnivores, but could be found in humans as well. D. immitis adults can be found mainly in heart and pulmonary arteries and D. repens in subcutaneous tissue. Most of the infected dogs are latent carrier of the parasite so final Dirofilaria infection is confirmed in most cases accidentally. Increasing migration of infected animals, vector spread, global warming and changes in human activity are leading to an increase in the number of infected animals and humans.

Aim. The aim of this study was to determine the microfilaria presence including the nematode species, and antibody presence which, all combined, will show the exposure of dogs to Dirofilaria sp. nematodes and therewith reveal the potential reservoirs of infection for susceptible hosts.

Material and methods. This study was conducted on 72 blood samples. The presence of microfilaria was proven with modified Knott's test, and their measurements revealed D. repens while additional information about a host's contact with this nematode was revealed using "in-house" developed indirect immunofluorescence antibody test.

Results. The results of this survey showed higher seroprevalence than microfilaria prevalence in the same dog population. Higher prevalence was also in animals that spend most of their lives outside the house.

Conclusion. A higher percentage of infected animals are animals who live outdoors. It is important to note

that the Knott's test and indirect immunofluorescence are two methods that add up each other and could be used in the routine diagnosis of dirofilariosis. Due to the proven high exposure of dogs to D. repens, seropositive dogs should be monitored clinically and treated in case of microfilaria presence in blood to prevent the possible infection of other susceptible hosts including humans.

DIF3

FIRST CASE OF OCULAR DIROFILARIASIS IN PATIENT FROM SLOVENIA: A CASE REPORT

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Background. Nematoes of the genus *Dirofilaria* are vector-borne filarial parasites that affect domestic and wild canids and are transmitted by several species of mosquitoes. *Dirofilaria repens* usually causes a non-pathogenic subcutaneous infection in dogs and is the principal agent of human dirofilariasis.
Case report. We describe a case of ocular dirofilariasis in a 69-year-old male patient from the region of Prekmurje in Slovenia (North-East of Slovenia). The patient presented with red eye and foreign body sensation in the eye. He was referred to an ophthalmologist. The patient did not travel abroad in the last few years, he does not own pets or cattle, he did not have contact with animals, he lives in an apartment, he never had any allergic or other reactions after a mosquito bite, he did not notice any changes on other parts of his body. Further examination was performed and revealed segmental conjunctival injection. Underneath the conjunctiva an approximately 7- 8 cm long, thin, moving structure was found. Application of local anaesthetic and removal of the parasite followed. The parasite was sent to a parasitological laboratory where it was morphologically analysed. Microscopically, the cuticle of the parasite had longitudinal ridges supporting the diagnosis of *D. repens* dirofilariasis. The definitive identification was done by *D. repens*-specific PCR. To our knowledge, this is the first case of ocular dirofilariasis in a patient from Slovenia.
Conclusion. There is evidence that *D. repens* has spread from the endemic areas of southern Europe to northern Europe. Climate change affecting mosquito vectors and the facilitation of pet travel seem to have contributed to this expansion. We assume that in the future we could see more similar cases also in the region of Slovenia.

DIVERSITY OF ECHINOCOCCUS AND OTHER TAENIIDS

ECHINO1

ASSESSMENT OF THE HUMAN RISK EXPOSURE TO *Echinococcus multilocularis* VIA SOIL AND CARNIVORE FAECAL ANALYSES IN URBAN VEGETABLE GARDENS USED FOR GROWING FRESH PRODUCES

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Background. Soil contamination by helminth eggs is particularly problematic in areas where humans may come into contact with soils polluted by domestic and/or wild carnivore faeces. This is particularly true in vegetable garden where human can be contaminated by raw vegetable consumption and through contact with soil. This study aimed to provide information on the deposit of *Echinococcus multilocularis* definitive host faeces in urban vegetable gardens and its surroundings, from an endemic parasite area in France (East region). Indeed, in France an average of 40 human cases are declared each year at the National Reference Centre for Echinococcoses, with over 60% of these cases in the Eastern part of France. The disease remains lethal in untreated cases. The level of *E. multilocularis* contamination in soil and copro-samples collected was determined in these high-risk zones for human exposure.
Material and Methods. Six vegetable gardens, all enclosed, located in the city of Besançon (France) were sampled for soil and faeces in April and September 2019. All fox, cat and dog faeces found were collected and identified for the host species by real-time quantitative PCRs. The occurrence of *E. multilocularis* in these soil and copro-samples was investigated after zinc chloride flotation and specific real-time quantitative PCR, combined to PCR inhibitor presence test.
Results. At least one scat was found in each of the six urban vegetable gardens, for a total amount of 28 faeces collected. All fox faeces were gathered at the immediate outside edges of the gardens, while those of dogs and cats were inside. Among the fox faeces collected, one sample was tested positive for *E. multilocularis* DNA (1/5, 20.0%). No *E. multilocularis*-positive soil sample (0/240) was detected among the six urban vegetable gardens, all enclosed.
Conclusion. The presence of *E. multilocularis* was reported for the first time in the Besançon city, located in the highly endemic area of France, indicating a potential parasitic risk in the immediate vicinity of these urban gardens devoted to household consumption.

EVPC – EUROPEAN VETERINARY PARASITOLOGY COLLEGE SYMPOSIUM

EVPC1

COMPARISON OF RECENTLY DEVELOPED COMMERCIAL MOLECULAR WORKFLOW FOR THE DETECTION OF CAUSATIVE AGENTS OF FASCIOLOSIS AND PARAMPHISTOMOSE WITH THE ROUTINE MICROSCOPIC METHOD IN THE CATTLE BREEDING IN FRANCE

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Background. Fasciolosis and Paramphistomose in the cattle breeding have revealed, over the course of research over the past 20 years, a hidden aspect that we do not know about. The multiple pathogenic effects of *F. hepatica* and their real impact on inflammatory and immune reactions make the great fluke not tolerable. Animal infections are commonly diagnosed by the sedimentation and faecal egg-counting methods. However, this method is time-consuming and operator dependent. Therefore, the DNA extraction and real-time PCR analysis have the potential for a preferred standardized diagnostic solution, allowing higher sensitivity, increased throughput, reproducibility, with the benefit of species confirmation and increased practicability.
Objectives. The objective of this study is to compare the newly developed commercial extraction and real-time PCR *Fasciola hepatica* & *Calicophoron daubneyi* detection kit with one of the routine laboratory sedimentation and faecal egg-counting methods.
Material and Methods. DNA extraction and PCR amplification was done following the protocol of commercial kit Bio-T kit® *Fasciola hepatica* & *Calicophoron daubneyi* (BioSellal). Sedimentation, flotation, and egg-counting were carried out in respect of the classical Mac Master derived technique in the one of routine veterinary diagnostic laboratory. The last one was considered as the reference method.
Results. For *Fasciola hepatica* the obtained diagnostic sensitivity and specificity are about 87.5%; 93.45% respectively, and 94.4%; 56% respectively for *Calicophoron daubneyi*.
Conclusion. A slight lack of sensibility was observed for *Fasciola hepatica* detection that can be explained by the sampling bias. The results obtained for the specificity of *Calicophoron daubneyi* detection can be traduced by the improved level of sensibility of PCR kit comparing to the reference method. The newly developed biomolecular detection method showed a good potential in terms of practicability and at least the same level of detection as the reference technique for both targets.

EVPC2

THE EFFECTS OF *Allium sativum, Artemisia absinthium l., Cucurbita pepo, Coriandrum sativum, Satureja hortensis l. AND Calendula officinalis* ON THE EMBRYOGENESIS OF *Ascaris suum* EGGS DURING AN EXPERIMENTAL (IN VITRO) STUDY

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Background. *Ascaris suum* is present in traditionally managed indoor herds and on industrialized farms, especially in old fatteners and sows. Increasing resistance against antihelmintics redirected the research towards alternative, traditional therapies, medicinal plants included.
Objectives. This study comparatively evaluated the *in vitro* effects of *Allium sativum*, *Artemisia absinthium L., Cucurbita pepo, Coriandrum sativum, Satureja hortensis L.* and *Calendula officinalis* on inhibition of *A. suum* egg hatching and larval development.
Material and Methods. *A. suum* eggs were collected from randomly sampled of traditionally maintained swine faeces. In 3 ml cell culture plates, the egg suspension (ES, $8x10^3$/ml) was divided in two control (C) (1C -

1ml ES + 1 ml distilled water, 2C- five plates of 1ml ES + 1ml ethanol of 70%, 35%, 17.5%, 8.75%, and 4.375%, respectively) and six experimental groups. The experimental (E, 1-6) groups included ES + each alcoholic plant extract (10%, 5%, 2.5%, 1.25%, 0.625%). Both C and E were performed in quintuplicate. All groups were incubated at 27 °C for a total of 21 days, *A. suum* eggs being examined after 2, 14 (L1) and 21 (L2/L3) days.
Results. The efficacy of all tested plants, when compared to the control groups increased with concentration. Anti-embryogenic effects on the *A. suum* eggs were expressed by all plants, with more pronounced influence of the *A. sativum, A. absinthium*, *C. pepo* and *S. hortensis L* extracts at all tested concentrations.
Conclusion. *A. sativum* and *A. absinthium* extracts showed the strongest antihelmintic activity; still, in-depth phytochemical studies are required to identify the compounds responsible for the antihelmintic properties of these species. To our best knowledge, this is one of the few ethnopharmacological reports on the antihelmintic activity of medicinal plants traditionally used for the treatment of *A. suum* infection in Romania.

Funding source: This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement № 816172.

EVPC3

MOLECULAR DETECTION OF PATHOGENS IN FLEAS COLLECTED FROM DOGS IN UZBEKISTAN: PRELIMINARY RESULTS

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Background. Fleas are known to be important vectors for various pathogens, some of them with zoonotic potential. Domestic dogs live in close contact with humans, exposing them at risks by carrying fleas into human settlements. The most common flea species parasitic in dogs in South-Est Asia are *Ctenocephalides felis* and *Ctenocephalides orientis*.
Aim. The aim of the present study was to evaluate the prevalence and diversity of fleas and associated pathogens by means of PCR, in Uzbekistan, Central Asia.
Material and Methods. Fleas were manually collected from dogs in different regions of Uzbekistan and preserved in ethanol. They were morphologically identified and molecularly tested for the presence of *Rickettsia* spp., *Bartonella* spp. and *Acanthocheilonema reconditum*.
Results. A total of 198 fleas were collected from 77 dogs from 5 different regions of Uzbekistan. The vast majority were from genus *Ctenocephalides* (88.38%) and the rest were *Pulex irritans* (11.11%). *Bartonella* spp. were detected in 4 fleas (2%) of genus *Ctenocephalides* that were collected from 3 dogs. Four fleas (2%) of the same genus were found to be infected with *A. reconditum* in 3 dogs. *Rickettsia* spp. were identified in 57 fleas (28.8%) of both genera, with *R. asembonensis* and *R. helvetica* being molecularly confirmed.
Conclusion. The presence of vector-borne pathogens was confirmed in fleas of dogs from Central Asia, advocating the importance of further surveillance on zoonotic bacteria.

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EVPC5

EPIDEMIOLOGY AND CLINICAL EXPRESSION OF CANINE BABESIOSIS IN DOBROGEA AREA, SOUTHEAST OF ROMANIA

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Background. Canine babesiosis is a significant tick-borne disease caused by large and small intra-erythrocytic protozoa of the genus *Babesia* (Apicomplexa: Piroplasmida) that affects dogs worldwide. An increasing

number of reported cases of canine babesiosis is noticed in the last decades in Romania, mainly in South-eastern areas.
Objectives. The present study aimed to provide an overview on the epidemiology and clinical expression of canine babesiosis in Dobrogea area (Southeast of Romania).
Material and Methods. A total of 306 client-owned dogs, of age from 2-month to 13-year old, were tested for presence of intra-erythrocytic piroplasms, by using Giemsa-stained thin blood smear microscopic examination.
Result: Of the tested dogs, 27.8% (95%CI: 22.83-33.16) were positive for large piroplasms. *Babesia*–infected dogs displayed different clinical presentation: mild (38.8%; n=33), moderate (29.4%; n=25), and severe disease (31.8%; n=27). Additionally, based on clinico-pathological changes, 38 (44.7%) dogs were diagnosed with uncomplicated babesiosis, while 34 (40.0%), and 13 (15.3%) dogs showed complicated babesiosis with a single organ dysfunction, and complicated babesiosis with multiple organ dysfunctions (MODs), respectively. Age appeared a risk factor for severe disease (mean age=5.8 years) and MODs (mean age=6.8 years). Dogs with uncomplicated babesiosis demonstrated a higher recovery rate (81.6%), while dogs with complicated babesiosis and severe disease showed the lowest recovery rate (38.5%). Good prognosis was associated with early diagnosis and early treatment.
Conclusions: The findings provide valuable information for better understanding of the epidemiology of canine babesiosis in Romania.

EVPC6

ANAPLASMOSIS IN GRAZING RUMINANTS IN GREECE: HIGH INDIVIDUAL AND FLOCK-LEVEL SEROPREVALENCE

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Background. *Anaplasma* species are obligate intracellular rickettsial pathogens transmitted by ticks affecting human and animals. In small ruminants, the main species causing anaplasmosis is *A. ovis* transmitted by *Rhipicephalus bursa* ticks. Although the disease appears to be widespread, the extent of the infection and the productivity losses remain poorly understood.
Objectives: To study the abundance of *Anaplasma* species infection in grazing ruminants in Greece.
Material and Methods: Overall, 185 farms, were enrolled in a cross-sectional study the West Macedonia Region of Greece. The farms were proportionally distributed to all 4 counties of the Region according to their farm density. From each farm 10-20 animals were examined for the presence of ticks and blood samples were taken. For the detection of antibodies against *Anaplasma* spp. samples were examined with indirect enzymatic immunoassay (ELISA) specific for *A. phagocytophilum*. Selective ELISA-positive *Anaplasma* samples coming from all infected flocks were further processed with molecular analyses. Ticks collected from the animals were identified to species.
Results. Overall, 91.7% of the farms were found infected with *Anaplasma* spp, with seroprevalences varying regionally (50% of the non-infected flock located in only one county). Seropositivity varied within flocks and on several occasions all animals per farm tested were found exposed to the pathogen. Twenty-eight positive samples that further analyzed by PCR and sequencing, revealed the presence of *A.capra/A.marginale/A*.ovis (18 samples) and *A.capra/A.ovis* (8 samples). Ticks identified were in their majority *R. bursa* followed by *Dermacentor marginatus, Hyalomma marginatum, H. scupense, R. turanicus, R. sanguineus s.l, Haemaphysalis sulcata* and *Ixodes ricinus*.
Conclusion. The study confirms the high prevalence of anaplasmosis in the area tested and the dominance of *A.capra/A.ovis.* Anaplasmosis should be considered as an important constraint of grazing ruminant production, and further efforts are needed to better understand its clinical importance and the need to apply proper control measures.

Funding source: COMPLETE project -INTERREG IPA II GR-AL

EVPC7

SUPPLEMENTARY SALT LICKS AS TRANSMISSION HOT-SPOTS FOR INTESTINAL PARASITES

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Background. Sheep farmers and reindeer herders place salt-lick stones on natural pastures to supplement their animals' nutrition. The farmers/herders also use these natural gathering spots for easier monitoring of their animals.

The supplementary salt-licks are also intensively used by other ruminant species, and act as congregation spots for e.g. moose, red deer and reindeer in addition to sheep. As a result, species which otherwise may not interact, often congregate around these salt licks, and often defecate while ingesting salt or salty soil.

Objectives. Examine soil and faecal samples for occurrence of endoparasites to elucidate potential for inter- and intraspecific disease transmission through soil, aiming to establish the role and impact of salt licks as hot-spots for transmission of infectious diseases, using endoparasites in soil and animals as a proxy.

Material and Methods. Topsoil was collected in the runoff direction from the salt licks as well as control spots, and faecal samples were collected from wild reindeer grazing in the same areas. All samples were analysed using quantitative and qualitative traditional parasitological techniques.

Results. Analysis of samples revealed nematode (*Trichuris* spp, strongylid spp., Nematodirinae spp.) and cestode (*Moniezia* spp.) eggs as well as protozoan (*Eimeria* spp.) oocysts. The most prevalent and abundant eggs in the samples belonged to the duodenal nematode *Nematodirus battus*

Conclusion. The preliminary results indicates that salt-licks acts as cumulative accelerators on natural pastures for disease transmission both within and between wild and domestic ruminants, representing possible implications for domestic sheep and wild reindeer health management.

In addition, *N. battus,* a black-listed sheep nematode able to cause outbreaks of profuse diarrhoea resulting in stunted growth and mortality in grazing lambs seems to have established in wild reindeer as well, implying unknown health impacts for these vulnerable populations.

EVPC8

EFFICACY OF A SPOT-ON COMBINATION CONTAINING 1% W/V MOXIDECTIN AND 10% W/V IMIDACLOPRID IN THE TREATMENT OF *Troglostrongylus brevior* INFECTION IN CATS

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Background. Troglostrongylosis caused by *Troglostrongylus brevior* has become a major respiratory disease in populations of domestic cats from Europe. Infections with *T. brevior* are often severe and can be fatal, mostly in kittens and young cats. Nevertheless, treatment options for cat troglostrongylosis are still limited.

Objectives. This study investigated the efficacy of a spot-on combination containing 1% w/v moxidectin and 10% w/v imidacloprid (Advocate® for cats, Elanco Animal Health) for the treatment of the infection caused by *T. brevior* under laboratory conditions.

Material and Methods. Twenty domestic cats were infected with 100 third-stage larvae (L3) of *T. brevior* each on Study Day 0. The cats were randomly allocated to one of the two study groups, i.e., Group 1 (G1), left untreated, and Group 2 (G2), treated two times at a 4-week interval, i.e., on SDs 26 and 54, with Advocate® for cats at the minimum recommended dose. The primary efficacy criterion was the number of viable adult stages counted at necropsy on SDs 64-65. The faecal shedding of first-stage larvae (L1) was also evaluated in treated and untreated cats.

Results. No adult worms were found at necropsy of treated cats. Adult *T. brevior* were found in 4/10 G1 cats. The experimental infection was, however, considered successful as all the cats enrolled in the study shed larvae during the study. None of the G2 cats shed larvae after the first treatment until the end of the study.

Conclusion. The present results indicate that Advocate® spot-on solution for cats is an efficacious option for treating cat troglostrongylosis.

Funding source: This study has been supported Bayer Animal Health (now part of Elanco Animal Health).

FISH PARASITOLOGY

FISHP1

SURVEY ON THE *Anisakis* spp. SENSIBILISATION IN SOUTHERN ITALY OPERATORS OF THE FISHERIES SECTOR

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Background. The larval forms of *Anisakis* sp. can cause severe allergic symptoms in humans. Recently, it has been hypothesized a correlation between exposure to this parasite and fish sector professional activity. This because the surface antigens would be expressed also by the inactivated larvae and could induce sensitization through skin contact or inhalation with consequent rhino conjunctivitis, asthma, anaphylaxis, and dermatitis.
Objectives. Determine the sensitization to *Anisakis* allergens in subjects of fish sector in Southern Italy using a diagnostic algorithm with high sensitivity and specificity based on Immunoblot and Basophil Activation Tests (BAT).
Material and Methods. The study included 123 workers potentially at risk from contact with *Anisakis* allergens of Sicily. The subjects were subjected to the specific IgE dosage for *Anisakis* spp. and cross-reactive allergens. The subjects were also tested by BAT and Immunoblot to complete the diagnostic algorithm proposed.
Results. Among the fish sector operators, the results showed a positivity of 7.4% in cooks/food preparation workers, 6.3% in street vendors, 4% in fish industry employees and 3.3% in the fishermen. Among the professionally exposed subjects tested with Immunoblot, a processing fish operator with clinical signs, was negative for both the IgE assay for *Anisakis*, tropomyosin and Ascaris and Immunoblot. Two workers with no clinical manifestations referable to *Anisakis* allergy showed a greater response for *A. pegreffii* extracts by Immunoblot analysis.
Conclusion. The data obtained showed that the most exposed workers were cooks, followed by the street vendors probably due to a more continuous contact and the non-use of PPE. The results obtained highlight the need of a diagnostic algorithm that includes successive cascade tests with respect to the skin prick test and the specific IgE dosage for the *Anisakis* allergy diagnosis.

Funding source: The present work was funded by the Italian Ministry of Health (RC IZS SI 02/20 - ALLERGIA AD ANISAKIS COME MALATTIA PROFESSIONALE: APPROFONDIMENTI DIAGNOSTICI NEL SETTORE DELLA TRASFORMAZIONE DEI PRODOTTI DELLA PESCA.

FISHP2

METAZOAN PARASITES OF MORMYRID FISHES FROM SELECTED LOCALITIES IN SOUTH AFRICA

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Background. Freshwater elephantfishes from the Mormyridae are diverse in size, shape and distribution. Mormyrids are widespread in Afro-tropical river systems and comprises 22 genera with 229 species. Few parasite records for mormyrids exist from South Africa leading to long term research on the bulldog, *Marcusenius macrolepidotus* and Southern Churchill, *Petrocephalus wesselsi*.
Material and Methods. Fish were collected by electro-shocking at selected localities in the Limpopo River System. Parasites were preserved using standard techniques. Some samples were collected in 96% ethanol for future molecular work.
Results. The following parasites were recorded from *M. macrolepidotus*: monogeneans *Mormyrogyrodactylus gemini* and *Gyrodactylus* sp. (skin); *Bouixella* sp. 1 and *Archidiplectanum* sp. (gills); digeneans diplostomid metacercariae from the eyes, cranial cavity and muscle (from both host species) and clinostomid metacercaria (body cavity, recorded from both host species); cestode *Ichthyolepis africana* (intestine); nematode

Procamallanus laeviconchus (stomach from both hosts) and *Contracaecum* larva (body cavity); pentastomid *Sebekia wedli* infective stage larva (body cavity); copepod *Afrolernaea mormyroides* (gills) and *Neoergasilus japonicus* (skin). Besides those mentioned, *Bouixella* sp. 2 was recorded from the gills of *P. wesselsi* indicating lower species richness.

Conclusion. There is still much research to be done with regards to parasite diversity of mormyrid fishes considering the number of species and their distribution. Molecular analysis of parasites should be included to accurately determine the diversity of parasites from these interesting hosts. The two hosts co-existed in several localities but did not share many parasite species.

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FISHP3

ULTRASTRUCTURE OF THE EGG ENVELOPES AND EARLY EMBRYOS OF *Rohdella amazonica* (TREMATODA: ASPIDOGASTREA)

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Background. Detailed examination of embryonic structures, including eggshell construction, provides insight into the life cycles, adaptations, and phylogenetic affinities of parasitic helminths.

Objectives. Egg structure and early embryonic development of the aspidogastrean, *Rohdella amazonic*a, a basal trematode, were studied by transmission electron microscopy (TEM) to gain insight into functional, developmental, and phylogenetic characteristics.

Material and Methods. Gravid worms were removed from the intestine of naturally infected banded puffer fish *Colomesus psittacus*, collected from the Bay of Marajó, Paracauari River (Pará, Brazil), and processed by standard TEM methods.

Results. By the time of pronuclear fusion, the fertilized zygote is already enclosed in a thick electron-dense pre-operculate eggshell and an underlying layer of vitellocytes that fuse into a vitelline syncytium as they are still secreting their shell granules. When cleavage commences a small number of macromeres move to the area between just underneath the eggshell, where they fuse to form a single syncytial embryonic envelope. Simultaneously, the smaller blastomeres continue to divide as they maintain contact with each other, but remain separate from the vitelline syncytium. Concurrent with these cellular changes, a thickened knob expands at one pole of the eggshell and begins to form an opercular suture. By the time the operculum is fully formed, the vitelline syncytium has mostly degenerated, while the smaller blastomeres have become cohesive as a single mass that precedes the differentiation and morphogenesis of the cotylocidium larva.

Conclusion. The general pattern of cleavage and eggshell formation resembles that of other trematodes and polylecithal cestodes, but the single embryonic envelope has been reported only in a few basal taxa. The only other aspidogastrean studied in detail to date is very similar, indicating close phylogenetic affinity and conservatism within this basal neodermatan group.

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FISHP4

LATE EMBRYOGENESIS AND MORPHOGENESIS OF THE COTYLOCIDIUM LARVA OF *Rohdella amazonica* (TREMATODA: ASPIDOGASTREA)

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Background. Aspidogastrea, a basal taxon of Trematoda, is regarded as being among the most primitive of neodermatan flatworms. Understanding the developmental biology and larval morphology of its members might lead to better understanding of the phylogenetic relationships among this and more derived groups such as the Digenea and Cestoda.
Objectives. Late stages of embryogenesis and morphogenesis of the cotylocidium larva of *Rohdella amazonic*a, an aspidogastrean parasite of fish, were studied to reveal the functional and developmental ultrastructure of the larva, as well as phylogenetically relevant characteristics of the embryos and larvae.
Material and Methods. Gravid worms were removed from the intestine of naturally infected banded puffer fish *Colomesus psittacus*, collected from the Bay of Marajó, Paracauari River (Pará, Brazil), and processed by standard methods of transmission electron microscopy (TEM).
Results. During late cleavage and rearrangement of the blastomeres, the vitelline syncytium that plays a role in eggshell formation and nutrient provision to the embryo completes its apoptotic degeneration as the embryonic mass grows substantially. Early larval morphogenesis involves cellular positioning that defines antero-posterior polarity of the differentiating larva. Progressing through larvigenesis, the anterior end forms a muscular oral sucker surrounding the mouth, which leads inward into the pharynx and expanding digestive cavity. At the posterior end, a large disc forms as a precursor to the eventual ventral disc. The fully formed cotylocidium, still within the eggshell, is flexed ventrally, bringing the two poles into near juxtaposition. The neodermatan tegument with outwardly projecting small microvilli becomes fully formed, as two grandular regions, myocytons, and a protonephridial system occupy the rest of the body's interior.
Conclusion. The ultrastructural features described here are very similar to those reported for *Aspidogaster limacoides* from fish and *Cotylogaster occidentalis* from molluscs, but differ from the more diverse miracidia of digeneans, which have been studied more extensively.

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FISHP5

A NOTE ON THE OCCURENCE OF *Pallisentis ussuriensis* (KOSTYLEV, 1941) (ACANTHOCEPHALA) FROM THE BROWN BULLHEAD (*Ameiurus nebulosus*) IN BOSNIA AND HERZEGOVINA

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Background The investigation of fish parasites in Bosnia and Herzegovina in recent years became significant, and therefore new records of parasitic Acanthocephala and their hosts are important for better understanding of fish parasitology and pathology. Acanthocephalans are obligate parasites of vertebrates, mostly of fish. There is limited knowledge about the diversity of fish-parasitizing Acanthocephala in Western Balkans.
Material and Methods Fish were sampled in Sava River by net fishing, anesthetized and then dissected. Adult acanthocephalans were found in the intestines and/or pyloric cecea of the fish in 6 of 15 brown bullhead. Light microscopy studies on the worm and SEM on attachment structures (hooks and spines) were performed.
Results Acording to light microscope and SEM studies on proboscis hooks indicated 3-4 rows of curved, sclerotized hooks, arranged alternately.

Conclusion New acanthocephalan parasite is reported for the first time from Bosnian fishes. *Pallisentis ussuriensis* may have been introduced into the country through the importation of grass carp from Far East, where this parasite was first described and is presumed to be naturally occurring.

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FISHP6

SILVER CATFISH SERVING AS INTERMEDIATE PARASITE HOST

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Schilbe intermedius (Silver catfish) is a freshwater fish species from Schilbeidae and occurs abundantly in most dams and river systems in the Limpopo Province of South Africa. This fish was examined as potential intermediate host for heteroxenous parasites. Parasitological surveys were conducted between 2009 and 2018 at five different dams within the Limpopo River System. The hosts (n = 485) were collected using gill nets and conventional fishing gear and dissected for larval endoparasites. All parasites removed were fixed and preserved according to standard methods for each group. Seven species, belonging to four different parasite groups were collected during this study. Trematodes included encysted *Clinostomum ukolii* metacercariae from the body cavity, *Diplostomum* type 1 from the eye, *Diplostomum* type 2 from the swim bladder and an unidentified digenean larvae encysted in the body cavity. Cestodes were represented by the gryporynchid larva, *Paradilepis scolecina*, imbedded in the intestinal wall. The nematode *Contracaecum* sp. were the most dominant larval parasite and recorded from the body cavity. Pentastomid larvae from the genus *Alofia* were only recorded in fish from the Phalaborwa Barrage occupying different areas within the body cavity. *Schilbe intermedius* thus acts as intermediate host for a number of parasites making use of piscivorous birds and crocodiles as definite hosts.

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FISHP7

TEMPORAL STABILITY OF POLYMORPHIC ARCTIC CHARR PARASITE COMMUNITIES REFLECT SUSTAINED DIVERGENT TROPHIC NICHES

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Introduction: Polymorphic Arctic charr *Salvelinus alpinus* populations frequently display distinct differences in habitat use, diet and parasite communities. Changes to the relative densities and composition of fish communities has the potential to alter the habitat-niche of Arctic charr populations. This study investigated whether the restocking of a native species and the unintentional introduction of an exotic fish species altered the diet and parasite communities of a polymorphic Arctic charr population.

Material and Methods Differences in the parasite community, diet and stable isotopes ($\delta^{13}C$, $\delta^{15}N$) were evaluated among three Arctic charr morphs (piscivore, benthivore and planktivore) from Loch Rannoch, Scotland, before and after the fish community was modified.

Results. The three Arctic charr morphs displayed distinct differences in parasite communities, diet and stable isotopes before and after the fish community was modified. However, the piscivorous morph increased fish consumption, and the planktivore morph consumed more zooplankton after this period. Parasite taxa-richness increased over the same period, with the establishment of four new trophically transmitted taxa.

Native parasite prevalence and infection intensity also increased in all charr morphs.
Conclusion: Overall, the Loch Rannoch Arctic charr population has maintained its distinct polymorphic diversity through the time despite changes in the fish community. This indicates that restocking of a native fish species may only induce minor shifts in the parasite community and diet of Arctic charr morphs.

FISHP8

EUROPEAN EEL INFECTION WITH *Anguillicola crassus* IN POLISH WATERS 2014 - 2020

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Background. The swim bladder nematode *Anguillicola crassus* is a common parasite of European eels (*Anguilla anguilla*). The level of eel infection in Polish waters was unknown.
Objectives. The aim of the studies was to check the level of eel infection in the Polish EEZ, southern Baltic Sea.
Material and Methods. Fish has been examined each year between 2014 and 2020. Parasitological analysis focused on the presence of nematode *A. crassus* has been performed (the total number of investigated eels was 2819).
Results. The total number of found parasites was 15681. The correlation between infection intensity and host length, Fulton condition factor, age of the fish, area and time of sampling have been analyzed. The prevalence and intensity of infection have been calculated. Intensity of infection varied from 1 to 95 parasites per fish. We observe decreasing value of the mean prevalence of *A. crassus* infection: from 72-73% in 2014-2015 to 53% in 2019-2020. Mean prevalence of infection reported previously from Polish waters in 2000-2002 was 73.6-76.2%.
Conclusion. During last two years we observe lower level of eel infection with the swim bladder nematode.

FISHP9

PRESENCE OF THE LIVER NEMATODE PARASITES AND DIET OF SHORTHORN SCULPIN (*Myoxocephalus scorpius*) FROM POLISH WATERS

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Background. Shorthorn sculpin (*Myoxocephalus scorpius*) characterizes by a relatively sedentary lifestyle. Presence of the nematodes in the muscle tissue of shorthorn sculpin and Baltic cod (*Gadus morhua*) have been studied in fish collected along the Swedish coast a few years ago, but no attention was paid to nematodes observed on or in the intestines or livers. In general, sculpin were less infected than cod, taking into account the abundance and prevalence of parasites. The current level of cod infection with Anisakidae nematodes in Polish waters is well known, but no studies focused on shorthorn sculpin were conducted.In case of predatory fish the main way of its infection with nematode parasites is via eating the infected preys.
Objectives. The aim of our study was to assess the presence of Anisakidae nematodes in the livers as well as diet composition of shorthorn sculpin from north-west Polish waters.
Material and Methods. Samples have been collected during survey in November 2020. Standard ichthyological analyses of 37 fish were performed onboard and livers were frozen for further parasitological investigation. Thawed livers were digested in artificial digestive juice. All parasites were collected and identified on the base of anatomo-morphological features.
Results. *Contracaecum* sp. nematode parasites have been detected in 13.5% of investigated fish. Diet composition was studied on the basis of stomach content analysis. Among food items the most abundant were *Crangon crangon, Bylgides sarsi* and *Gammarus* sp. All found preys were parasitologicaly inspected for the presence of nematodes.
Conclusion. Due to our best knowledge, the present study is the first attempt to describe the current status of parasitological infection with Anisakidae nematodes and diet of shorthorn sculpin in Polish waters.

FISHP10

EUROPEAN PERCH *Perca fluviatilis* FROM ESTUARY OF THE VISTULA LAGOON (POLAND): MUSCLE TISSUE PARASITES AND DIET

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Background. European perch (*Perca fluviatilis*) in Polish inland and coastal waters belong to the most important fish species for fisheries and ecosystem function. It is also eagerly chosen by consumers. During recent years there were reports from the anglers about the presence of parasites in the muscle tissue of these fish in the Vistula Lagoon. That arouse concern of consumers health.
Objectives. The aim of our studies was to describe the parasite composition and diet of European perch from the Vistula Lagoon.
Material and Methods. Fish (total length 10-25cm) were caught by an angler in the estuary section of one of the lagoon's tributaries - the Nogat River in June 2020 and transported to the laboratory for further analysis. During standard ichthyological analyses of 42 fish the viscera and muscle tissue were inspected for the presence of parasites. The unskinned fillets were digested in artificial gastric juice to revel the presence of parasites.
Results. During visual inspection two fish infected with *Camallanus lacustris* have been detected. Digestion revealed more infected fish. The prevalence of infection with the nematode was 21,43%, and intensity of infection 1-6 parasites/fish. Even 10cm long fish were infected. To determine the diet composition of European perch, the stomach content of each perch was analyzed. Stomachs of 81% of fish were empty. The dominant prey was *Gammarus* sp. Previous studies focused on the parasite fauna of European perch from the Vistula Lagoon were conducted between 1994 and 1997 and the infection level was on much lower level (prevalence 3,5 % and intensity of infection 1-2 parasites/fish).
Conclusion. The prevalence of European perch infection with nematode parasite in Vistula Lagoon increased during last 25 years. Presence of the nematodes in the muscles tissue of fish is discouraging for the consumers.

FISHP11

DIVERSITY OF MONOGENEAN PARASITES (PLATYHELMINTHES) ACROSS THE AFRICAN CONTINENT: A SOUTH AFRICAN CASE STUDY

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Background: Monogeneans are a diverse parasitic group with approximately 5500 species in 750 genera known worldwide. In Africa, 470 species in 35 genera have been described from fish hosts. Given the high diversity of freshwater fish species in Africa (more than 3,000 spp.), one can expect the same for their monogenean parasites.
Material and Methods: From March 2012 to February 2017, various fish species were sampled during field surveys at 10 localities across four South African provinces (Limpopo, North-West, Northern Cape and KwaZulu-Natal). Collected hosts were screened for the presence of monogenean parasites and any found were fixed in glycerin-ammonium picrate for morphometric analyses and in ethanol for molecular characterisation.
Results: Eleven fish species of four families, Claridae (1), Cichlidae (4), Mormyridae (2) and Cyprinidae (4) were collected and a total of 30 monogenean species belonging to seven genera were recorded from them during the present study. The most recorded species were those of *Gyrodactylus* (13), followed by *Enterogyrus* (6), *Quadriancanthus* (4), *Macrogyrodactylus* (3), *Bouxiella* (2), *Archidiplectanum* (1) and *Dactylogyrus* (1). Out of 30, 11 species are new to science and are in the process of being described. Of the fish hosts, *Clarias gariepinus* was sampled at most of the localities and harboured the most monogenean species (15) followed by *Tilapia*

sparrmanii (4). The smaller sized cyprinids from the genera *Enteromius*, *Labeo* and *Pseudobarbus* showed the importance of smaller hosts for discovering parasite diversity as each of the hosts had undescribed species either of genus *Gyrodactylus* or *Dactylogyrus*.
Conclusion: The present findings show that detailed investigations of monogeneans in a single country, can dramatically increase our knowledge on the distribution and diversity of fish parasites in Africa. Future studies should include even more hosts from diverse families, especially families with high numbers of smaller size species such as the Cichlidae and Cyprinidae.

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FISHP12

THE INFECTION OF SPRAT WITH NEMATODE PARASITES: FROM KIEL BIGHT TO THE CENTRAL BALTIC SEA

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Background. Sprat (*Sprattus sprattus*) is a key fish species in the Baltic Sea ecosystem. It is one of the most important fish species in the pelagic food webs, where it is a major prey of piscivorous predators such as cod, salmon and marine mammals. However, sprat may not only be a source of energy and nutrients, but infected fish might also be a source of parasitic infection for their consumers. Preliminary studies in Danish and Polish waters indicated that sprat was infected with larval stages of the anisakid nematode *Contracaecum osculatum*, a parasite that is also heavily infesting cod (*Gadus morhua*) and grey seals (*Halichoerus grypus*) in the Baltic. However, these local studies were limited in spatial coverage.
Objectives. As previous studies on *C. osculatum* in Baltic cod found increasing infection from the western to the central Baltic, the aim of the present study was to investigate the current spatial differences in prevalence and abundance of anisakid nematodes in sprat on a broader scale.
Material and Methods. Sprat samples (whole fish) were collected in five different areas on a west-east transect from Kiel Bight to the southern Gotland Basin during surveys in first quarter 2020. Fish were visually inspected for the presence of nematodes following standard ichthyological analyses.
Results. We present and discuss the results in relation to previous findings, where increasing levels of infection with nematode parasites, especially with *C. osculatum*, have been observed during the latest decade in many Baltic Sea fish species, concurrent with an increase in abundance of grey seals, the final host in the life cycle of *C. osculatum*.
Conclusion. Sprat as the most important fish species in the pelagic food webs of the Baltic Sea, might play a role of transmitter of the nematode parasites.

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FISHP13

LIVER NEMATODES AND DIET OF SALMON (*Salmo salar*) FROM POLISH WATERS, SOUTHERN BALTIC SEA

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Background. Atlantic salmon (*Salmo salar*) is at the top of the trophic pyramid in the Baltic Sea, in areas without sea mammals. It is also important species for Baltic sea fisheries, willingly chosen by consumers. However, the occurrence of potentially zoonotic nematodes arouse the food safety and human health concerns. The level of Baltic salmon infection with Anisakidae parasites is unknown. The high level cod (*Gadus morhua*) (also piscivorous, predatory fish) infection with these zoonotic parasites is recently observed in the Baltic Sea area. Diet of fish is not only the source of nutrients, but may show the way of infection with parasites.

Objectives. The aim of our studies was to check the presence of Anisakidae nematodes in the livers and diet of Baltic salmon from Polish sea waters.
Material and Methods. Samples have been collected during 2020. Standard ichthyological analyses of 120 fish were performed and livers were frozen for further parasitological investigation. Thawed livers were digested in artificial digestive juice. All parasites were collected and identified on the basis of anatomo-morphological features. A subsample of parasites have been identified using molecular methods.
Results. *Contracaecum osculatum* nematode parasites have been detected in 13.33% of investigated fish. Diet composition was studied on the basis of stomach content analysis. Among food items the most abundant were fish: sprat *Sprattus sprattus* and three-spined stickleback *Gasterosteus aculeatus*, while invertebrates were represented only by *Mysis mixta*.
Conclusion. Baltic Sea sprat have been previously found infected with *Contracaecum osculatum*, therefor it is probably the main source of salmon infection with that nematode parasite.

Funding source: This work was supported by own research fund of *National Marine Fisheries Research Institute* (DOT21 ParaSalmon).

FISHP14

COMPARISON OF ANISAKIDAE NEMATODES INFECTION OF LIVERS OF COD (*Gadus morhua*) FROM SWEDISH AND POLISH WATERS OF THE BALTIC SEA

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Background. Cod, *Gadus morhua*, important for Baltic Sea fisheries, is one of the fish species that is most heavily infected with nematodes. Increasing level of cod's liver parasitic infection has been previously reported in Danish and Polish waters. Conversely, there is a lack of knowledge about cod's liver infection level in Swedish waters.
Objectives. The aim of our study was to evaluate the prevalence, intensity of infection and species composition of nematodes in the liver of cod caught in Swedish waters and compare it with Polish data.
Material and Methods. Samples of cod were collected during bottom trawl surveys in Swedish and Polish waters (February and November 2015; February and November 2016; November 2017). During this period, in total 1793 livers of cod from Swedish and 2210 from Polish waters were analysed for the presence of parasites. Livers were digested in artificial stomach juice. All parasites were collected and identified on the basis of anatomo-morphological features. Subsample of parasites was identified using molecular methods.
Results. The most abundant nematode parasite was *Contracaecum osculatum*. Molecular identification of nematode parasites confirmed also the presence of *Pseudoterranova decipiens*, *Anisakis simplex* and *Hysterothylacium aduncum*. Generalized linear models (GLMs) were applied to analyse the prevalence and intensity of cod infection with *C. osculatum*. Year, quarter and area of sampling as well as sex and total body length of fish were statistically significant.
Conclusion. The increasing prevalence of infection in cod livers was observed from southern (Polish waters) to northern areas of sampling (Swedish waters surrounding Gotland island). High level of cod's liver infection with *C. osculatum* could be the result of the increasing number of the grey seal *Halichoerus grypus*, which is one of the most important final hosts in the life cycle of this parasite species in the Baltic Sea.

Funding source: This work was supported by own research fund of *National Marine Fisheries Research Institute* (FBW PolParaCod 2019-2021).

FISHP15

ANISAKID NEMATODES INFECTING LIVER OF COD (*Gadus morhua*) FROM THE NORWEGIAN SEA

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Background. Atlantic cod (*Gadus morhua*) belong to the most important commercial fish species on the world, not only because of its flesh, but also liver (used for consumption or production of liver oil). However, the occurrence of potentially zoonotic nematodes in that organ arouse the food safety and human health concerns.
Objectives. The aim of our studies was to explore the presence, intensity of infection and distribution of the nematodes of the different genera of Anisakidae in the liver of *G. morhua* from the Norwegian Sea.
Material and Methods. Cod from two fishing areas: FAO IIa1 (n = 36) and FAO IIa2 (n = 52) were investigated for the presence of parasites in the liver. After digestion in artificial gastric juice all parasites found were collected and identified to the genus level. The subsample of parasites was identified using molecular methods.
Results. In FAO IIa1 94.4% of livers were infected with Anisakidae nematodes, while in FAO IIa2 – 86.5%. Identification on the basis of anatomo-morphological features of nematodes revealed the presence of representatives of *Contracaecum, Anisakis* and *Pseudoterranova* genera. Molecular identification of nematodes (gene cox 2 analysis) revealed the presence of *Pseudoterranova bulbosa* in both sampling areas.
Conclusion. In the liver of cod from the Norwegian Sea the *Contracaecum* sp.*, Anisakis* sp. and *Pseudoterranova bulbosa* were found.

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FISHP16

HOW DIFFER THE DISTRIBUTION OF ANISAKID NEMATODES IN THE MUSCLE TISSUE OF COD (*Gadus morhua*) FROM THE NORWEGIAN SEA?

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Background. Atlantic cod (*Gadus morhua*) is one of most important commercial fish species on the world. Consumers prefer its mild flavour and dense, flaky white flesh. However, the occurrence of potentially zoonotic nematodes of the genera *Anisakis* and *Pseudoterranova* in cod muscle tissue arouse the food safety and human health concerns.
Objectives. The aim of our studies was to explore the presence, intensity of infection and distribution of the nematodes of the different genera of Anisakidae in the muscle tissue of *G. morhua* from the Norwegian Sea.
Material and Methods. Samples were collected during commercial surveys in March 2017 in fishing areas FAO IIa1 (n = 50) and FAO IIa2 (n = 56). After ichthyological analysis, the unskinned flesh of each fish was divided into three parts – anterior ventral (belly flaps), dorsal fillet and caudal fillet – and examined using a white-light transilluminator. All parasites found were collected and identified to the genus level and subsample was identified using molecular methods.
Results. Higher prevalence of infection with *Anisakis* than with *Pseudoterranova* in the musculature of cod from both fishing areas was found. In FAO IIa1, a lower prevalence of infection with *Pseudoterranova* was recorded (14%) than in FAO IIa2 (~39%), whereas the opposite was found with *Anisakis* (88% and ~55%, respectively).
Conclusion. The two parasite genera were distributed differently in cod muscle tissue: most *Anisakis* larvae were present in the belly flaps (predominantly the left side), while *Pseudoterranova* spp. were dispersed with descending frequency in belly flaps, dorsal fillet and caudal fillet.

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FISHP17

HELMINTHS OF THREE-SPINED STICKLEBACK *Gasterosteus aculeatus* LINNAEUS, 1758 IN THE OPEN WATERS OF NORTH-WESTERN PACIFIC

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Three-spined stickleback, *Gasterosteus aculeatus* Linnaeus, 1758, is a common fish in the epipelagic layer of the north-western Pacific. Being anadromous, it spends the initial stage of the ontogeny and the spawning period in fresh waters. In total, 759 specimens of three-spined stickleback were caught in 2018-2019 from RV Professor Kaganovsky in the Bering Sea, the Sea of Okhotsk and open waters of north-western Pacific off the Kuril Islands. The first part of our study, based on the 214 specimens collected in 2018, is already published (Gordeev, Sokolov, 2020). Here we present the results of its second part. Some of the dissected sticklebacks were infected by juvenile nematode *Anisakis* sp., cestodes *Pelichnibothrium speciosum*, *Bothriocephalus scorpii*, and *Dibothriocephalus dendriticum*, as well as trematode *Bunodera mediovitellata*. The species affiliation of cestode plerocercoids was confirmed with the help of genetic methods: sequencing of rDNA 28S and cox1 genes. Anadromous fish usually lose their parasites during the transition from freshwater to the sea and back. In particular, this phenomenon has been shown for salmon *Oncorhynchus* spp., which also inhabits the Far East. However, although the sticklebacks examined in our study were caught at a distance of up to 500–600 km from the shore, their parasitic fauna was mostly represented by species of clearly freshwater origin (*B. mediovitellata* and *D. dendriticum*). Infection by coastal *B. scorpii* was irregular throughout the area. Our study showed that the infection of three-spined stickleback differed significantly from that of all the other examined teleost species in the study area. It harboured mostly freshwater and coastal helminths, while truly marine parasites such as *P. speciosum*, which heavily infects other teleosts in north-western Pacific, had very low values of both prevalence and infection intensity. These are the first data on the helminths of *G. aculeatus* so far in the open ocean.

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FISHP18

ELEVATED INFECTION WITH *Contracaecum osculatum* IN COD *Gadus morhua* FROM THE SOUTHERN BALTIC SEA AND ITS NEGATIVE EFFECT ON CONDITION AND MORTALITY OF THE FISH

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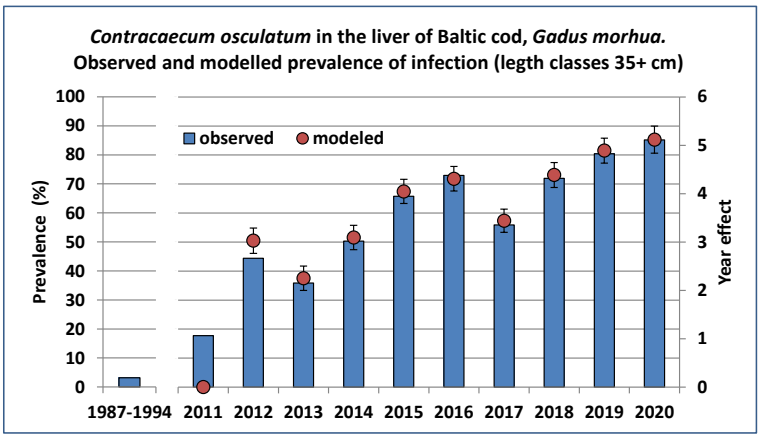
Background. In the last century, parasitic infection with *Contracaecum osculatum* (Nematoda: Anisakidae) affected Baltic cod only marginally. In the period of 2011–2020 the prevalence of infection increased markedly and dispersed to the entire area of the southern Baltic.

Objectives. The aim of this study was to quantify the prevalence and intensity of the infection of cod with nematodes that occur in the liver of fish and to investigate the association between the infection and condition of cod.

Material and Methods. Cod *Gadus morhua* from the southern Baltic Sea were sampled between 2011–2020 and investigated for the presence of nematodes in the liver. Generalised Linear Models (GLMs) were applied to analyse the prevalence and intensity of cod infection with nematodes and to investigate the association between the Fulton's Condition Factor (FCF) and the intensity of infection.

Results. The prevalence of infection with *C. osculatum* larvae is much higher compared with previous studies undertaken over the past few decades. Remarkable increase in the infection level of cod was reported in 2011 and there was a further increasing trend in subsequent years (2012-2020). FCF decreased significantly with an increasing numbers of parasites in the liver.



Conclusion. The presence of nematodes in the liver of cod negatively affects the condition of fish and may increase mortality of large and heavily infected individuals. Elevated infection of cod is associated with an increase in numbers of the grey seal (*Halichoerus grypus*) in the Baltic Sea, which are final hosts for *C. osculatum*.

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FISHP20

DIVERSITY AND ACTIVITY OF SUGAR TRANSPORTING GENES OF *Anisakis simplex s. s.* L3 AND L4 LARVAE, *IN VITRO* ANALYSIS

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Background. The larvae of genus Anisakis nematodes responsible for disease known as anisakiasis in humans. *Anisakis simplex* is as a model organism due to the possibility of *in vitro* culture of both larval L3 and L4, different functions of the digestive system an additional advantage is the fact that only these stages have been identified as a pathogenic factor in humans. In the present study we characterized the GLUT-like transcripts in *A. simplex* and by investigating the relationship it with glucose.

Material and Methods. L3 and L4 larvae of *A. simplex* s.s. was cultured *in vitro* according to Iglesias et al. (2001) using medium RPMI-1640 with glucose at concentration of range from 0.1 to 20 mg/ml for 12 h and 24 h. The larvae from each sample were used for total RNA isolation and biochemical analyses. The gene expression levels (mRNA) of five facilitated glucose transporters (FGT 1, FGT 2, FGT 3, FGT 5, FGT 9) and sugar transporter (SWEET 1) was determited by the Real-time PCR. Gene expression profiles were calculated using the comparative Pfaffl method (2001).

Results. We obtained full-length sequences of 5 putative GLUT (glucose transporter)-like genes (*FGT1, FGT2, FGT3, FGT5, FGT9*) and *SWEET1* from *A. simplex*. The mRNA expression of glucose transporters in L3 larvae after 12 hours of sugar stimulation, correlates with a decrease in glucose content, which may indicate a lack of response to an external stimulus and only in the L4 larvae a positive response was recorded.

Conclusion. Analysis of the expression glucose transporters of *A. simplex*, as a representative of parasitic nematodes, will affect the better understanding of the biology of parasitic nematodes and will allow finding ways to overcome the diseases caused by them.

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FISHP21

FEEDING HABITS SHAPE INFECTION LEVELS BY PLEROCERCOIDS OF THE TAPEWORM *Triaenophorus crassus* IN MUSCLE OF A SYMPATRIC PAIR OF WHITEFISH IN AN OLIGOTROPHIC LAKE

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Background. Sympatric pair of whitefish belonging to the *Coregonus lavaretus* complex inhabits Teletskoye Lake (West Siberia, Russia). This pair is formed by a small "plankthivorous" – *C. l. pravdinellus* (Dulkeit, 1949) (total body weight is up to 40 g at 7 years old) and large "benthivorous" – *C. l. pidschian* (Gmelin, 1789) (total body weight is around 150 g at the same age). Such different feeding strategies have to be reflected on the structure of parasite communities of these whitefish. *Triaenophorus crassus* is one of common muscle parasite of whitefish in Teletskoye Lake. This cestode is transmitted via food webs and infested in whitefish muscles.
The main aim of the present study was to estimate the relation between different feeding habits of the 'benthivorous' and 'planktivorous' whitefish and the infection level of *T. crassus* plerocercoids.
Material and Methods. Fish were collected in 2017 and 2019–2020 (August-September) in Teletskoye Lake (51°79'N; 87°30'E) using gill-nets. For parasitological analysis, we collected 146 and 101 individuals of the 'benthivorous' and 'planktivorous' whitefish, respectively.
Results. For the 'benthivorous' whitefish the prevalence, intensity and abundance of *T. crassus* ranged from 22.4% to 51.9%, 1.9–2.8 and 0.4–1.3, respectively, whereas the same indices for the 'planktivorous' one ranged from 94.7% to 97.5%, 4.2–4.8 and 4.0–4.7, respectively. The level of intensity of infection and abundance of *T. crassus* in muscle was relatively stable among studied years for both forms. The level of prevalence was higher in the years 2019 and 2020 than in 2017 for the 'benthivorous' form, whereas for the 'planktivorous' form this index did not change during the studied years.
Conclusion. We can conclude that *T. crassus* can be an indicator of trophic niche specialization and reflects changes in whitefish habitat selection.

Funding source: This research was supported by the Russian Foundation for Basic Research (grant № 19-34-60028).

FISHP22

DIVERSITY OF *Diplostomum* (DIGENEA: DIPLOSTOMIDAE) IN FISH FROM WESTERN SIBERIA ACCORDING TO DNA BARCODES

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Background. Trematodes of the genus *Diplostomum* are important fish pathogens. The data on their diversity in water bodies of Siberia are inconsistent, which may be due to the complexity of species identification. This study is aimed at identifying the species diversity of metacercariae of *Diplostomum* spp. from fish of two largest lakes in Western Siberia based on analysis of partial *cox*1 mtDNA gene sequence.
Material and Methods. In total, the nucleotide sequences of 77 metacercariae from ten fish species (*Perca fluviatilis*, *Sander lucioperca*, *Esox lucius*, *Cyprinus carpio*, *Carassius gibelio*, *C. carassius*, *Rutilus rutilus*, *Leuciscus leuciscus*, *L. idus, Abramis brama*) from Chany Lake and 30 metacercariae from six fish species (*Coregonus lavaretus*, *A. brama*, *L. leuciscus*, *P. fluviatilis*, *Cottus sibiricus*, *Lota lota*) from Teletskoye Lake were obtained. The *cox*1 gene region was amplified using the primers described by Steenkiste et al. (2015). Received sequences were used for molecular identification of the isolates using the BLAST program.
Results. Three species of trematodes of the genus *Diplostomum* have been recorded in fish from Chany Lake: *D. spathaceum*, *D. pseudospathaceum*, and *D. baeri*. *D. pseudospathaceum* was recorded in all studied fish species, *D. spathaceum* in eight species (*Perca fluviatilis*, *Sander lucioperca*, *Cyprinus carpio*, *Carassius gibelio*, *C. carassius*, *Rutilus rutilus*, *Leuciscus leuciscus*, *L. idus*) whereas *D. baeri* was found only in *P. fluviatilis*. In the fish of Teletskoye Lake, in addition to the above three species of metacercariae, *D. mergi* (*L. lota*), *D.* sp. 1 (*C.*

sibiricus), for which there were no reference sequences in the GenBank database, and *D*. sp. 2 (*C. lavaretus*), which has more than 99% identity to sequences from metacercariae from northern Norway.
Conclusion. Thus, 7 species of trematodes of the genus *Diplostomum* were recorded in the fish of the two largest lakes in Western Siberia.

Funding source: This work was supported by the Russian Science Foundation, project № 19-74-10054

FISHP23

FIRST MOLECULAR DATA OF THE PARASITIC CRUSTACEANS GENUS *Salmincola* (COPEPODA: LERNAEOPODIDAE) FROM ASIAN PART OF RUSSIA

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Background. Parasitic crustaceans from genus *Salmincola* are widespread over the area of Holarctic ectoparasites and infest different salmonids in marine and freshwater ecosystems. Unfortunately, there is a few data regarding to their diversity in water bodies of Russia based on molecular analysis. The present study is the first attempt to identify several parasitic crustaceans from Siberia and Kamchatka regions using 28S rDNA gene sequence.
Material and Methods. Ectoparasitic crustaceans were collected from fins, skin, gill, and nasal fossae of whitefishes (genus *Coregonus*) in the area of Teletskoye and Baunt lakes (Siberia) and from mouth of charr (genus *Salvelinus*) and kokanee salmon (genus *Oncorhynchus*) from Kronotskoye Lake (Kamchatka). The 28S gene region was amplified using the primers described by Reumont et al. (2009). Phylogenetic reconstruction was performed using the Bayesian inference approaches with MrBayes v.3.2.1.
Results. A total of 20 specimens of *Salmincola* spp. from studied salmonids of Siberia and Kamchatka were examined. Based on 28S gene sequences, the studied parasitic crustaceans were distributed among five species-level clades: *S. carpionis* (charr), *S. edwarsii* (kokanee salmon), *S. extumenses* (whitefish from Baunt Lake), *S. lavaretus* (whitefish from Teletskoye Lake), and *S. extensus* (whitefish from Baunt Lake).
Conclusion. All studied parasitic crustaceans were well identified using 28S rDNA gene sequence.

Funding source: This work was supported by the Russian Science Foundation, project № 19-74-10054

FOODBORNE AND WATERBORNE PARASITES: CHANGING CLIMATE, CHANGING TRENDS, CHANGING PARASITES

FWB1

LOW PREVALENCE OF *Toxoplasma gondii* INFECTION IN MERINO BLACK AND MERINO WHITE LAMBS IN THE REGION ALENTEJO, PORTUGAL

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Background. *Toxoplasma gondii* is one of the most common zoonotic parasites worldwide. Human infection is particularly dangerous in its congenital form and in people with immunological deficiency. Toxoplasmosis may be acquired through the consumption of raw or undercooked meat containing *T. gondii* tissue cysts and lamb meat is generally considered a main source of human infection.
Objectives. This study aimed to determine the prevalence of T. *gondii* infection in Merino lambs in the region Alentejo.
Material and Methods. Blood samples were collected between September 2019 - January 2021 from 330 Merino Black and Merino White lambs aged 5-12 months on 22 farms in the Alentejo region. A total of 15 animals were sampled per farm. All sheep were reared in extensive production systems having access to large areas of pastures (32-1146 ha). The presence of specific antibodies to *T. gondii* was assessed using a commercial indirect enzyme-linked immunosorbent assay (ID Screen Toxoplasmosis Indirect Multispecies). A flock was considered positive if at least one animal tested positive by ELISA.
Results. Of the total sample, 3 lambs tested positive for *T. gondii* antibodies (0.91%; 95% CI: 0.31–2.64%). Seropositive lambs were aged between 7-8 months. The prevalence of positive flocks was 13.64% (95% CI: 4.75–33.3%).
Conclusion. The low level of exposure of Merino lambs to *T. gondii*, may be explained by the young age of animals, taking into account the persistent character of infection. Other reason for the low prevalence may be the extreme hot and dry conditions observed in the Alentejo region during the summer period that could compromise the survival of *T. gondii* oocysts. Considering the slaughter age of 90-120 days, the consumption of meat from Merino lambs reared in extensive production systems in the Alentejo region may represent a low risk of human infection in Portugal.

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FWB2

SCOPING REVIEW ON THE PROGRESS OF VACCINATION AGAINST FASCIOLOSIS: PAST, PRESENT AND FUTURE PERSPECTIVES

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Background. Fasciolosis is a worldwide foodborne trematodosis caused by platyhelminthes from the family Fasciolidae. Its treatment is based on the use of triclabendazole. Nevertheless, its continued use has propagated the emergence of resistance against this drug. For that reason, the development of an effective vaccine is of paramount importance. Although numerous vaccination trials have been described in the literature, optimal and reproducible levels of protection have not been reached to date.

Objectives. The aim of this study is to systematically review the vaccine trials against *Fasciola* spp. carried out during the last 20 years in order to scope this body of literature, identify knowledge gaps and clarify concepts.
Material and Methods. A systematic search based on PRISMA-ScR guidelines was conducted to identify vaccine trials against fasciolosis from 1 January 2000 to 31 December 2020. Multiple key words were used to search for articles using the following strategy: ("fasciol*"[All Fields] OR "liver fluke"[All Fields]) AND ("vaccin*"[All Fields] OR "immunogen*"[All Fields] OR "protect*"[All Fields]) AND 2000/01/01:2020/12/31[Date - Publication]. Resulting publications were screened for eligibility according to previously established criteria and data extracted and analysed.
Results. Of the 1205 publications identified, 76 studies containing 134 vaccine trials were included in the subsequent analyses. The obtained results showed considerable heterogeneity among trials with 34 different vaccine candidates, 10 hosts' models, 10 administration routes and 26 adjuvants assayed. Most of these trials tested proteins from the adult stage of the parasite (74%). Obtained protection percentages ranged from 23.1 to 97%, although up to 48.5% of trials did not reach significant reduction of parasite burdens.
Conclusion. High variability in the protection rates and partial success have been reported in the published vaccination studies against fasciolosis. Therefore, homogeneous criteria should be established, as well as other approaches, including the use of juvenile parasite proteins, explored for future action and research.

FWB3

EXPLORING THE EARLY STEPS OF HOST INVASION BY *Fasciola hepatica* JUVENILES: *EX VIVO* MODEL AND QUANTITATIVE PROTEOMIC APPROACH

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Background. Fasciolosis comprises a global health issue at a livestock level, together with a human zoonotic disease in different areas of the world. Although *Fasciola hepatica* life cycle has been extensively explored, many features of the early interaction with its definitive vertebrate hosts still need to be addressed.
Objectives. The aim of the study is to reproduce the migration of *F. hepatica* newly excysted juveniles (FhNEJ) through the host intestinal barrier and characterize the proteomic changes that the parasite undergoes during this process.
Material and Methods. A murine *ex vivo* model was set up, in which FhNEJ were directly injected into small intestine portions. After that, migrating juveniles were recovered and analysed by Sequential Window Acquisition of all Theorical Mass Spectra (SWATH-MS), a label-free quantitative proteomic approach that allows simultaneous identification and quantification of complex protein samples that has rarely been used in the field of parasitology.
Results. After an incubation time of 2.5 hours around 22% of injected FhNEJ were recovered for proteomic characterization. SWATH-MS allowed to identify up to 120 FhNEJ differentially expressed proteins, revealing changes in key processes of the early stages of *F. hepatica* infection such as proteolysis, antioxidant responses or alteration of the host defence mechanisms.
Conclusion. The proposed model successfully replicated the invasion of the host intestinal wall by FhNEJ, allowing to further explore their antigenic repertoire in the early phase of the infection. Moreover, the described proteomic methodology will provide new insights for vaccine development against *F. hepatica.*

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FWB6

GENETIC CHARACTERIZATION REVEALS EVIDENCE FOR AN ASSOCIATION BETWEEN WATER CONTAMINATION AND ZOONOTIC TRANSMISSION OF A *Cryptosporidium* sp. FROM DAIRY CATTLE IN WEST BENGAL, INDIA

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Background. *Cryptosporidium* sp., an enteric parasite with zoonotic potential, can infect wide range of vertebrate hosts, including humans. Transmission occurs via fecal-oral route. Disease is self-limiting in immunocompetent individuals, but can be life-threatening among malnourished and immunocompromised patients. Determining the source of infection and the mode of transmission is crucial for the control of cryptosporidiosis.

Objectives. To identify the source of *Cryptosporidium* infection in farm workers in a new endemic zone and the zoonotic potential of the etiological agent.

Material and Methods. Fresh fecal samples from farm workers and bovine calves; and water samples from nearby water bodies around the farms were collected. All samples were screened by conventional microscopy (Kinyoun staining), enzyme linked immunosorbent assay (ELISA) and Polymerase Chain Reaction (PCR). *Cryptosporidium* isolates were genetically characterized based on two housekeeping genes i.e., 18SrRNA and hsp70. LD analysis of polymorphic site at 18SrRNA locus of our study isolates were carried out to investigate the possibility of genetic recombination event.

Results. We identified that dairy cattle can be a potential source of *Cryptosporidium* infection for humans. Unprecedented high infection rate of *Cryptosporidium* was identified- 22% in calves and 19% among farm workers. We identified four different species- *C. parvum, C. ryanae, C. bovis* and *C. andersoni,* with *C. ryanae* being the prevalent one with a new host. Majority of the cases (animals and humans) carried *C. ryanae,* indicating its virulence. Evidence for genetic recombination was only identified in *C. ryanae* for the first time.

Conclusion. Our findings of zoonotic transmission of *Cryptosporidium* spp, suggest a serious public health risk for the farm workers and villagers living in close proximity.

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GIS1

ZOONOTIC DISEASES IN THE RUSSIAN ARCTIC: THE STUDY OF ENVIRONMENTAL SUITABILITY TO ANTHRAX AND LEPTOSPIROSIS IN THE CURRENT AND FUTURE CLIMATE

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The study assesses an aggregate of environmental and socio-economic conditions associated with the historical distribution of two re-emerging zoonotic diseases – anthrax and leptospirosis – in the Russian Arctic. A database of climate indicators obtained by interpolation of weather stations' observations within the study area was created to support the study. An ensemble of climate models under the CMIP5 experiment was applied to project those indicators by 2100 assuming no change in human behavior with regard to greenhouse emissions (RCP8.5 scenario).

We applied Maximum Entropy ecological niche modelling approach (Maxent) and used officially recorded historical burials of animals died from anthrax as presence locations. Maxent model suggested a most significant influence of soil type and pH, yearly maximum and mean air temperatures, annual precipitation at temperatures below 0°C and vegetation intensity on the suitability to *Bacillus anthracis*. Modeling with projected climate data demonstrated a dramatic increase of suitability across the most part of the study area, resulted from the rise of air temperatures and consequent thawing of permafrost that enables release of conserved anthrax soil foci.

We used livestock leptospirosis data for 2000 – 2019 and employed the Forest-based Classification and Regression algorithm to explore the relationships between the cumulative leptospirosis incidence per unit area by municipal districts (G-rate) and a set of socio-economic, landscape, and climatic factors. Socio-economic variables related to human and livestock population densities, and agricultural development were found to be the most important, while climate and landscape factors demonstrated a significantly lower influence with nearly similar contributions of mean yearly precipitation and air temperature and number of days with above-zero temperatures. A projection using the future climate data suggested an up to 4.4-fold climate-related increase in the G-rate.

The findings may be used to improvement the regional system of anti-epizootic measures with regard to the studied diseases.

Funding source: The study was supported by the Russian Foundation for Basic Research (Grant 18-05-60037 "Medical-Geographic modeling the space-time changes of environmental and socially significant diseases under the conditions of changing climate and economic development of the Russian Arctic").

HELMINTH IMMUNOMODULATION AND INTERACTIONS WITH THE MICROBIOME

HIMM1

SEEING THE WHOLE: LIGHT SHEET MICROSCOPY ELUCIDATES THE BIOLOGY OF SCHISTOSOMES WITHIN THE SPINAL CORD

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Background. Light sheet fluorescence microscopy (LSFM) might be a method of choice to display tissue helminths *in toto* as it enables the visualization of whole organs. Furthermore, it allows to quantify and demonstrate the distribution of immune cells in the affected tissue.
Objectives. We adopted LSFM, a new method in the field of helminth-parasite interactions, to explore migration of the neurotropic schistosome *Trichobilharzia regenti* within the spinal cord of mice. Specifically, we employed comprehensive 3D imaging to follow the parasite distribution and host immune response.
Materials and Methods. MHC-II-EGFP knock-in mice were infected with *T. regenti* and the thoracic spinal cord was isolated 0, 7, and 14 days post infection (dpi). The samples were cleared using the CUBIC method and visualized by Zeiss Lightsheet Z.1. The LSFM data were supported by transcriptomic analysis, flow cytometry, and immunohistochemistry.
Results. The LSFM analysis revealed that the spinal cord invasion augmented the expression of MHC-II, especially 14 dpi. Surprisingly, the MHC-II+ cells were localized not only in the close vicinity of the migrating schistosomula but also throughout the entire spinal cord, including perivascular areas. The MHC-II+ cells around the schistosomula were partly represented by Iba1+ cells (i.e., microglia/macrophages). The upregulation of the MHC-II pathway was confirmed by transcriptomic and flow cytometry analyses of the spinal cord.
Conclusion. LSFM facilitated the depiction of the cellular immune response in the whole organ. Indeed, we revealed that *T. regenti* infection activated the immunocompetent cells in the whole spinal cord, not only around the parasite. Based on our experiments, we assume that LSFM is a promising method for a better understanding of host-parasite interactions.

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HIMM2

TRANSCRIPTOME-DRIVEN EXPLORATION OF *Trichobilharzia regenti* (SCHISTOSOMATIDAE) NEUROINVASION IN MICE: MECHANISMS OF THE IMMUNE RESPONSE AND PATHOGENICITY

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Background. Helminth neuroinfections severely impact human health. However, the host-parasite interplay within the nervous tissue often remains poorly understood, partly due to the unavailability of suitable experimental models.
Objectives. We explored mechanisms of the host immune response and helminth-induced neuropathogenicity in mice infected with the neurotropic schistosome *T. regenti*, which represent a promising model in the neuroinfection research.
Material and Methods. We employed a multiscale approach, including behavioural testing of *T. regenti*-infected mice, histological and transcriptomic analyses of their spinal cords, nervous tissue microdissections, flow cytometry or immunohistochemistry.

Results. *T. regenti* schistosomula invaded mostly the spinal cord where they induced eosinophilic meningomyelitis culminating 14 days post infection. Flow cytometry and transcriptomic analysis confirmed massive activation of the immune response in the infected spinal cords. Intriguingly, we recorded striking upregulation of M2 markers (*Arg1*, *Chil3*/*Ym1*) and arginase-1 also dominated among the proteins found in the close vicinity of the migrating schistosomula. Hence, the previous concept of microglia/macrophages actively fighting against the schistosomula needs to be reconsidered as they rather help in tissue repair. Next, we evaluated the pathological sequelae of *T. regenti* neuroinvasion. While no significant demyelination or neuronal apoptosis were noticed, we observed a substantial transcriptomic disruption of neurophysiological signalling pathways, which could explain the motor deficits observed in the hindlimbs of infected mice.
Conclusion. The comprehensive characterisation of *T. regenti* neuroinvasion broadens the range of animal models available to study pathogen-related neuroinflammatory processes. A complex insight into their diversity is a prerequisite for the development of better protective measures, treatment strategies, and diagnostic tools.

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HIMM3

IL-33 HAS A DETRIMENTAL ROLE IN THE PROGRESSION OF ALVEOLAR ECHINOCOCCOSIS IN A MOUSE MODEL

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Background: In alveolar echinococcosis (AE), the *Echinococcus multilocularis* metacestode develops in the liver, where an appropriate Th1/Th17 response may lead to its clearance. In susceptible patients, the immune response switches to a Th2 phenotype during late stage of infection. The role of the pleiotropic cytokine IL-33, described to drive immunity towards a Th2 response, is not known in AE. This study aimed at describing the role of this cytokine during AE, using an *in vivo* mouse model.
Material and Methods: Wild-type (WT, n=6) and IL-33 -/- (KO, n=9) C57Bl/6J mice were infected by intraperitoneal injection of *E. multilocularis* metacestodes and euthanized 4 months after (late stage of infection). Immunophenotyping by flow cytometry was performed on peritoneal, liver and spleen cells. Expression of cytokine genes in liver was quantified by RT-qPCR and serum cytokines were quantified using the LEGENDplex™ multiplex assay.
Results: The median weight of lesions was significantly lower in the KO (5.9g) versus the WT (9.5g) group (Wilcoxon test $p<0.05$). Peritoneal cell infiltrate was more prominent in the KO group ($p<0.05$), with a similar composition. Significantly lower proportions of $CD11b^{+}Gr1^{Hi}$ ($p<0.01$) and $CD11b^{+}Gr1^{Lo/-}$ ($p<0.05$) myeloid cells were observed in livers of KO mice, together with higher proportions of $TCD4^{+}$ ($p<0.01$), $TCD8^{+}$ ($p<0.05$), NKT ($p<0.05$), and a higher $CD4^{+}/CD8^{+}$ ratio ($p<0.05$). In the liver of KO mice, IL-17F ($p<0.01$), IL-1β, IFN-γ and IL-12p35 genes were induced (not significant), and IL-1RN was repressed ($p<0.01$). Thus, the KO group had an improved Th1/Th17 serum cytokine profile.
Conclusion: In this applied AE mouse model, hepatic changes are observed even in absence of liver lesion. In the IL-33-KO group, we observed a hepatic immune environment adapted to parasite clearance, and a better control of its peritoneal development. These data highlight a detrimental role of IL-33 during AE.

HIMM5

INTERACTION BETWEEN HELMINTH PARASITES AND THE HAEMOSTATIC SYSTEM OF THEIR HOSTS: A SCOPING REVIEW

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Background. Parasites have evolved a great variety of strategies to establish and survive in their hosts. Since the 1950s, a great amount of research has postulated that one of these strategies could be the interaction between parasite molecules and the haemostatic system, the mechanism responsible for forming (coagulation) and degrading (fibrinolysis) blood clots in vertebrates. Nevertheless, this body of evidence has never been systematically analysed.

Objectives. This study aimed to systematically review the available evidence regarding the molecular interaction between helminth parasites and the host haemostatic system in order to update concepts, identify knowledge gaps and contribute to future research.

Material and Methods. A systematic search following PRISMA-ScR guidelines was conducted in online databases to identify all relevant articles published until 31 December 2019. Publications were screened for eligibility and data extracted and analysed according to previously established criteria.

Results. Of the 6498 articles initially identified, 101 studies published between 1956 and 2019 were included in the subsequent analyses. The obtained results revealed 33 species of helminth parasites (mainly *Ancylostoma caninum*, *Schistosoma mansoni*, *Dirofilaria immitis* and *Haemonchus contortus*), whose capability to interact with the haemostatic system had been shown. In 65 of 101 studies (64%), this interaction was attributed to an anticoagulant or pro-fibrinolytic potential of the parasite, which was related to different parasite mechanisms such as blood-feeding, migration, invasion, evasion and pathogenesis. The main effects of these interactions were the prolongation of the coagulation time, together with the degradation of fibrinogen and the inhibition of activated coagulation factor X (coagulation pathway), as well as the binding and activation of plasminogen (fibrinolysis). Among different antigens, serpins and enolases were the main parasite molecules responsible for the anticoagulant and pro-fibrinolytic purposes, respectively.

Conclusion. A great diversity of helminth parasites possesses similar strategies to interact with the haemostatic system of their hosts, mainly resulting in anticoagulant and pro-fibrinolytic effects, which suggest potential parasite mechanism to survive and evade host responses.

HOST-PARASITE INTERACTIONS

HPI1

LOCALIZATION OF THE PROTEINASE INHIBITORY ACTIVITY IN THE FISH CESTODE *Eubothrium rugosum*

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Background. Parasite infestations are among the factors affecting the activity of fish gut enzymes. Changes in the activity of the host digestive enzymes in the infected fish may be linked, inter alia, to inhibition of the host proteolytic enzymes.
Objectives. The study was aimed to determine the localization of the inhibitory ability towards trypsin and chymotrypsin in tapeworms *Eubothrium rugosum*.
Material and Methods. The tapeworms were isolated from the intestine of burbot, *Lota lota*. To separate the tegumental brush border (TBB), the worms were treated with Triton X-100 and subjected to differential centrifugation followed by the enzyme assays. To assess the inhibitory ability, we determined trypsin and chymotrypsin activities before and after the incubation with various worm preparations.
Results. Alkaline phosphatase activity in the brush border (BB) fractions was more than 3 times higher than in the tapeworm homogenate obtained after Triton X-100 treatment. No differences were found in the alkaline phosphatase activity among the various centrifugation fractions. Both the BB fractions of *E. rugosum* and its excretory/secretory products released into the incubation medium significantly inhibit trypsin and chymotrypsin activities. Only trace trypsin- and chymotrypsin-like activities were noted in the homogenized worms lacking BB.
Conclusion. Methodologically, the study findings mean that not only the active fractions of the disrupted BB, but the whole worms can be used for exploring proteinase inhibitors.

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INTERNATIONAL PROJECTS IN PARASITOLOGY

INTP1

OVERVIEW OF IMPACT: STANDARDISING MOLECULAR DETECTION METHODS TO IMPROVE RISK ASSESSMENT CAPACITY USING *Cryptosporidium* spp. IN READY-TO-EAT SALADS

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Background. In recent years, several cryptosporidiosis outbreaks in association with the consumption of fresh produce have been reported across Europe. Currently, a fast, reliable and standardisable molecular assay for testing of protozoan parasites in fresh produce is lacking.

Objectives. The IMPACT project aims at capacity building and exchange of knowhow amongst various research laboratories and improvement of risk assessment capacity for food safety in Europe. The goal of the project is to provide guidance for artificial contamination studies and to validate a real-time PCR assay for the detection of *Cryptosporidium* in ready-to-eat salad as a model organism for other food-borne protozoa.

Material and Methods. Current procedures for spiking and detection of *Cryptosporidium* oocysts in fresh produce were reviewed, a market survey of oocyst suppliers was conducted and expert opinions were obtained. An SOP based on an 18S qPCR assay for the detection of *Cryptosporidium* in salad leaves was implemented in two laboratories. The optimised SOP along with video-tutorials were disseminated to four partner laboratories for validation.

Results. A guidance document on spiking of salad leaves with *Cryptosporidium* oocysts was established. Feedback from four partner laboratories where the SOP was implemented was used to fine-tune the protocols. Validation of the resulting SOP by a ring trial in ten food-testing laboratories is planned. The hands-on experiences of the participating laboratories will be analysed via a detailed questionnaire on the performance of various aspects of the protocol under standard operating conditions.

Conclusion. The proposed SOP for *Cryptosporidium* spp. can be extrapolated for the detection of infective oocysts of other protozoans in food matrices. The final SOP will be disseminated using the network of NRLs in Europe, EFSA focal points, COST Action Euro-FBP network, and the OHEJP network. IMPACT enables exchange of knowledge between EU participants contributing to the strengthening of food testing networks.

Funding source: EFSA Partnering Grant GP/EFSA/ENCO/2018/03 – GA03

6th INTERNATIONAL WORKSHOP ON ARCTIC PARASITOLOGY – IWAP 6.0e

IWAP1

ACANTHOCEPHALANS OF TELEOST FISHES IN THEWEST ANTARCTIC MARINE ECOSYSTEMS

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Background. The life cycles of acanthocephalans of teleost fishes are associated with crustaceans, fish, birds and marine mammals and can be used as indicators of the state of Antarctic marine ecosystems.This is important in the context of climate changes and anthropogenic pressure in Polar regions.

Objectives. The species diversity, parameters of infection, co-occurrence of acanthocephalanspecies of Antarctic teleost fish were studied and analyzed in this study.

Material and Methods. During 2014–2019, 159 specimens of teleost fishes of 6 species were examinednear the Ukrainian Antarctic Station "AkademikVernadsky", Galindez Island, Argentine Islands, West Antarctica. Helminths were collected manually, fixed in 70% ethanol and identified by morphology.

Results. Acanthocephalans of 11 species of 4 genera were found in Antarctic fish. Teleost fishes are the definitive hostsforacanthocephalans of 6 species: *Metacanthocephaluscampbelli*, *M. dalmori*, *M. Johnstoni*, *M. rennicki*, *Echinorhynchuspetrotschenkoi* and *Aspersentismegarhynchus*.For the acanthocephalans of 5 species of the genus *Corynosoma*: *C. bullosum, C. evae, C. hamanni, C. pseudohamanni* and*C. Shackletoni* teleost fishes are the paratenic hosts.*Corynosomapseudohamanni* and *C. evae*were found in all studied fish species. The greatest species diversity and infection rates were observed in three fish species: *Nototaeniacoriiceps*, *Parachaenichthyscharcoti*and *Chaenocephalusaceratus*.The rarest acanthocephalan species were *A. megarhynchus*, *E. petrotshenkoi* and *C. shackletoni*.

Conclusion. This study revealed high species richness of acanthocephalans in benthos teleost fishes in Argentine Islands, West Antarctica.

Funding source: This study was supported by the National Research Foundation of Ukraine (project № 2020.02/0074)

IWAP2

FROM MUMMY, WITH LOVE - IMPACT OF *Babesia microti* INFECTION ON THE INITIATION AND COURSE OF PREGNANCY IN BALB/C MICE

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Background. Vertical transmission is the least understood route of *Babesia microti* transmission in humans and rodents. The course of vertically transmitted babesiosis in offspring is highly dependent on the phase of the infection in mothers, and may differ between host species.

Objectives. The aim of this study was to evaluate the success of vertical transmission in the murine model of congenital babesiosis.

Material and Methods. The first set of experiments involved two groups of female mice infected with *Babesia microti* before mating: inseminated on the 7th day (group A) and after the 40th (group B) day post infection. A second set of experiments involved pregnant females infected with *B. microti* during the first (group C) and second (group D) trimesters. Ultrasound examinations were used to monitor the progress of pregnancy. Blood smears and PCRs were used for the detection of *B. microti*. Pathology was assessed histologically.

Results. Successful development of pregnancy was only recorded in females mated during the chronic phase of the infection (group B). The success of vertical transmission of *B. microti* in this group was 63%. No evidence of pregnancy was detected in females mated during the acute phase of infection (group A) or on

the 4th day of pregnancy (group C). In the group infected on the 12th day of pregnancy (group D), numerous complications, including loss of pregnancy and stillbirths, were recorded. During the acute phase of infection, parasitaemia was lower in pregnant females compared to infected non-pregnant control females.

Conclusion. Acute *B. microti* infection prevents the initiation of pregnancy and embryonic development if it occurs during the first trimester, and causes severe complications in foetal mice in the second and third trimesters of pregnancy. Chronic *B. microti* infection has no detrimental impact on the initiation and development of pregnancy, but results in congenital infection of the offspring.

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KMOTT1

HEPATIC HISTOPATHOLOGICAL FINDINGS IN MULES SEVERELY INFECTED WITH LIVER FLUKES (*Fasciola hepatica*)

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Background. Fascioliasis is a zoonotic disease caused by liver flukes transmitted by freshwater lymnaeid snails. Among equines, donkey and horse reservoir roles have been highlighted in human endemic areas. However, infection in mules has received very limited research, and too little is known about the pathogenicity caused by *Fasciola hepatica* in this host.

Material and Methods. Two mules that died of natural cause in an Andean locality of Mendoza, Argentina, were necropsied. Their livers were inspected and dissected for the classification of macroscopic lesions. Samples of liver tissue were fixed in 10% formalin and histological sections stained with haematoxylin–eosin.

Results. After inspection, 20 and 97 fasciolid flukes were found in each liver. The liver infected by the lower burden of flukes showed no lesions or pathological changes upon macroscopic exam. The other liver showed blunt lobe borders, Glisson capsule with moderate opacity, parenchyma with multifocal subcapsular haemorrhages and firm and stringy organ consistency. In both livers, resistance was perceived upon the parenchyma incisions, probably due to fibrosis, and there was mild thickening of the bile ducts with an increased bile viscosity. Histology of the low-burden liver led to a diagnosis of mild lymphocytic hepatitis. The high-burden liver led to a diagnosis of chronic hepatitis, with areas of acute inflammation and pronounced interstitial fibrosis.

Conclusion. *F. hepatica* may severely affect mule health. Before death, the high-burden mule showed an evident out-of-condition and emaciated aspect, presenting severe chronic and acute lesions.

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KMOTT2

COPROLOGY AND MORPHOLOGY OF *Fasciola hepatica* EGGS FROM INFECTED MULES IN ANDEAN LOCATIONS FROM ARGENTINA

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Background. Fascioliasis is a zoonotic disease caused in the Americas by *Fasciola hepatica*. Among reservoirs, donkeys and horses have been highlighted in human endemic areas. However, little is known about the epidemiological importance of mules.

Material and Methods. Faeces from 208 mules were obtained from two Andean locations in Mendoza, Argentina: Aconcagua 81; Uspallata 127. Samples were stored at 4 °C, and then analysed using the Lumbreras sedimentation technique. *Fasciola hepatica* eggs obtained from mule faeces were measured under a calibrated microscope, including egg length (EL), egg width (EW), egg shape (EL/EW ratio) and egg size (EL*EW).
Results. In Aconcagua, 32 (39.5%) were positive for *F. hepatica* (mean epg of 3.6 ± 2.5), while in Uspallata 31 out of 127 mules (24.4%) were positive (mean epg of 4.7 ± 8.7). The prevalence difference was significant (Fisher's test, p=0.0298). According to the amount of faeces produced by an adult equine (22.7kg/day), a mule may be releasing 2270–794,500 (mean 101,242) fasciolid eggs to the environment on a daily basis. A total of 179 eggs from 63 infected mules were measured (mean EL of 136 ± 10.5 µm, mean EW of 78.2 ± 6.7 µm).
Conclusion. *Fasciola hepatica* egg measurements found in the Argentinian mules fit well with the egg size from donkeys in Bolivia and Egypt. The high prevalences in the two mule groups and the estimated amounts of eggs/mule/day highlight the epidemiological importance of these equids.

Funding source: Argentina: ANPCyT (project № PICT-2017-1361); Universidad Juan A. Maza (project №s 1163/18 and 576/18). Spain: AECID (project № 2017/ACDE/001583); IAEA (Animal Production and Health Section, project № RLA5049); Plan Estatal de Investigación Científica y Técnica y de Innovación (Health Research project № PI16/00520); RICET (project № RD16/0027/0023); PROMETEO Program (project № 2016/099); Universidad de Valencia (project № 2017/01).

KMOTT3

LIFE SPAN OF THE LYMNAEID VECTOR *Galba truncatula* FROM THE NORTHERN BOLIVIAN ALTIPLANO HUMAN FASCIOLIASIS HYPERENDEMIC AREA UNDER EXPERIMENTAL CONTROLLED CONDITIONS

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Background. Fascioliasis is a worldwide freshwater snail-borne zoonotic parasitic disease caused by *Fasciola hepatica*. A human fascioliasis endemic area is located in the Northern Bolivian Altiplano, at 3,820- 4,100 m altitude, besides the valley of La Paz city.
Material and Methods. Collected living lymnaeids of the only vector species *Galba truncatula* were used for experimental procedures. Non-infected lymnaeids were arranged in breeding boxes containing 2000 ml fresh water and maintained under controlled conditions of 20/20, 25/10, 16/9, 22/5 °C day/night; 90 and 65% relative humidity; and a 12 h/12 h light/darkness photoperiod in precision climatic chambers in which day sunlight was of 3500 lux. The water was changed weekly and lettuce added ad libitum.
Results. Life spans of lymnaeids from different localities differed significantly ($P<0.05$). The life span varied up to a maximum of 10-12 months depending on locality origins and experimental conditions. The longest was observed in lymnaeids from the locality of Kallutaca under day/night temperatures of 22°C/5°C.
Conclusion. Longevity in *G. truncatula* usually varies between 6 and 12 months, although up to 17 months have been reported elsewhere. Altiplanic *G. truncatula* snails fit well within the aforementioned extremes. Aestivation and hybernation modify lymnaeid longevity and explain the longer longevities observed in long-term laboratory adapted altiplanic *G. truncatula* kept in breeding boxes. These results also fit well with epidemiological data indicating that the infection risk for humans and livestock in the Altiplano covers the whole year.

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KMOTT4

TRANSMISSION CAPACITY OF *Fasciola hepatica* INFECTING MULES FROM ANDEAN LOCATIONS IN ARGENTINA

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Background. In the Americas, fascioliasis disease is caused by *Fasciola hepatica* and transmitted by lymnaeid snail species from the *Galba/Fossaria* group. The capacity of mules as reservoirs of *Fasciola hepatica* has been highlighted recently. However, to assess the role of mules in fascioliasis transmission, the transmission capacity of fasciolids from mule origin remains to be analysed.

Material and Methods. Due to morphological similarities, rDNA ITS-1 and ITS-2 were analysed to characterize a lymnaeid population from Uspallata, Mendoza, Argentina. Fasciolid eggs from mule faecal samples were kept in darkness at 24°C for 14 days, and then exposed to light to stimulate hatching. Snails reaching 4 mm in length were placed individually in Petri dishes at 22 °C and exposed to one miracidium. The presence and quantification of metacercariae in each Petri dish was observed from day 30 onwards.

Results. The ITS-1 and ITS-2 sequences of lymnaeids from Uspallata were identical to the *Galba truncatula* haplotypes from Bolivia and Chile. Of 22 monomiracidially infected snails, eight (36.4%) shed cercariae. Three snails continued shedding cercariae after 70-90 days post-infection. The mean total cercarial shedding capacity was 54.3 cercariae/snail ± 84.8.

Conclusion. The infectivity of miracidia from mule origin, the number of cercariae/snail and the cercarial shedding period longer than 70 days fit the enhanced transmission pattern in *F. hepatica*/*G. truncatula* at very high altitude in Bolivia. These features indicate that the mule is able to maintain the *F. hepatica* cycle independently.

Funding source: Argentina: ANPCyT (project № PICT-2017-1361); Universidad Juan A. Maza (project №s 1163/18 and 576/18). Spain: AECID (project № 2017/ACDE/001583); IAEA (Animal Production and Health Section, project № RLA5049); Plan Estatal de Investigación Científica y Técnica y de Innovación (Health Research project № PI16/00520); RICET (project № RD16/0027/0023); Universidad de Valencia (project № 2017/01) and Programa de Ayudas de la Generalitat Valenciana y Fondo Social Europeo, 2019 (ACIF/2019/182).

MALARIA

MAL1

EXPERIMENTAL STUDY ON SUSCEPTIBILITY OF COMMON EUROPEAN SONGBIRDS TO *Plasmodium elongatum* (LINEAGE pGRW6)

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Background. *Plasmodium elongatum* (lineage pGRW6) is a widespread avian malaria parasite, causing severe disease in non-adapted hosts. This parasite lineage is distributed globally however, it has never been reported in Common starlings *Sturnus vulgaris* or Common crossbills *Loxia curvirostra*, extensively sampled European songbirds.
Objectives. The aim of this study was to test the hypothesis that birds of these two species might be resistant to the pGRW6 infection.
Material and Methods. Lineage pGRW6 was isolated from a naturally infected Eurasian reed warbler *Acrocephalus scirpaceus* and inoculated into groups Common starlings and Common crossbills. Experimental and control groups were maintained in controlled conditions and examined every 4 days using light microscopy. Haematocrit value and body mass were monitored throughout the experiment. At the end of the experiment (44 days post exposure), samples of internal organs were collected for histological examination for possible presence of phanerozoites.
Results. All control birds remained uninfected. Experimental starlings were resistant to the infection. All exposed crossbills were susceptible and survived until the end of this study. Prepatent period was 12–16 days post exposure. Light parasitaemia (< 0.7%) developed in all exposed crossbills, and only few phanerozoites were seen in bone marrow cells of the infected birds. Significant changes were reported in haematocrit value but not body mass in the exposed crossbills compared to controls.
Conclusion. *P. elongatum* (pGRW6) is of low virulence in Common crossbills and is unable to develop in Common starlings. Low virulence in Common crossbills is likely due to low ability of this parasite lineage to develop phanerozoites resulting in light damage of bone marrow cells. The global distribution of this parasite might be due to low virulence in wild adapted avian hosts, which survive this infection and serve as reservoirs host for non-adapted birds in whom this infection is often lethal.

Funding source: Research Council of Lithuania (Award № MIP-045/2015) and European Social Fund (Project № 09.3.3-LMT-K-712-01-0016).

MAL2

NEW CHLOROQUINE-FERROCENE HYBRIDS UPGRADED WITH AZATHIA HETEROCYCLE AS PROMISING ANTIPLASMODIAL AGENTS

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Background. As malaria treatment is impeded by increasing resistance to conventional drugs, discovering new antimalarial compounds that can overcome parasite resistance has emerged as a global scientific goal.

Objectives. The current study examined the antimalarial potential of twelve chloroquine(CQ)-ferrocene hybrids with additional azathia heterocycle, designed not only to target the cause of infection (*Plasmodium* parasites) but also to treat too extensive immune response that could lead to disease pathogenesis.
Material and Methods. *In vitro* evaluation of the antimalarial activity of the compounds was performed using the colorimetric lactate dehydrogenase assay in cultures of both 3D7, CQ-sensitive (CQ^S) and Dd2, CQ-resistant (CQ^R) strains of *P. falciparum*. Compounds were first screened at a concentration of 1000 nM (three replicates per compound for each strain) and those that showed a minimum of 50% parasite growth inhibition were further titrated to determine their IC_{50} value. CQ was used as a positive control. Before evaluation of the antimalarial potential of the selected compounds, all were tested for cytotoxicity on rat peritoneal macrophages and shown nontoxic at a concentration lower than 10 µM.
Results. Four (F1, F2, F3, and F7) of the twelve investigational compounds inhibited the growth of the CQ^R *P. falciparum* strain by 50% at a concentration of 1000 nM, whilst none exerted this effect against both strains. When titrated to determine IC_{50} values, two compounds F1 (171 nM) and F3 (260 nM) were more active than CQ (287 nM) against the CQ^R strain, whilst F2 (350 nM) and F7 (446 nM) did not surpass CQ.
Conclusion. Better *in vitro* activity against the CQ^R strain of two compounds is highly relevant, since the primary goal of new treatment options is to overcome parasite resistance. The next step in this study would be to evaluate their anti-inflammatory effects in several *in vitro* macrophage-based models.

MIGRANTS AND MIGRATING PARASITES

MIGR1

MOLECULAR EPIDEMIOLOGY OF BLASTOCYSTIS AMONGST SYRIAN REFUGEE COMMUNITIES LIVING IN NORTH LEBANON

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Background. Several geographical regions around the world such as the Middle East remain yet poorly explored regarding the molecular epidemiology of *Blastocystis*.
Objectives. We performed the first epidemiological survey ever conducted in the Syrian refugee population living in North Lebanon. Since molecular data regarding *Blastocystis* epidemiology were already available from the local Lebanese population, our second aim was to compare the subtypes (STs) and genotypes identified in the Syrian and local populations to investigate the circulation of the parasite between both communities.
Material and Methods. A total of 306 stool samples were collected from Syrian refugees living in 26 Informal Tented Settlements (ITS) subjected or not to Water, Sanitation and Hygiene (WASH) interventions in North Lebanon, then screened for the presence of *Blastocystis* by real-time polymerase chain reaction targeting the small subunit RNA gene followed by subtyping.
Results. The overall prevalence of the parasite reached 63.7% and *Blastocystis* colonization was not significantly associated with gender, age, symptomatic status, abdominal pain or diarrhea. In contrast, WASH intervention status of ITS was identified as a risk factor for infection. Among a total of 164 subtyped isolates, ST3 was predominant, followed by ST1, ST2 and ST10. No particular ST was reported to be associated with age, gender, symptomatic status or WASH intervention status of ITS. Intra-ST diversity of ST1 to ST3 was low suggesting large-scale anthroponotic transmission. Moreover, few ST1 to ST3 genotypes were common to the Syrian refugees and host populations.
Conclusion. The high prevalence observed in the Syrian cohort highlights the active circulation of the parasite in this population in link with poor sanitation conditions. *Blastocystis* is mainly transmitted through the inter-human route in the Syrian cohort and the circulation of the parasite between the refugee and host communities remains limited because of reduced contact between these two populations.

MIGR2

EVALUATION OF THE ALLPLEX™ GASTROINTESTINAL PANEL – HELMINTH(I) ASSAY FOR THE DETECTION OF HELMINTHS AND MICROSPORIDIA IN STOOL SAMPLES

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Background. Recently, the first commercial multiplex PCR detecting helminths, the Allplex™ Gastrointestinal Panel – Helminth(I) (GIPH) assay (Seegene), was introduced on the market. It targets *Ancylostoma duodenale*, *Ascaris* spp., *Enterobius vermicularis*, *Hymenolepis* spp., *Necator americanus*, *Strongyloides* spp., *Taenia* spp., *Trichuris trichiura* and 2 Microsporidia genera, *Enterocytozoon* and *Encephalitozoon*. This study aimed at comparing it to classical diagnostic methods.
Material and Methods. Stool samples (N=110) were selected from a prospective collection of routine samples analyzed from 2016 to 2020 at the Rennes University Hospital (France). All were positive for helminths (MIF concentration, Baermann/Harada-Mori methods) or for microsporidia (PCR), and an aliquot was stored at -80°C. This collection included: 8 *Ascaris* spp., 11 *E.vermicularis*, 13 hookworms, 21 *H.nana*, 35 *S.stercoralis*, 5

T.saginata/asiatica, 11 *T.trichiura*, 9 *E.bieneusi* and 1 *E.intestinalis*. Samples were suspended in FecalSwab™ medium and analyzed with the Allplex™ GIPH assay following manufacturer's instructions, using the MICROLAB® STARlet IVD (Hamilton) and CFX96 (Bio-Rad) devices. False negative results were re-tested with bead-beating pretreatment prior to extraction.

Results. Concordance was perfect for *Taenia* spp. (N=5) and microsporidia (N=10). False negative results were observed in 54% (6/13), 34% (4/11) and 20% (7/35), for hookworms, *E.vermicularis* and *Strongyloides* spp detection, respectively. For these targets, pretreatment poorly improved the results. *T.trichiura* detection was critically low without pretreatment, as only 9% (1/11) of the samples were positive, but reached 91% (10/11) with bead-beating pretreatment. Mechanical lysis was also needed for *Ascaris* spp. and *Hymenolepis* spp. to reduce false negative results from 1/8 and 1/21, respectively, to none for both.

Conclusion. Overall, with an optimized extraction process, the Allplex™ GIPH assay allows the detection of numerous parasites with roughly equivalent performance to that of microscopy, except for hookworms. However, the multiplex panel is not fully adapted to migrants and travellers, as it includes opportunistic Microsporidia but not *Schistosoma* spp.

Funding source: The PCR reagents were purchased from the Seegene Company at reduced price, but the firm did not take part in the writing of the manuscript, nor its submission.

MIGR3

ON CUTANEOUS LEISHMANIASIS AND TERTIAN MALARIA TRANSMITTED BY MIGRANTS

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Background. In the last decade, the number of migrants arriving from Asia and Africa, who come and leave our country (Bulgaria) on their way to Western Europe through the Balkan Peninsula, has not subsided. Cutaneous leishmaniasis and tertian malaria are transmissible parasitic diseases imported mainly from people coming from Afghanistan.

Material and Methods. Among migrants it was observed 36 cases of cutaneous leishmaniasis and 14 cases of tertian malaria for a period of 5 years. The etiological diagnosis was made in the laboratory of parasitology at the Medical University.

Results. It was diagnosed 36 cases of cutaneous leishmaniasis (with single skin ulcers, disseminated forms or in the stage of scarring). These patients were treated with fluconazole with good effect. All cases of malaria were late recurrences of tertian malaria, which was acquired in the country of origin. They were admitted to hospital and treated with chloroquine and primaquine. In addition to the characteristic fever, only two patients had anemia. In all diagnosed and treated cases severe splenomegaly was observed by ultrasound.

Conclusion. The presence of phlebotomies in the country provides a favorable opportunity for local distribution of cutaneous leishmaniasis, although such cases have not been registered so far. The presence of *Anopheles* mosquitoes in South Bulgaria is a prerequisite for epidemic spread of tertian malaria, if there is no timely diagnosis and radical treatment, especially in the period from April to October. Given the conditions of observed global warming, this risk period will be extended.

A ONE HEALTH APPROACH TO MANAGE PARASITIC INFECTIONS

OH1

COLLECTION OF PROTOCOLS FOR THE DETECTION AND CHARACTERISATION OF SHIGA TOXIN-PRODUCING *Escherichia coli* (STEC), ENTEROTOXIGENIC *E. coli* (ETEC), *Cryptosporidium* spp. AND ANTIMICROBIAL RESISTANCE (AMR) IN *Salmonella* AND *Campylobacter*

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Background. OH-Harmony-CAP (One Health Harmonisation of Protocols for the Detection of Foodborne Pathogens and antimicrobial resistance [AMR] Determinants) is a 3-year project that is part of the One Health European Joint Programme. It aims to 1) collect information on current capabilities, capacities, adaptabilities and interoperabilities, at both the National Reference Laboratory (NRL) and the primary diagnostic level, across Europe by developing an in-depth OHLabCap survey; and 2) to quantitatively describe current and best practices and to develop harmonized protocols.

Aim. The Work-Package (WP) 4 of OH-Harmony-CAP is devoted to the collection, assessment and ranking of protocols for the detection and characterization of model organisms, with the aim to propose harmonised procedures. The selected protocols under study are detection and characterization of Shiga toxin-producing *Escherichia coli* (STEC), Enterotoxigenic *E. coli* (ETEC), *Cryptosporidium*, and determination of AMR in *Salmonella* and *Campylobacter*.

Methods. Protocols were collected from different networks (OH-Harmony-CAP partners, NRLs and other laboratories). Groups of experts for each model organism were formed. Evaluation tables summarizing protocols, and a method for ranking them were developed.

Results. Overall, 23 institutions submitted their laboratory protocols. The files shared included different types of documents, such as standard operating procedures, published works, standard methods, and general information on the protocols applied. The groups of experts filled in the evaluation tables designated to each pathogen. The next step is a comparison of the collected protocols and proposing a harmonized procedure for each model organism.

Funding source: This work is part of OH-Harmony-CAP project, funded by the European Union's Horizon 2020 Research and Innovation programme under grant agreement № 773830: One Health European Joint Programme.

OH2

HUMAN FASCIOLOSIS – A NEGLECTED, EMERGING PARASITIC DISEASE IN EUROPE

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Background. Fasciolosis is a One Health problem. Globally, the distribution of human fasciolosis (HF) caused by *Fasciola hepatica* reflects the distribution of animal fasciolosis. In Europe, autochtonous cases of HF have

recently been reported, including one case in Denmark and two cases in Belgium. In non-endemic areas, clinicians may not be familiar with this infection, and there is usually a long disease duration before the diagnosis. Identification of cases is challenged by the absence of pathognomonic symptoms as well as unavailability of diagnostics. We therefore speculate that the actual number of HF cases could be much greather than that reported.

Material and Methods. Here, we analysed published data on animal and human fasciolosis in the region of former Yugoslavia (Bosnia and Herzegovina, Serbia, Croatia).

Results. Fasciolosis is not notifiable, which is why it is difficult to estimate the incidence. Animal fasciolosis caused by *F. hepatica* is enzootic to at least some parts of the region. Sheep and cattle are the main reservoir of *F. hepatica*, and the intermediate host is *Lymnaea truncatula*. Over the two last decades, numerous reports have demonstrated the presence of *F. hepatica* in small and large ruminants in the region. Between 1928 and 1964, 25 cases of HF were reported, mostly from Croatia. Esteban *et al*. (1998) reported of 2,951 cases of HF in Europe over a 25-year period, including 4 cases in the region.

Results. The re-emergence of HF observed over the most recent years could be explained by climate change, changes in food habits, migrations, and resistance in anthelminthic therapy. This new trend requests increasing awareness among clinical microbiologists and infectious disease specialists with regard to autochthonous HF, and should be addressed using a One Health approach.

This paper have been presented in part at the XXII/XXIII Symposium of Epizootiologists and Epidemiologists, Belgrade, Serbia, 26 - 28 April 2021.

OH3

THE NECESSITY OF MONITORING RODENT HELMINTH COMMUNITIES IN LIGHT OF THE ONE HEALTH APPROACH

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Background. Because of globalization and urbanization, humans more frequently come into contact and cohabitate with wild animals, which leads to the possibility of pathogen transfer. Rodents commonly live near humans and domestic animals, and are well known natural reservoirs of zoonoses, including those caused by helminths. Climate changes can lead to zoonoses in previously non-endemic areas or in previously uninfected host species.

Material and Methods. Host samples were collected in areas such as picnic grounds, weekend settlements, and arable land. These are the places where humans and domestic animals can encounter infected animals or a contaminated environment, leading to pathogen transmission.

Results. During the study period, 11 host species from the families Muridae and Cricetidae were registered. Helminths were present in each host species, and five of them were infected with parasites of medical and veterinary importance. The total species diversity of helminths in Serbian rodents consists of 36 identified species. Seven of them have confirmed zoonotic potential: *Mesocestoides lineatus*, *Hymenolepis diminuta*, *H. nana* (*fraterna*), *Taenia martis*, *Hydatigena taeniaeformis*, *Calodium hepaticum* and *Moniliformis moniliformis*. The dominant helminths regarding the number of infected host species and occurrence sites were *H. diminuta*, *H. nana* (*fraterna*) and *M. lineatus*.

Conclusion. Human travel and commerce over large distances facilitates the spread of parasites and their hosts into areas where they were previously absent. Additionally, the encroaching of human settlements into natural habitats, coupled with climate change, leads to parasites invading new hosts, including humans. All the above necessitates regular monitoring of rodent populations, the parasites they carry, and their environment. Results of these analyses must be made available to physicians and veterinarians as evidence of parasite presence in a given area, which can allow experts to anticipate the occurrence of parasitic diseases in humans, livestock and/or pets.

OH4

PRELIMINARY STUDY ON THE PRESENCE OF ARGs IN *E. coli* STRAINS ISOLATED FROM STRAY CATS

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Background. Antibiotic resistance (AR) is now considered a zoonosis that needs to be addressed by a One Health approach that considers animal, human and environmental health interdependent. Several studies have shown that antibiotic-resistant bacteria and resistance genes are everywhere in nature. *Escherichia coli* is one of the microorganisms that can acquire and transfer resistance genes and, as it is part of the intestinal microflora of animals and humans, is also considered as an indicator of the evolution of antibiotic resistance. The study of the presence of bacterial strains harbouring AR genes (ARGs) in stray cats would be interesting to investigate the role of these animals to disseminate AR in the territory.

Objectives. The aim of our works was to investigate the presence of ARGs in *E. coli* isolated from rectal swabs of stray cats.

Material and Methods. For *E. coli* isolation, 48 rectal swabs collected in cats from one shelter and different colonies located in a province of northern Italy (Monza Brianza) were seeded on McConkey agar. Subsequently, the presence of ARGs was assessed by PCR, following previously published protocols.

Results. From 48 rectal swabs analysed, *E. coli* were isolated in 19 (39.6%). 85% of these harboured one or more of the genes investigated: 12/19 carried the bla_{TEM}, 9/19 the *tet(A)* and 3/19 the *sulIII* genes. No strains were found to harbour the bla_{CTXM} and *qnrS* genes. Only one cat in which *E. coli* was isolated had an history reporting recent antibiotic treatment (doxycycline and amoxicillin plus clavulanic acid).

Conclusion. These preliminary data show the presence of *E. coli* harbouring ARGs in stray and shelter cats that rarely have received antibiotic treatment. Following a One Health approach, further studies will be conducted both to deepen the knowledge of antibiotic resistance in microorganisms from these animals and to study their resistance mechanisms.

OTHER ECTOPARASITES: FROM BIOLOGY TO CONTROL

ECTO4

BIODIVERSITY AND WEST NILE VIRUS: UNDERSTANDING THE ECOLOGY OF VIRAL CIRCULATION IN NORTHEASTERN ITALY

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Background. The recent spread of West Nile Virus (WNV) has been related to changes in environmental conditions, leading to increases in its vectors abundance and distribution. The virus has become endemic in northeastern Italy since 2008, with cases re-occurring in non-overlapping areas. The study aims at exploring the effect that biodiversity of hosts and vectors might have on the occurrence of WNV.
Material and Methods. Indices of biodiversity were calculated for wild birds and mosquitoes for 2010-2018, using population data available at the Italian League for Bird Protection and information collected during WNV entomological surveillance. A Maximum Entropy approach was used to study the effect of variations of mosquito and bird diversity on the geographical pattern of WNV occurrence.
Results. Significantly higher Shannon-Wiener's diversity indices for wild birds were obtained for 2015 and 2018 with respect to 2012 ($p=0.04$). Variations in the relative abundance of the five dominant species indicated fluctuations in the bird community throughout the study period. *Cx. pipiens* always resulted being the most dominant mosquito species; mosquito diversity was significantly higher for 2017 vs 2014 only ($p=0.03$). Bird diversity had limited effects on WNV presence; while lower mosquito diversity was associated with increasing occurrence of the virus.
Conclusion. The fluctuations in bird diversities indicated potential changes in the migratory patterns of wild birds and/or variation in the local community structure, although with limited impact on the probability of finding the virus. The inverse effect of mosquito diversity on the pattern of occurrence of WNV stressed the importance of *Cx. pipiens* as vector, although the results also suggested that other mosquito species could be involved in WNV dynamics. The study is still at a preliminary stage; the results lead to future extensive analyses including higher spatial and temporal details also accounting for the prevalence of WNV in wild birds.

ECTO5

TICKS VERSUS ECOSYSTEM ENGINEERING OF HEAVY-WEIGHT ANIMAL SPECIES

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Background: Recent studies indicate that change of landscape, corelating with microclimatic properties, can greatly influence tick populations. Ecosystem engineers, species which play a significant role in the active change of habitat they inhabit, can be the agents of such change. We are studying the impact of some of such ecosystem engineer species on tick populations co-inhabiting the same areas.
Objectives: To assess the overall influence of animal-induced changes of landscape on the tick populations in selected study sites inhabited by semi-feral, large, hoofed animal species.
Material and Methods: Since May 2019, a quantitative study of live tick samples is ongoing. The ticks are collected by flagging on selected transects in semi-feral enclosures in central Czech Republic during the seasons of high tick activity. Each study site covers the area of approximately 300 m^2. The selected transects include both the pastured landscapes with currently active animal herds and the non-pastured landscapes where the animals are not yet allowed to enter, and which are kept in a wild state.

Results: There are preliminary results available. From the 634 collected live ticks: 83 (13%) were located on pastured, engineered landscapes with active animal herds and 551 (87%) were located on the non-pastured, wild landscapes. Initial statistical analysis has been carried out using the paired sample T-test with with α=0.05; df=21; t=7.2878 and p<0.0001. This result supports the hypothesis that there is a substantial statistical difference in the means of tick numbers between the individual landscapes.
Conclusion: The preliminary results indicate that the influence of landscape change, induced by large animal activity, on the populations of live ticks is indeed substantial. This information could be useful for future preventative measures against tick overpopulation or for greater understanding of the complex tick ecology in European fragmented landscape.

ECTO8

MONITORING OF INVASIVE MOSQUITO SPECIES IN PALERMO (ITALY)

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Background. Culicides are vectors of high health relevance being able to transmit several pathogens, including zoonotic agents.
Objectives. Monitoring was carried out to investigate the introduction of mosquito invasive species and assess the risk of indigenous spread of viruses transmitted by these species.
Material and Methods. Entomological catches were carried out on a fortnightly basis from April to November 2016 in 5 sites in the metropolitan City of Palermo (Port of Palermo, Falcone-Borsellino Airport, Orleans Ornithological Park, a plant nursery in an urban context and a Seaside Club near Palermo), through BG-sentinel traps, Universal Trap and ovitraps. Culicides identification was carried out by morphological keys, PCR Barcoding and sequencing. Non-parametric tests were used for data analysis.
Results. A total of 201 adult and 740 egg catches were collected. The following species were found: *Culex pipiens*, *Culiseta longiareolata* and *Aedes albopictus*, this last one representing the only detected invasive species. All the species followed a similar trend in all the monitored sites, with an increase of mosquito density from spring to summer, followed by a reduction in autumn. In the Orleans and Vivaio sites, *Ae.albopictus* species was found to a greater extent than *Cx.pipiens*. Eggs also followed the same trend with a significant peak in the summer season.
Conclusion. The analysis showed an increased risk for autochthonous spread of new pathogen during the summer season due to the greater density of vectors, coinciding with the higher tourist flows that could facilitate the introduction of new viruses and with the intensified outdoor activities of people which increases exposure.
The high vector density also in the Orleans and plant nursery sites also suggests expanding the monitoring network in sites where conditions are particularly favourable for vector colonization.

Funding source: Financed by CCM 2014 and RC IZS SI 03/19 – Italian Ministry of Health

ECTO9

DISTRIBUTION OF *Aedes (Stegomyia) aegypti* AND *Aedes (Stegomyia) albopictus* (DIPTERA: CULICIDAE) AND THEIR CONTROL IN RUSSIA

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In the last 20 years, two tropical species of mosquitoes - *Aedes (Stegomyia) aegypti* (L.) and *Aedes albopictus* (Skuse) actively settled in Americas, Australia, and Europe. Both species are vectors of many arboviral diseases, such as yellow, dengue, Chikungunya, and Zika fevers. In the territory of the former USSR *Ae. aegypti* mosquitoes found since 1911 in the settlements of the Black Sea coast (Abkhazia and Georgia) and existed there until the 1950-1960s. After the extermination measures *Ae aegypti* mosquitoes recovered and were identified in Sochi in 2000-2004. *Ae. albopictus* mosquitoes was first registered in Russia (Sochi) in 2011. Currently, this species has distributed along the Black Sea coast of Abkhazia to Crimea and also settled inland from the coast to the

altitude of 600 meters above sea level. These two species are synanthropic and live in settlements, both rural and urban. It is shown that *Ae. albopictus* exhibits higher ecological plasticity at egg, larval and adult stages in relation to temperature and other environmental factors than *Ae. aegypti*. An integrated control program for these species has developed. For the first time in Russia, a series of experiments carried out to establish the susceptibility level of *Ae*. *aegypti* and *Ae. albopictus* to insecticides (OPs, carbamates and pyrethroids), as well as biological preparations based on *Bacillus thuringiensis israelensis*. A complex of pest control measures against these mosquito species has been developed, in both places, where larvae and pupae are breeding, and the habitats of adult mosquitoes, depending on the degree of their anthropogenicity. A list of compounds effective against these mosquito species has compiled. The invasion of *Ae. aegypti* and *Ae. albopictus* into new regions requires constant monitoring of their population size, identifying and eliminating potential habitats, reducing their populations to the minimum and protecting people from their attacks.

ECTO10

COMPLEXITY OF WEST NILE VIRUS VECTOR *Culex pipiens* POPULATION DYNAMICS IN NORTHEASTERN ITALY

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Background. In the last decade, West Nile Virus (WNV) showed an increasingly wider spread in Italy and in particular in the northeastern Regions. Due to the complexity of the environment-host-vector-pathogen interaction, and the incomplete understanding of the epidemiological pattern of the disease, WNV occurrence can be hardly predictable in time and space. The analyses of ecological drivers affecting the vector population are central in the study of WNV transmission dynamics.

Material and Methods. The variability in *Culex pipiens* population dynamics was analyzed using environmental and climatic data collected through the WNV entomological surveillance program in the northeastern Italian Regions. The study was based on a series of generalized linear mixed models accounting for an Information-Theoretic approach and model-averaging algorithms, to assess the relationship between seasonal mosquito growth rates and intrinsic and extrinsic predictors. The approach allowed identifying the most significant combinations of variables outlining the *Cx. pipiens* population dynamics, also indicating the level of uncertainty of the results.

Results. Several environmental and climatic factors had significant impact on the mosquito population dynamics, with population density (used as proxy for intraspecific competition) and length of daylight being the predominant drivers. The results obtained for each year were then compared to detect any inter-annual difference in coefficients magnitude, sign, and significance. These outcomes indicate that different combinations of factors might have distinctive, sometimes divergent, effects on mosquito population dynamics.

Conclusion. This study stressed the marked complexity in the response of mosquito populations to combinations of extrinsic and intrinsic drivers; in fact, short-term fluctuations in climatic/environmental parameters had divergent effects of population dynamics. A more realistic acquaintance of the mechanisms driving mosquito population variations could be paramount to improve WNV surveillance activities and to implement effective early detection programs.

THE MICROBIOME OF PARASITES AND ROLE IN DISEASES

MBIOM1

PRELIMINARY CHARACTERIZATION OF THE MICROBIOTA OF ADULT *Fasciola hepatica* FROM BOVINE AND OVINE HOSTS

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Background. *Fasciola hepatica* is a foodborne zoonotic parasite, which is maintained in an indirect life cycle between an invertebrate host (snails) and a vertebrate host (mainly large-size herbivorous species commonly raised as livestock). The juvenile worms are released in the vertebrate host duodenum after metacercariae ingestion, and rapidly penetrate the gut wall to migrate towards their definitive location, the intra-hepatic biliary ducts. During this migration, *F. hepatica* may interact not only with the host tissues, but also with its microbial communities, as it has been shown for other helminths. Nevertheless, to the best of our knowledge, the *F. hepatica*-associated microbiota has never been explored.

Objectives. This study aimed to characterize the bacterial communities associated with the adult stage of *F. hepatica*, and analyze the potential influence of the hosts species in its composition.

Material and Methods. *F. hepatica* adults were isolated from bovine and ovine hosts. Each worm was longitudinally divided in two halves; one portion was incubated with antibiotics and washed in sodium hypochlorite, while the other half was left untreated. Total DNA was isolated from each sample and prokaryotic 16S rRNA gene amplicon sequencing (V3-V4 region) was performed on the MiSeq platform (Illumina). Sequence data were processed using QIIME2 and statistical analyses performed with MicrobiomeAnalyst.

Results. Rarefaction curves showed that all microbial diversity was captured and maintained following data curation. Retained sequences were assigned to 4 bacterial phyla and 18 genera, whilst a substantial percentage remained unclassified (53,47%±8,40, at phylum level). The phylum *Firmicutes* was the most abundant in all samples (39,31%±8,17), followed by *Proteobacteria* (7,12%±2,56). At genus level, *Brevibacillus* (23,17%±4,77) and *Bacillus* (15,98%±3,63) were predominant in all samples, followed by *Methylobacterium/Methylorubrum* (3,60%±1,29) and *Escherichia/Shigella* (2,1%6±2,78). Notably, microbial communities clustered by host species using PCA, whilst antibiotic/bleach treatment showed little effect on overall microbial profiles. Moreover, microbial richness was significantly higher in specimens collected from cow compared to those from sheep, although no significant differences were detected in alpha diversity (Shannon index) between groups.

Conclusion. Our preliminary results point towards a possible link between the microbiota of *F. hepatica* adults and their vertebrate hosts. However, further investigations using additional replicates are needed to confirm these findings and assess their biological meaning.

MBIOM4

GUT MICROBIOME DIVERSITY IN RELATION TO *Blastocystis* AS A SINGLE OR MULTI-PARASITIC INFECTIONS

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Background. Microorganisms including bacteria, fungi and protozoa co-occur and continuously evolve in the human gut since millions of years. Variations in the composition of the human gastrointestinal microbiome have been postulated to effect gut health conditions.

Objectives. To get the full picture of gut microbiome diversity in correlation to *Blastocystis* spp. either as a single parasite or with multi-parasitism.
Material and Methods. Parasite-positive stool samples were collected from Egyptians with gastrointestinal (GI) symptoms. Amplicon sequencing of DNA extracted from stool samples for gut bacteria (targeting the 16S rRNA gene), gut eukaryotes, parasites and fungi (targeting the 18S rRNA gene and internal transcribed spacer (ITS) sequence) was done for bacterial and eukaryotic profiling, through analysis of BION data with graphical presentation- principal coordinate analyses (PCoA). Shannon' s diversity index was used as a measure of diversity, as well as the diversity of both the bacterial and eukaryotic diversity in correlation with patient related data, including age, residency (urban/rural), stool consistency and gastrointestinal (GIT) symptoms.
Results. There is a higher Shannon bacterial diversity in *Blastocystis*–positive patients with or without *Entamoeba* parasites than *Blastocystis*-negative individuals. There is an interesting variation with statistical significance when comparing bacterial and eukaryotic diversity in relation to age (child/adult) of patients, GIT symptoms, particularly more in patients with abdominal pain than patients with diarrhea.
Conclusion. Based on our findings, *Blastocystis*-associated protozoa and/or fungi may have a role in the presence of the bacterial diversity or vice versa. Among study individuals, patients' age and GIT symptoms may have a role in both bacterial and eukaryotic diversity. Experimental animal research to investigate whether there is a certain group of bacteria or metabolites critical to *Blastocystis* is essential to understand the full picture of gut microbiome.

MBIOM5

CHANGES IN FAECAL MICROBIOTA OBSERVED IN BALB/C INTERFERON-GAMMA KNOCK-OUT MICE UPON ORAL INFECTION WITH *Toxoplasma gondii* OOCYSTS

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Background. Microbial eukaryotes are increasingly recognized as potentially important modulators of microbiome communities. Little is known regarding the potential interplay between a systemic *Toxoplasma gondii* infection and the microbiota of the gastrointestinal tract.
Material and Methods. We investigated the microbial diversity of six BALB/c interferon-gamma knock-out mice before and after oral infection with *T. gondii* oocysts. Forty-five faecal samples (mean no. of samples/mouse, 7.5; range, 5−8), collected every other day starting three days before the infection, were subject to DNA extraction and amplicon-based next-generation sequencing of 16S and 18S ribosomal DNA for detection and differentiation of bacteria, parasites and fungi.
Results. Preliminary comparison of results from faecal samples before and after the infection indicates a relatively stable faecal microbiota at the phylum level, with the Firmicutes: Bacteroidetes ratio changing from 1.6 to 2.6 and an increased abundance of *Bacteroides* and *Parabacteroides* starting five days after infection. DNA of *T. gondii* was not detected in the faecal samples.
Conclusion. The observations from this study add to the knowledge on *T. gondii* infection and microbiome of the gastrointestinal tract.

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MBIOM6

MICROBIAL COMMUNITY STRUCTURE ASSOCIATED WITH *Coregonus lavaretus* AND CESTODES PARASITIZING THEIR DIGESTIVE TRACT

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Background. Sympatric pair of whitefish which inhabits Teletskoye Lake in the Altai Mountains has a different feeding habits. *C. l. pravdinellus* feeds on zooplankton, whereas the diet of *C. l. pidschian* is based on benthic prey. The aim of the study was to identify composition and structure of microbial community of the digestive tract of sympatric pair of whitefish from Teletskoye Lake (West Siberia, Russia) and their gut parasites *Proteocephalus* sp.
Material and Methods. Fish and cestodes were collected in the north part of Teletskoye Lake (51.79 N; 87.30E). Microbial communities associated with the cestodes from gut of *C. l. pidschian* and *C. l. pravdinellus* were studied using next-generation high-throughput sequencing of the 16S ribosomal RNA genes.
Results. The dominant phyla in associated microbiota of the cestodes extracted from intestine of *C. l. pidschian* and *C. l. pravdinellus* were represented by Proteobacteria and Tenericutes, respectively. At the genus level the microbiota of cestodes parasitizing the intestine of *C. l. pidschian* were dominated by *Rickettsiella*, *Mycoplasma*, and unclassified bacteria from Aeromonadaceae and Enterobacteriaceae families. The microbiota of cestodes parasitizing the intestine of *C. l. pravdinellus* were dominated by *Mycoplasma* and *Acinetobacter*.
Conclusion. These findings expand our current knowledge regarding the relationships between microbial communities of gut parasites (*Proteocephalus* sp.) and their sympatric host (whitefish).

Funding source: This work was supported by the Russian Science Foundation, project № 19-74-10054

MBIOM7

MICROBIAL COMMUNITY STRUCTURE IN A HOST-PARASITE SYSTEM: THE CASE OF PRUSSIAN CARP AND ITS PARASITIC CRUSTACEANS

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Background. The aim of the present study was to investigate the microbial community of skin mucus of infected and uninfected Prussian carp caused by parasitic crustaceans from the genus *Argulus foliaceus* and *Lernaea cyprinacea* in an eutrophic lake with parallel studying of associated microbiota of their parasites and environmental compartments.
Material and Methods. Prussian carp *Carassius gibelio* (Linnaeus 1758) and their ectoparasites were collected in the area of Malye Chany Lake in west Siberia (Russia, 54036’56.3’’N, 78012’5.9’’E). Associated microbiota of skin of Prussian carp and ectoparasites were investigated by sequencing of the V3, V4 hypervariable regions of 16S rRNA using Illumina MiSeq sequencing platform.
Results. In the microbial community associated with the parasitic crustaceans *Argulus* sp. and *Lernaea* sp., along with representatives of the normal microbiota, there were identified microorganisms that could be potential agents of infectious diseases in fish (*Flavobacterium* sp., *Aeromonadaceae* sp., *Corynebacterium* sp. and *Streptococcus* sp.). Each parasite is characterized by a specific structure of its associated microbiota, which, apparently, may indicate their role as vectors of different infectious disease. Significant perturbation of dominant microbiota of skin mucus of unhealthy fish in comparison with healthy fish was registered (ADONIS, $p \leq 0.05$).
Conclusion. Results from these studies indicate that ectoparasites have the potential to alter skin microbiota, which can play a possible role in transmission of secondary bacterial infection in fish, caused by pathogenic bacteria.

Funding source: This work was supported by the Russian Science Foundation, project № 17-74-10054

MBIOM8

THE GUT MICROBIOTA OF *Cystidicola farionis* PARASITIZED THE SWIM BLADDER OF THE CHARR *Salvelinus schmidti* IN LAKE KRONOTSKOE (KAMCHATKA, RUSSIA)

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Background. The aim of the present study was to analyze the bacterial diversity associated with the gut and body of *Cystidicola farionis* parasitizing the swim bladder of the different morphs of the nosed charr *Salvelinus schmidti*.

Material and Methods. *Cystidicola farionis* from the nosed charr *Salvelinus schmidti* were collected in the littoral zone of the Lake Kronotskoe. Associated microbiota of the gut microbiota of *Cystidicola farionis* were investigated by sequencing of the V3, V4 hypervariable regions of 16S rRNA using Illumina MiSeq sequencing platform.

Results. The common dominant microbiota of the gut and body of nematode were represented by *Aeromonas*, *Pseudomonas*, *Shewanella*, and *Yersinia*, while the associated microbiota of the swim bladder of the nosed charr was dominated by *Acinetobacter*, *Cetobacterium*, *Pajaroellobacter*, *Paracoccus*, *Pseudomonas*, *Shewanella*. By comparing the associated microbiota of nematode parasitizing the different morphs of the nosed charr the difference in richness estimates (number of OUT's and Chao1) were revealed between the N1g and N2 morphs.

Conclusion. For the first time the microbial communities of the nematode gut and body, as well as microbiota of the swim bladder of fish were analyzed using a next-generation sequencing approach. Moreover, increasing our knowledge of gut-associated microbiota of fish nematode parasites will help to elucidate more details of microbe-parasite relationships.

Funding source: This work was supported by the Russian Science Foundation, project № 19-74-00104

PROTOZOAN INFECTIONS IN LIVESTOCK AND THEIR CONTROL – ZOONOTIC AND ANIMAL HEALTH ASPECTS

PROTLS1

EXPLORATION OF NOVEL NUCLEOSIDE ANALOGUES AS PROMISING TREATMENT FOR VETERINARY TRYPANOSOMIASIS

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Background. Animal African Trypanosomiasis (AAT) is a widespread disease caused by *Trypanosoma spp.* and has devastating effects on animal husbandry due to the scarcity of efficient drugs and development of drug resistance, emphasizing the need for novel treatment options.
Objectives. We previously identified tubercidin analogues as highly potent trypanocides with curative activity in both stage 1 and stage 2 of mouse models of Human African Trypanosomiasis (HAT). We now provided an in-depth evaluation of new nucleoside analogues *in vitro* and *in vivo* against a broad range of AT species.
Material and Methods. A library subset of 93 antitrypanosomal nucleosides was evaluated for cytotoxicity and *in vitro* activity against relevant AT species *i.e. T. brucei*, sensitive and isometamidium (ISM)-resistant *T. congolense*, *T. vivax*, *T. evansi* (type A and B) and *T. equiperdum*. *In vitro* metabolic stability and *in vivo* activity in the late curative mouse models were evaluated for selected 'lead' compounds.
Results. Our analyses resulted in the identification of 4 promising compounds with confirmed *in vitro pan*-AT activity (Table 1), all metabolically stable in the target species (*i.e.* bovine, horse). Among these nucleoside analogues, analogue 4 was highly active in *T. vivax*, *T. congolense*, *T. evansi* and *T. brucei* late curative mouse models.

Table 1. *In vitro* screening results of confirmed nucleoside leads [IC_{50}(µM)]

Compound	MRC-5	PMM cytotoxicity	*T. brucei*	*T. vivax*	*T. congolense*	*T. congolense* *ISM-R*	*T. evansi (type A)*	*T. evansi (type B)*	*T. equiperdum*
Analogue 1	>64	>64	0.04	0.06	0.07	0.26	0.03	0.03	0.1
Analogue 2	>64	>64	0.49	0.004	0.04	0.43	0.76	0.01	0.04
Analogue 3	>64	>64	0.03	0.03	0.18	0.14	0.1	0.01	0.02
Analogue 4	>64	>64	0.03	0.05	0.1	0.18	0.03	0.01	0.02

Conclusion. We evaluated the *in vitro* and *in vivo* activity of novel antityrpanosomal agents against a broad range of AT species. Analogue 4 represents a potent chemotherapeutical candidate for treatment of animal trypanosomiasis.

PROTLS2

COMPARATIVE STUDY OF THE SEROLOGICAL TESTS USED FOR THE DIAGNOSIS OF *Toxoplasma gondii* INFECTION IN DOMESTIC PIGS

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Background. *Toxoplasma gondii* is the third most important foodborne parasite worldwide. It is estimated that one third of human global population is chronically infected and that the consumption of *T. gondii* cysts through raw or undercooked pork meat is one of the main infection sources for humans. Thus, surveillance is recommended to implement control measures and prevent the infection in domestic pigs. Nevertheless, the diagnostic performance of the techniques used is not always known, which can lead to misdiagnosis.
Objective. To accomplish a comparative study of the most widely used serological tests in pigs.
Materials and Methods. Panels of sera from experimentally (n=202) and naturally (n=244) infected pigs were analysed by three commercial ELISAs (IDScreen®, PrioCHECK™ and Pigtype®). A tachyzoite based Western Blot was also employed with sera from experimentally infected pigs. The reference criterium used to classify sera as positive or negative was the result obtained by the majority of the tests and diagnostic performance was determined by TG-ROC analyses.
Results. All ELISAs and the Western blot showed moderate to perfect agreement (*k*=0.632-0.903) with differences observed in Se and Sp values for the cut-offs suggested by the manufacturers (IDScreen®: AUC=1, Se=98%, Sp=100%; PrioCHECK™: AUC=0.99, Se=100%, Sp=75%; Pigtype®: AUC=1, Se=99%, Sp=99%; WB: Se=92%, Sp=100%) with sera from experimental infections. However, the agreement notably decreased (*k*=0.363- 0.738) and the Se of IDScreen® was reduced (IDScreen®: AUC=0.96, Se=73%, Sp=100%; PrioCHECK™: AUC=0.96, Se=100%, Sp=72%; Pigtype®: AUC=1, Se=98%, Sp=97%) with sera from naturally infected animals.
Conclusion. The differences detected in the diagnostic performance among tests and regarding the panel of sera used evidence the need of harmonizing the serological tests routinely employed in pigs in order to obtain comparable and reliable results. Two additional in-house tests will be included in the comparative study (ELISA and IFI) and all tests will be readjusted if needed.

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PROTLS3

COMPARATIVE PROTEOMICS OF *Megatrypanum* SPECIES

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Background. The species of *Trypanosoma* are spread worldwide. According to the mode of transmission, they are divided into two large groups, stercorarian and salivarian trypanosomes. Members of numerous stercorarian group except the causative agent of Chagas disease, *Trypanosoma (Schyzotrypanum) cruzi*, are usually apathogenic for their hosts. Several stercorarian trypanosome species, all belonging to the *Megatrypanum* subgenus, were isolated in Croatia: *Trypanosoma melophagium* from sheep ked, *T. theileri* from cattle and *Trypanosoma* sp. from red deer. Although apathogenic and members of different subgenus than *T. (Schyzotrypanum) cruzi*, they belong to the same group of trypanosomes. With the development of technology, protein markers for pathogenic trypanosome species have been identified.
Aim. As there are no data about the host's immune response i.e. the appearance of antibodies in hosts infected with apathogenic species, the aim of this study was to compare the antigen from three different trypanosome species belonging to the *Megatrypanum* subgenus, determine their similarity, i.e. difference, and compare the obtained results with proteins of trypanosomes whose genomes were sequenced and stored in protein databases. The obtained results will provide insight into the probable reasons of their apathogenicity for their hosts.
Material and methods. Proteomic profile of stercorarian trypanosomes from Croatia was analyzed by two-dimensional gel electrophoresis (2DE) and mass spectrometry (precisely MALDI TOF/TOF) of the *in vitro* grown trypanosomes.
Results. Proteomic analysis showed marked difference between analyzed trypanosome flagellates in 283 protein spots. Based on the obtained MS and MS/MS spectra, databases were searched using Mascot version

2.1. revealing 268 low score and 15 successfully identified proteins of pathogenic trypanosomatid species: *Trypanosoma cruzi*, *Trypanosoma equiperdum*, *Trypanosoma brucei gambiense* and *Leishmania major*.
Conclusion. Comparative proteomic analysis of stercorarian trypanosome species isolated in Croatia revealed similarity with pathogenic species from the family Trypanosomatidae: *Trypanosoma cruzi*, *Trypanosoma equiperdum*, *Trypanosoma brucei gambiense* and *Leishmania major*.

PROTLS4

Giardia duodenalis PREVALENCE IN CATTLE IN LATVIA

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Background. *Giardia duodenalis* is a protozoa which is most often associated with water-borne outbreaks and calves are one of the main carriers of this parasite. Cattle farming is the main agricultural field in Latvia, thus it is important to assess the prevalence of *Giardia* in cattle in Latvian.
Material and Methods. A total of 32 dairy herds were visited and faecal samples from 973 cattle aged 1 day to 12 years old were collected *per rectum* during March – December, 2020. Animals were divided in 3 age groups (1-90, 91-730 and above 731 days old). A questionnaire was designed to collect information about the animal and herd management. The fluorescent microscopy for antibody labelled cyst/oocyst detection was used.
Results. Overall prevalence of *Giardia* in cattle was found 8.4% and at least one Giardia shedding animal was found in 87.5% of the herds. The significantly higher ($p<0.00001$) prevalence was observed in the youngest (16.4%), followed by the middle (6.8%) and the oldest (2.7%) animal age group. There were no significant differences ($p=1.4$) observed between male (11.7%) and female (8.0%) animals. In 16.0% of the animals diarrhoea was associated with *Giardia* infection ($p<0.001$). It was observed that in 100.0% of the infected herds, organic manure for field fertilization was used, while 10.7% of infected herds had some type of waterbody (such as lake or river) in the pasture used by cattle.
Conclusion. *Giardia* is commonly found in Latvian cattle herds and the highest prevalence in calves aged 1-91 day was found. Diarrhoea was significantly associated with *Giardia* infection in cattle. Field fertilization with organic manure could be a potential source of infection.

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PROTOZOA IN FOOD & ENVIRONMENT – METHODS USED IN DIFFERENT ENVIRONMENTAL MATRICES (WATER, SOIL, VEGETABLES, SHELLFISH…)

ENVIRO1

ASSESSMENT OF INDUSTRIAL FREEZING PROCESS EFFICIENCY ON *Cryptosporidium* OOCYSTS IN FOODS USING AN *IN VITRO* METHOD BASED ON CELL CULTURE COUPLED TO REAL-TIME qPCR

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Background. *Cryptosporidium* is one of the leading causes of gastroenteritis globally and is responsible for foodborne and waterborne outbreaks. For the foodborne transmission route, fresh produce (leafy greens, aromatic herbs) are the most reported foods in outbreaks worldwide. Since 2016, a standardized method is available to detect *Cryptosporidium* spp. in fresh leafy greens and berry fruits (ISO 18744). Such standards are essential to improve exposure assessment to *Cryptosporidium* in food. However, the proposed method suffers from some drawbacks including the inability to differentiate infective oocysts from dead ones. An *in vitro* method has been proposed to characterize the infective character of oocysts in lambs lettuce (Kubina et al., 2021). It implies: i) the seeding of cells' cultures directly with oocysts recovered from food; ii) the spontaneous excystation and release of sporozoites by the deposited oocysts; iii) the ability of the released sporozoites to penetrate, infect the cells and proliferate, as measured by real-time qPCR after 48h-culture (CC-qPCR).

Objectives. To assess if this method could be applied to other food matrices and could be used to determine the efficacy of representative industrial freezing process.

Material and Methods. Frozen matrices (parsley, raspberries) were artificially contaminated with different amounts of infective oocysts and the detection thresholds of the CC-qPCR assay were assessed. Artificially contaminated fresh matrices (10^5 oocysts/sample) were freezing-processed and the survival of the parasites after processing was characterized.

Results. The CC-qPCR based-method was able to reliably highlight the infective character of up to 20 oocysts initially seeded onto frozen parsley and raspberries, recovered and deposited on cells'culture (parasite proliferation in 100% of samples). Industrial freezing process was efficient to inactivate the parasites, although for some process, in case of high contamination level, some infective parasites could remain.

Conclusion. Freezing is an efficient control measure allowing to reduce contamination of fresh foods by *Cryptosporidium* oocysts.

Funding source: This work is supported by the Region Normandie and the UMT ACTIA PROTORISK within the "Surgelpro" project, which is promoted by Valorial, an agri-food competitiveness cluster.

ENVIRO2

DETECTION OF ANTIBODIES AGAINST SPOROZOITE CCp5A ANTIGEN REVEALS THE SOURCE OF INFECTION FROM TOXOPLASMOSIS OUTBREAK IN SÃO PAULO BRAZIL

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Background: Brazil has registered over the past 20 years the occurrence of several outbreaks related to the ingestion of oocysts in food, soil, or water. From March to May of 2019, a sudden increase of serologically diagnosed cases of acute toxoplasmosis was noted in São Paulo city. This unusual frequency was informed to the local Epidemiological Surveillance (COVISA/SP). The notification of all acute toxoplasmosis cases led to an outbreak identification involving 83 cases. The outbreak investigation aiming to identify the source of contamination and included PCR analysis of samples from the local food (vegetables, spices, fruits, meat)

as well as water. However, the presence of *T. gondii* was not detected in the implicated possible sources of infection.

Material and Methods: twenty-eight sera samples were collected from patients on the occasion they sought the eye care service from the Federal University of São Paulo. The presence of antibodies against CCP5A (*T. gondii* sporozoite antigens) was evaluated by ELISA in sera samples of those patients.

Results: individuals ophthalmologically evaluated presented with fever (93%), severe myalgia (78%) persistent headache (77.5%), and the onset of swelling in lymph nodes, especially in the neck region. Five patients presented with Retinochoroiditis (RC) between 1 and 5 months after exposure. From the 28 serum samples analyzed in the present study, 93% were *T. gondii* IgM positive and 100% were IgG positive, 82% were positive for IgG-CCp5A. All the patients with RC presented antibodies against CCP5A

Conclusion: The oocyst-borne nature of this outbreak was revealed by the presence of antibodies against CCP5A in the patient's sera. The discrimination for different routes of *T. gondii* transmission in people has important consequences for future epidemiologic studies including that investigating pathogenicity in the context of the route of infection which remains to be clarified.

PARASITE TAXONOMY, SYSTEMATICS AND PHYLOGENY IN THE MOLECULAR ERA

SYST1

DIVERSITY AND TAXONOMY OF EURASIAN *Triaenophorus* spp. (CESTODA, BOTHRIOCEPHALIDEA: TRIAENOPHORIDAE) BASED ON PARTIAL *cox*1 mtDNA GENE SEQUENCES

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Background. Cestodes of the genus *Triaenophorus* are common parasites of different fishes (esocids, percids, salmonids, etc.) in the Holarctic. Taxonomic models of different authors, based on morphological and ecological-biogeographic characters, suggest the presence of two to five species of this genus in Eurasia. Our study is focused on the verification of the most correct model from among three different competing taxonomic models that exist for these pikeworms. We have applied molecular genetic data based on partial cox1 mtDNA gene sequences since the classical morphological methods of research do not provide an adequate solution in favor of one of them.
Material and Methods. Samples were collected from a vast area from the northwest of Russia to the Russian Far East. The species affiliation of the cestodes was annotated in accordance with Kuperman's description (1968, 1973). The c*ox*1 gene was amplified using the primers described by Steenkiste et al. (2015). Phylogenetic reconstruction within the genus *Triaenophorus* was performed using the Bayesian inference approaches with MrBayes v.3.2.1.
Results. A total of 61 specimens of *Triaenophorus* spp. from different fish species and waterbodies of Eurasia were examined. Based on *cox*1 gene sequences, the studied pikeworms were distributed among five species-level clades: *T. amurensis*, *T. crassus*, *T. meridionalis*, *T. orientalis* and *T. nodulosus*. The posterior probability values for all species-level clades obtained by the BI method were not less than 0.95. The species-level haplogroups are distinctly separated in the haplotype network.
Conclusion. From this work it can be concluded that there are significant genetic differences among the five species of the genus *Triaenophorus* which are taken into account by the taxonomic model of Kuperman (1968): *T. amurensis*, *T. crassus*, *T. meridionalis*, *T. nodulosus* and *T. orientalis*. Thus, these five species previously described are recognized as valid in accordance with the genetic analyses from this study.

Funding source: This research was supported by the Russian Foundation for Basic Research (project № 19-34-60028)

SEE DIROFILARIA & OTHER EMERGING VECTORBORNE ZOONOSES

SEEDIF1

AN AUTOCHTHONOUS CASE OF FELINE THELAZIOSIS IN NORTH MACEDONIA

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Background. The Oriental eye worm, *Thelazia callipaeda* (Spirurida: Thelaziidae) is a zoonotic nematode vectored by lacrimophagous drosophilid flies of the genus *Phortica*. The primary hosts are the wild and domestic canids, with natural infections reported in felids, mustelids, and lagomorphs. Domestic dogs and cats are the most important reservoirs of *T. callipaeda* for human infections, although cats are not considered as a typical host because of their cleaning habits.
Material and Methods. Two whitish, filiform parasites were collected from the right eye of a stray cat that was referred to the University Veterinary Hospital at the Faculty of Veterinary Medicine in Skopje in May 2021 with unilateral conjunctivitis and epiphora. After local anesthesia, the parasites were removed, washed in physiological saline solution, fixed in 70% ethanol and sent to the Laboratory for parasitology and parasitic diseases at the Faculty of Veterinary Medicine in Skopje for morphological identification. The parasites were identified by the position of the vulva in females (located in the anterior half of the body and anteriorly to the oesophageal-intestinal junction), and by the number and position of postcloacal papillae (5 pairs on the ventral surface of the body) and spicule shape and size in males (unequal spicules).
Results. The parasites (1 male and 1 female) were identified as *T. callipaeda*. The female was 1.52 cm long with body width of 410 µm, and the male was 1.09 cm long with body width of 380 µm.
Conclusion. To the best of our knowledge, this is the first report of *T. callipaeda* infection in a cat in North Macedonia. The finding indicates that there is a transmission cycle of *T. callipaeda* and that practitioners should include the Oriental eye worm infection amongst the differential diagnosis of ocular diseases in cats.

SEEDIF2

***Dirofilaria repens* IN DOGS IN SLOVENIA**

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Background. Until the last decade of the 20th century, dirofilariasis in dogs occurred mainly in southern European countries. The introduction of the Pet Travel Scheme in 2000 contributed to the spread of dirofilariasis by facilitating the movement of infected, microfilarial dogs from endemic areas throughout Europe. The prevalence of *Dirofilaria repens* in dogs in Slovenia has not been studied so far. Therefore, the aim of the present study was to estimate the prevalence of *D. repens* in dogs in Slovenia.
Material and Methods. A statistically representative number of 465 dogs older than one year and born in Slovenia were recruited between April and October 2018. Epidemiological data were collected and blood samples were taken. Real-time PCR was performed on all samples to detect filarioid DNA, and *D. repens*- and *D. immitis*-specific real-time PCRs were performed on positive samples. Blood samples from 446 dogs were tested for *Dirofilaria* spp. using a modified Knott's test. Descriptive statistics were used to characterise the sample.
Results. Three out of 465 (0.64 %) dogs tested positive for *D. repens* by species-specific real-time PCR, whereas *D. immitis* DNA was not detected. Two of the three PCR-positive dogs were also positive in the modified Knott's test. Two of the three positives never travelled outside the country suggesting autochthonous infection.
Conclusion. We conclude that the prevalence of *D. repens* in Slovenian dogs is rather low. Detailed epidemiological mapping of dirofilariasis is important to develop a rational approach to prevention of the disease and thus reduce the risk of human infections. We believe that the results of our study add important data to the European epidemiological map of the disease.

SEE TOXOPLASMOSIS

SEETOX1

SEROPREVALENCE OF HUMAN TOXOPLASMOSIS IN BULGARIA AND CLINICAL PRESENTATION OF THE DISEASE

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Background. Toxoplasmosis is one of the most common parasitic infections in Bulgaria. The diagnostic algorithm includes serological tests performed in specialized laboratories.

Objectives. Aim of the study was to establish the seroprevalence of toxoplasmosis in the country and its clinical presentation for 5-year period.

Material and Methods. Data from the annual reports of the Regional Health Inspectorates (RHI) in the country and from the National reference laboratory (NRL) Diagnosis of parasitic diseases were used. The different classes of antibodies were determined by ELISA and CLIA methods, and in NCIPD an IgG avidity test was also applied.

Results. For the period from 2016 to 2020 a total of 58,700 individuals from all regions of the country were tested for toxoplasmosis and the established average seropositivity was 18%. Due to the fact that only congenital toxoplasmosis is subject to a mandatory registration and notification, detailed data on the different groups of patients with clinical form of the disease were available only at the NRL Diagnosis of parasitic diseases. During the studied period in the NRL were examined 709 persons, of which 304 pregnant women, 69 with lymphadenitis, 50 with suspected ocular form, 19 with fever of unknown origin and 136 were tested prophylactically. With evidence of recent infection were 27% of pregnant women, 20% of patients with lymphadenitis, 8% of those with ocular involvement and 13% of prophylactically tested. Low IgG avidity was established in 31% of pregnant women with evidence of recent infection. Presence of latent infection was found in 20% of all examined in NRL.

Conclusion. Our study shows average levels of IgG seroprevalence among the Bulgarian population slightly lower than the average levels for Europe and the need for examination of the pregnant women because there is a significant hidden incidence of toxoplasmosis.

Funding source: The work is supported by the Bulgarian National Science Fund (project № KP-06-H-43/2/27.11.2020) under the “Competition for financial support for basic research projects – 2020”.

SEE VECTORS AND VECTOR-BORNE PATHOGENS

SEEVEC1

TICK BITE MARKERS IN EXPOSED PEOPLE – PROMISING RESULTS OF THE TICK SALIVARY AV422 PROTEIN

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Background. Ticks, as vectors of number of pathogens, are spreading, bringing more people at risk of infection. Tick-borne diseases pose an increasing health threat, and reliable confirmation of tick bite would be helpful in their diagnostics and screening.
Objectives. The aim of this study was to assess the antigenicity of AV422 – tick salivary protein, in people exposed to ticks, which can be potentially useful in clinical practice and epidemiological studies.
Material and Methods. Sera were sampled from individuals professionally exposed to ticks: two groups of military personnel from Serbia, who differed in recently reported tick bite (group I, n=48, with; group II, n=19, without), exposed mainly to *Ixodes ricinus*, and farm workers from Crete, Greece, with suspicion of a Rickettsial disease, exposed mainly to *Rhipicephalus sanguineus* (group III, n=9). Western blot analysis was conducted with recombinant AV422 as antigen, derived from the coding sequence of *I. ricinus*. Seroreactivity on membrane strips containing purified r*Ir*AV422 was detected by chemiluminescent visualization.
Results. The presence of specific anti-AV422 IgG antibodies was shown in 62.5% (30/48), 57.9% (11/19), and 66.7% (6/9) of the individuals tested within study groups I, II and III, respectively.
Conclusion. The results from this study reveal AV422 as an immunogenic tick salivary protein in persons exposed to different ticks. They also constitute a promising starting point for further investigations on the protein's characteristics and the potential applicability on general population at risk.

Funding source: This work was supported by the Ministry of Education, Science, and Technological Development, Republic of Serbia (Project № 173006; Contract № 451-03-9/2021-14/200015)

SEEVEC2

DISTRIBUTIONAL CHANGES OF THE SNAIL *Bulinus truncatus*, INTERMEDIATE HOST OF *Schistosoma* spp., IN THE FACE OF CLIMATE CHANGE

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Background. Climate change affects the distribution of many vector-borne diseases through its impact on life history traits of disease agents and their intermediate hosts. Species distribution models are useful tools to infer current and future distributions of host and pathogen species, and improve the prediction of disease risk. Correlative models accurately predict current species distributions but lack the capacity to forecast distributions. Experimental data on species' tolerance limits embedded in so-called mechanistic models increases the accuracy of forecasts considerably. Schistosomiasis is such a vector-borne disease where more detailed information on both intermediate host and parasite ecology are needed to support accurate forecasts of disease risk. Making these predictions remains difficult due to the unknown effects of rising temperatures and changing rainfall patterns on both snail intermediate hosts and parasites. Availability of ecological data differs greatly between abiotic factors (e.g. temperature, rainfall,…), snail species, and snail life stages. Furthermore, a lack of standardisation across studies impairs inter-study and interspecific comparability.
Objectives. Here, we focus on the snail *Bulinus truncatus,* intermediate host for both the human parasite

Schistosoma haematobium, causative agent of urinary schistosomiasis, and the bovine parasite *Schistosoma bovis*. We present the study setup were we subject *B. truncatus* snails originating from different geographic temperature zones to a range of temperatures in a common garden experiment. Life history parameters such as survival and growth are monitored closely during four months and a set of physiological parameters are measured. Additionally, a reduced representation sequencing technique is used to screen for genetic signs of local adaptation to climatic factors. The data are used to construct mechanistic species distribution models predicting the future distribution of *B. truncatus,* which can serve as a basis to assess schistosomiasis risk in the future.

TRICHINELLOSIS AND TRICHINELLA INFECTION IN SEE: CURRENT STATUS

TRICH1

INVASIVE SPECIES AS A HOST FOR *Trichinella* spp. NEMATODES IN CENTRAL EUROPE

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Background. Invasive species pose a threat to global biodiversity by displacing native animal species, limiting the food base or transmitting parasites. In Poland, the raccoon dog (*Nyctereutes procyonoides*) and the raccoon (*Procyon lotor*) are invasive species. The aim of the study was to determine the occurrence of *Trichinella* nematodes in both expansive alien species in Central Europe.
Material and Methods. Muscles samples (diaphragm, tongue, masseter and limb muscles) were collected post mortem from 113 raccoon dogs (Poland) and 164 raccoons (Poland, Germany, the Czech Republic). The muscles were tested separately using HCl–pepsin digestion. The larvae were counted and the intensity of the infection was expressed as the number of larvae per gram of muscle (LPG). Multiplex polymerase chain reaction (PCR) was used to identify the larvae at the species level.
Results. The presence of *Trichinella* larvae was confirmed in 6.7% of raccoons and in 39.82% of raccoon dogs. The presence of *T. britovi* in the raccoon dog, and *T. spiralis* and *T. pseudospiralis* in the raccoon were confirmed. The most larvae of *T. britovi* were located in the tongue, masseter and lower forelimb muscles among females, and in the tongue, lower forelimb and lower hindlimb muscles among males of the raccoon dog. The intensity of infection of *T. spiralis* in raccoon was low, therefore the predilection muscles could not be described. *T. britovi* isolates obtained from raccoon dogs are 100% identical with each other within partial sequence of CO1 gene.
Conclusion. The raccoon dog and the raccoon are hosts for different *Trichinella* species. The high prevalence of *T. britovi* in raccoon dogs, and *T. spiralis* and *T. pseudospiralis* in raccoons, as well as expansion of both species may cause an increase spreading of *Trichinella* nematodes in the sylvatic and domestic cycle.

Funding source: Muscle samples were collected within the National Science Centre, Poland, project № 2014/15/B/NZ8/00261 and Project Life +, № NAT/PL/428; the laboratory analysis were performed within the National Science Centre, Poland, project № 2017/25/N/NZ7/02625.

TRICH2

ROUTINE PROCEDURES AT THE INTERNATIONAL *Trichinella* REFERENCE CENTRE (ITRC) PRESERVES THE GENETIC VARIABILITY OF THE WILD STRAIN

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Background. The International *Trichinella* Reference Centre (ITRC) was created as a repository for *Trichinella* strains and source of materials and information for international research in 1988. The maintenance of strains is ensured by in vivo-passages in mice, however, knowledge about the long-term influence over generations of infections on the genetic richness and diversity of the wild strains was missing.
Objectives: to examine the effect of 30 years of in vivo passages in CD1 mice on the allele content and genetic structure of *Trichinella britovi* and *T. spiralis* reference strains maintained at the ITRC.
Material and Methods. A *T. britovi* (ISS107) and a *T. spiralis* (ISS160) reference strains, collected from wild hosts, have been preserved through serial passages in CD1 mice. Basing on single-larva microsatellite genotyping, analyses were performed on two levels: 1) by comparing larvae recovered from the oldest and the last generation of each *Trichinella* strains examined, 2) by comparing larvae recovered from individual mice in one experimental infection. Genetic diversities among samples were compared estimating allele frequencies and inferring their genetic structure by a Bayesian clustering analysis.

Results. The results show a substantial similar allelic content and genetic structure from the oldest to the most recent generation, for both *T. britovi* and *T. spiralis* isolates, with no evidence of lost genetic variability. Otherwise, the individual analysis of mice suggests that a genetic depletion from a generation to another, in absence of repooling of larvae after digestion, is possible and expected.
Conclusion. Routine procedures carried out at the ITRC allow the maintenance of the original genetic pool, despite human manipulation and artificial selection.

Funding source: This work was supported by the European Commission's Directorate-General for Health and Food Safety (DG SANTE) - European Union Reference Laboratory for Parasites, grant agreement № SI2.801980.

DIAGNOSIS AND EPIDEMIOLOGY OF VISCERAL LEISHMANIASIS

VL1

FELINE LEISHMANIASIS: SEROLOGICAL AND MOLECULAR DETECTION OF AN EMERGENT DISEASE IN A NON-ENDEMIC AREA OF NORTHERN ITALY

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Background. In recent decades feline leishmaniosis (FeL) has become an emerging disease, also in non-endemic areas for the canine infection.
Objectives. This study updates the epidemiological status for FeL in cats in northern Italy and compares results with previous studies of the same feline population. Co-infections with feline retroviruses FIV and FeLV were also investigated.
Material and Methods. Stray, shelter and owned cats from different cities in the Lombardy region of northern Italy, were prospectively randomly sampled between January 2020 and May 2021. A total of 255 cats were tested for *L. infantum:* 240/255 for antibodies by IFAT and 234/255 and 198/255 for *Leishmania* DNA by PCR on whole blood and lymph nodes, respectively. Rapid ELISA test was used to detect FIV or FeLV infection.
Results. Overall, 26/255 (10.2%) cats tested positive for *L. infantum*: in 8/26 cats Leishmania DNA was found in popliteal lymph nodes (leishmania/ml range from 15 to 60), 6/26 were PCR positive on whole blood (leishmania/ml range from 5 to 80) and 15/26 IFAT seropositive at titers ranging from 1:80 to 1:320 (Table 1). Two *Leishmania* infected cats were also FIV+FeLV coinfected, another was FIV positive and one was FeLV positive.

Table 1. Epidemiological data on feline leishmaniosis in studies performed in Lombardy region of northern Italy

Variable	Spada et al 2014	Spada et al 2016	Spada et al 2020	Current study
Years	2008-2010	2014	2016-2018	2020-2021
Population	233 stray cats	90 stray cats	117 stray cats	255 (160 stray, 43 shelter, 52 owned cats)
FeL overall prevalence	9.0%	12.2%	8.6%	10.2%
IFAT overall seropositivity	21/233 (9.0%)	11/90 (12.2%)	5/102 (4.9%)	15/240 (6.3%)
PCR overall positivity	0 (0.0%)	2 (2.2%)	5/115 (4.4%)	14/234 (6.0%)

Conclusion. A high prevalence of FeL was found in a non-endemic area of northern Italy, with an increasing trend in infection rates.

Funding source: C.U.P. H75H20000150001, Ministero della Salute, Ricerca Corrente IZS SI 08/20

VL3

DEVELOPMENT OF ENHANCED SENSITIVITY TOOLS TO MONITOR *Leishmania* INFECTION

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Background. Leishmaniases are neglected tropical diseases caused by an intracellular protozoan parasite transmitted by female phlebotomine sandflies. Each year this disease causes 30 000 deaths worldwide. Different forms exist, showing gradual severity: a cutaneous form conferred by *L. major*, a mucocutaneous form, and a visceral form conferred by *L. infantum*, which is fatal when left untreated. Today only few treatments are available, and they present issues of toxicity, high risks of relapse and emerging resistance.

Therefore, development of new treatments is necessary, and requires models allowing to monitor *Leishmania* survival.

Objectives. We propose the creation of eFFly-mCherry strains stably expressing enhanced bioluminescence coupled to mCherry red fluorescence to facilitate screening of new compounds against *Leishmania,* and to avoid necessity of multiple cultures for *in vivo* and *in vitro* detection.

Material and Methods. Electroporation of eFFly-mCherry constructs were realized on two species of *Leishmania* : *L. major* and *L. infantum*. Selection of parasites allowed to isolate single clones stably expressing luciferase and mCherry red fluorescent protein.

Results. Follow-up of growth and mortality by flow cytometry indicated eFFly-mCherry insertion did not alterate parasite development. Both fluorescence and bioluminescence were stably expressed in transformed strains of *L. major* and *L. infantum,* and signals decreased as mortality increased. Bioluminescence emission showed a strong correlation (R^2=0.9) with parasite concentration. Finally, red fluorescent parasites were observed inside primary murine macrophages with confocal microscopy, confirming parasites maintained their capacity to infect host cells.

Conclusion. Current treatments present multiple drawbacks, leading to the urgent need of new alternatives, which implies to set up new tools to perform efficient and fast screenings of drug libraries. The eFFly-mCherry reporter strains are a tool for rapid screening of anti-leishmanial compounds, and the possibility to use *L. major* and *L. infantum* presents an opportunity to rapidly screen for broad-spectrum anti-leishmanial compounds.

VL4

CROSSREACTIVITY BETWEEN *Leishmania infantum* AND *Leptomonas pyrrhocoris* ANTIGENS: IMMUNOFLUORESCENCE STUDY IN DOGS

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Background. The leishmaniases are a group of parasitic diseases caused by protozoa from genus *Leishmania*, transmitted to humans and animals by the bite of infected female phlebotomine sandflies. From the aspect of veterinary medicine the most important is canine leishmaniasis. In 2019, 98 countries and territories have become endemic for leishmaniasis. Serological tests are sensitive and easily demonstrate presence of antibodies in clinical canine leishmaniasis cases. Antigens are mainly obtained from *in vitro* cultures of parasites. It should be emphasized that there is a risk of infection with *Leishmania* when manipulating parasite cultures. Also, isolation of parasitic hemoflagelates from samples is sometimes difficult due to contamination of primary cultures with bacteria, fungi and yeasts. In order to simplify the procedure, the research was focused on the possibility of using alternative antigen sources. In diagnostics of leishmaniasis, starting from 1980s to the present, some of flagellates belonging to the family Trypanosomatidae were tested as sources of antigen: *Crithidia luciliae, Crithidia fasciculata, Leptomonas seymouri, Phytomonas serpens, Strigomonas culicis* and *Angomonas deanei*.

Aim. *Leptomonas pyrrhocoris* is a monoxenous trypanosomatid which parasitizes in the intestines of common insect from the family Pyrrhocoridae, firebug *Pyrrhocoris apterus*. Up to now this flagellate was not tested as a source of antigen in routine serology for canine leishmaniasis. The aim of this study was to prove its potential use.

Material and methods. *In vitro* cultured promastigotes of *L. infantum* and *L. pyrrhocoris* were used as a source of antigen for indirect immunofluorescence antibody test.

Results. The results of this survey showed high cross-reactivity level between two examined antigens.

Conclusion. Obtained results showed high potential in use of apatogenic *L. pyrrhocoris* promastigotes as alternative source of antigen for large scale serological screening of leishmaniasis in dogs.

VL5

EVALUATION OF RPMI-PY MEDIUM FOR *Trypanosoma cruzi* AND DIFFERENT *Leishmania* SPECIES

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Background. *Trypanosoma spp.* and *Leishmania spp.* are causal agents of a number of parasitic diseases. Culture media can be divided into 3 main categories: semisolid, biphasic, and liquid. While biphasic and semisolid culture media need blood, an important factor for the reproduction of parasites, most liquid media require fetal calf serum or erythrocyte lysate. A culture media RPMI-PY demonstrated a good performance in terms of time and parasitic load of *L. infantum* compared to other culture media.

Objectives. *In vitro* cultivation of parasites plays an important role in the study and treatment of the disease and to simulate the host environment, especially in an *in vitro* culture system can be extremely demanding, assuming one can actually determine all the relevant variables. The aim of the work was to evaluate the performance of RPMI-PY medium in different Leishmania species and also to evaluate in *T. cruzi* culture.

Material and Methods. The conventional Leishmania media used for the comparison are Evans' modified Tobie's medium (EMTM), RPMI 1640 medium, and peptone-yeast extract medium (PY) and RPMI-PY medium. *L. aethiopica, L. braziliensis, L. donovani, L. major, L. tropica, L. amazonensis* in promastigote forms, and a strain of *T. cruzi* were incubated at 24°C and were monitored by measuring the growth rate through the incubation period (3 days).

Results. Data regarding the growth rate and the enrichment curve were collected for all the different cultivation systems. Table 1 shows culture media data.

Table 1. Comparison of Leishmania and trypanosoma strains growth in 4 different culture media: classic media (RPMI, PY, EMTM) versus new culture media RPMI-PY

	RPMI			PY			EMTM			RPMI-PY		
	24 h	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**	**24h**	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**
L. major	2E+07	4E+07	1E+08	1E+07	2E+07	9E+07	8E+05	2E+07	1E+08	3E+07	8E+07	4E+08
L. tropica	4E+07	4E+08	2E+08	3E+07	2E+08	2E+08	2E+08	5E+08	3E+08	4E+07	3E+08	8E+08
l. brasiliensis	3E+06	2E+07	6E+07	2E+06	9E+06	3E+07	5E+07	2E+07	4E+08	2E+06	1E+07	3E+07
L. amazoniensis	5E+06	5E+07	1E+08	2E+07	4E+07	2E+08	2E+07	3E+07	5E+07	4E+06	6E+07	4E+08
T. cruzi	2E+07	3E+07	6E+07	2E+07	4E+07	5E+07	5E+06	1E+07	2E+07	2E+07	4E+07	7E+07

Conclusion. RPMI-PY is likely to be valuable additions to laboratory practice in light of the relatively simple recipes, general availability of the components, and in terms of suitability because rabbit breeding is not necessary and the costs are lowered and can be used for all Leishmania species and to cultivate *T. cruzi.*

WILDLIFE PARASITOLOGY

WILDP1

MOLECULAR EPIDEMIOLOGY OF TRICHOMONADS IN WILD WETLAND BIRDS IN THE NETHERLANDS

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Background. Severe granulomatosis in productive layers due to *Tetratrichomonas gallinarum* strain 13/16632 infection occurred in 2013 and 2017 on farms situated in a wetland area in the Netherlands. These outbreaks were mainly characterized by persistent increased mortality of hens and by a high within flock incidence of granulomas.

Objectives. Our aim was to evaluate the potential of wild wetland birds to act as a reservoir of virulent trichomonads such as *T. gallinarum* especially for chicken rearing farms.

Material and Methods. A prevalence survey on trichomonads was performed by analysing cloaca swabs of 526 birds belonging to 13 species of wetland birds. The number of birds sampled ranged from 1 to 275 per species. Birds were sampled at 15 locations, distributed over the Netherlands. DNA extracted from the cloaca swabs was subjected to a nested PCR assay using trichomonad specific primers targeting the ITS1 – 5.8S rRNA – ITS2 region. Positive nested PCR products were either cloned before sequencing or directly sequenced.

Results. Trichomonads were detected in nine bird species. The overall prevalence was 9% (47/526), while the prevalence in the five species of which a substantial number of birds were examined (at least 39 per species) ranged from 4 to 24%. Three trichomonad species were found: *T. gallinarum*, *Trichomonas tenax* and *Simplicimonas* sp. of which *T. gallinarum* dominated. The virulent *T. gallinarum* strain 13/16632 was not detected, but closely related strains were identified. Phylogenetic analysis revealed that all *T. gallinarum* isolates belonged to two clusters within lineage 15 of *Tetratrichomonas* lineages. All *T. tenax* isolates were identical and clustered with reference strains, while *Simplicimonas* sp. isolates showed large genetic diversity. Some isolates may represent a new species of the genus *Simplicimonas*.

Conclusion. We highlight that trichomonads are widespread and circulate abundantly amongst wetland birds, questioning, amongst others, its relevance for commercial poultry.

WILDP2

POSSIBLE INFLUENCE OF B CHROMOSOMES ON THE PREVALENCE AND ABUNDANCE OF INTESTINAL NEMATODE PARASITES OF THE YELLOW-NECKED MOUSE (*Apodemus flavicollis*)

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Background. B chromosomes are supernumerary chromosomes that have been discovered in over 1000 eukaryote species. They show remarkable variation in structure and behaviour, their only common trait being their conditional dispensability. The influence of these genetic elements on their carriers is still debated, with the two most accepted models of their maintenance being the parasitic and the heterotic model.

Objectives. The purpose of this study was to investigate the possible influence of B chromosomes on quantitative characteristics of intestinal nematode infection in the yellow-necked mouse (*Apodemus flavicollis*).

Material and Methods. A total of 305 mice were sampled across 18 localities on the territory of Serbia over five years. Every individual with more than 48 chromosomes was considered to have Bs. After dissection, intestinal nematodes were extracted and identified. An analysis of Bs influence on parasite prevalence and

abundance was conducted, using the exact unconditional test and generalized linear modelling respectively.
Results. Nine intestinal nematode species were detected in the host sample. In the total host sample, both prevalence and abundance of intestinal nematodes were greater in Bs carriers. Two nematode species, *Aspiculuris tetraptera* and *Mastophorus muris*, had significantly higher prevalence in the B+ subset of the host sample. Similarly, B chromosomes explained a significant proportion of abundance variation of the nematode species *Syphacia frederici*, with its abundance greater in Bs carriers.
Conclusion. While the results of this study show a possible connection between Bs presence and higher prevalence and abundance of intestinal nematodes, this finding is purely correlational. Previously published data indicate a complex effect of B chromosomes on host characteristics and survival through various molecular pathways. Bs carriers may reap the benefits of increased survival prospects as a by-product of the selfish behaviour of the chromosomes themselves, blurring the line between the parasitic and heterotic model.

WILDP3

SYMBIONTS OF BIVALVE MOLLUSCS OF THE KANDALAKSHA GULF AND THE ONEGA BAY OF THE WHITE SEA

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Background. The parasitological examination of bivalves is necessary for understanding life cycles realized at a particular area. We studied the symbiofauna composition of mass species of bivalve molluscs in the southern and western parts of the White Sea.
Material and Methods. During years 2009-2021 we collected bivalves from subtidal and intertidal zones at three areas in the Kandalaksha Gulf and three areas in the Onega Bay (separated by tens to hundreds of kilometres) (1-20 sampling sites at each area). 2499 specimens of 24 bivalve species were collected (8 to 393 specimens of each species). Mainly we focused on metazoan symbionts (also noting the presence of Protista).
Results. The 17 taxa of metazoan symbionts included representatives of 4 phyla. All taxa excluding parasitic Digenea are considered commensals. Digenean sporocysts and most commensals (except for "turbellaria") were host-specific, while digenean metacercaria used several species as hosts. Comparison of symbiofaunas of different bivalve species is represented as non-metric multidimensional scaling (Figure 1).

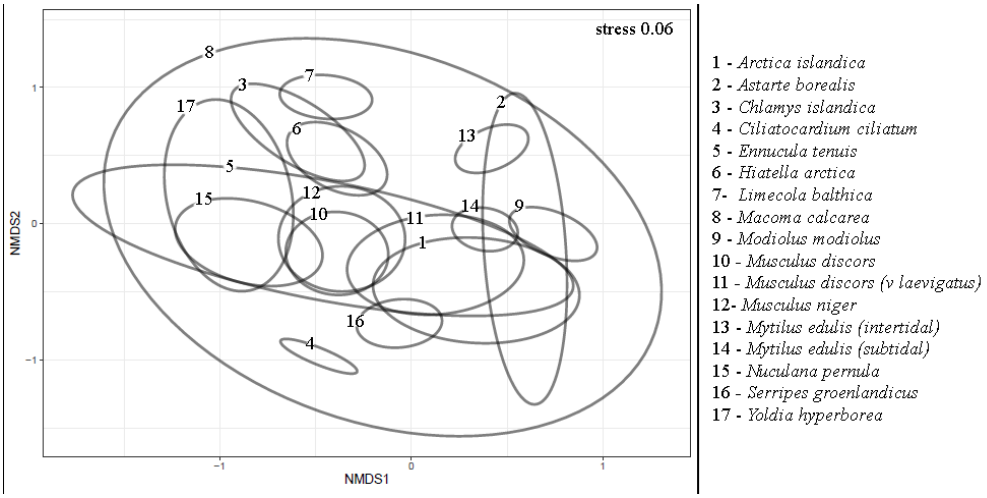

Figure 1. Non-metric multidimensional scaling (nMDS) ordination of the molluscs infected with turbellaria and trematoda based on the matrix of Bray-Curtis dissimilarities. Ellipses represent 95% confidence intervals of host species centroids.

Conclusion. The distribution of symbionts among the bivalve species was not uniform. The composition of symbiofauna of the studied bivalves was correlated with the host's phylogenetic position and ecology.

Funding source: The reported study was funded by Russian Science Foundation, the project № 19-74-10029.

WILDP4

Libyostrongylus douglassii (TRICHOSTRONGYLIDAE) IN OSTRICHES (*Struthio camelus*) IN PORTUGAL: CASE REPORT

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Gastrointestinal nematodes are a major health concern in captive ostriches. Nematodes of the genus *Libyostrongylus* are haematophagous and inhabit the ostrich's proventriculum. *Libyostrongylus douglassii* causes a disease known as rotten stomach, which may result in 50% mortality in young and occasionally adult birds. This study documents the detection of *L. douglassii* in a three year old ostrich at the Santo Inácio Zoo, Porto, Portugal.

The adult bird was transferred together with two approximately 1.5 year old ostriches from a wildlife park to the Zoo on the 14th April 2021. Following the finding of trichostrongylid eggs in faeces collected from the enclosure five days later, the three birds were treated with ivermectin (200mg/kg subcutaneously). The younger birds died 15 and 23 days after treatment without developing overt clinical signs besides apathy, prostration and dorsiflexion of the neck and were submitted to anatomopathological examination. The adult bird received two further ivermectin treatments within 1.5 months and faecal samples were collected 3 weeks post-treatment for coprological analysis. Faecal Egg Counts (FEC) were performed by the Mini-Flotac technique and coprocultures were set for morphological identification of L3 larvae. Histopathology revealed catarrhal haemorrhagic proventriculitis with distension of the glandular lumen and haemorrhagic erosion associated with the presence of nematodes. FEC were 880 EPG and 2430 EPG, respectively. Larvae recovered from coproculture were identified as *L. douglassii* based on the presence of a knob at the tip tail and the short size of the sheath tail.

While the occurrence of *Libyostrongylus* sp. had already been reported in Portugal, this is the first confirmation of the highly pathogenic *L. douglassii* species. The present data suggest a possible resistance of *L. douglassii* to ivermectin. More research is needed to assess the extent of infection and the status of anthelmintic resistance of this parasite in ostrich holdings in Portugal.

WILDP5

TAILED OR TAILLESS? ELUCIDATION OF THE LIFE CYCLE AND CERCARIAL DEVELOPMENT IN *Pseudozoogonoides subaequiporus* (DIGENEA: ZOOGONIDAE)

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Background. The most prominent morphological feature of the cercariae larvae of the Digenea is the tail—locomotory appendage used for transmission towards the next host in the life cycle. The family Zoogonidae is an exception. Unlike the vast majority of other digeneans, the zoogonid cercariae are tailless, they crawl on the seafloor to find and penetrate the second intermediate host—various slow-moving marine invertebrates. However, the genuine range of the intermediate hosts in these digeneans is poorly known since life cycles are described only for five species of the Zoogonidae.

Objectives. The goal of our work was to elucidate the life cycle of *Pseudozoogonoides subaequiporus* (Zoogonidae) and to describe it cercarial development.

Material and Methods. Definitive host of *P. subaequiporus* (Atlantic wolffish) and potential intermediate hosts (various gastropods and bivalves) were collected from the White Sea subtidal during summer-autumn of 2019–2020. Life cycle stages from the definitive and intermediate hosts were fixed for molecular and morphological studies.

Results. We found metacercariae in bivalve *Nuculana pernula*, which were morphologically similar with sexual adults of *P. subaequiporus*. We also discovered daughter sporocysts of unknown zoogonid species

in a gastropod *Neptunea despecta*. The sequences of ITS1 and 28S rDNA obtained from these life cycle stages and sexual adults of *P. subaequiporus* were identical. In the daughter sporocysts of *P. subaequiporus* we found underdevelopment cercariae with short tail primordium, formed tailless cercariae, and encysted metacercariae.

Conclusion. *Pseudozoogonoides subaequiporus* may adopt two different life cycle strategies: either three-host (*N. pernula*—*N. despecta*—Atlantic wolffish) or truncated two-host (*N. despecta*—Atlantic wolffish). The tail-bud forms during the embryogenesis of *P. subaequiporus* but disappears when cercariae become fully formed.

Funding source: The reported study was funded by Russian Science Foundation, the project № 19-74-10029.

WILDP8

"BODY SNATCHERS" OR HOW PARASITIC BARNACLES (RHIZOCEPHALA) MANIPULATE THEIR HOSTS

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Background. Parasitic barnacles **(**Rhizocephala) infect decapod crustaceans and are known for their amazing ability to take control over the host's body. They are able to change host's morphology, metabolism and even manipulate their behavior. However, until recently the exact mechanisms of these host-parasite interactions remained enigmatic.

Objectives. In our research we focused on the morphological description of specialized rootlets invading nervous ganglia of the host. We used a range of species from several families (Peltogastridae, Peltogasterellidae, Sacculinidae, Polyascidae)

Material and Methods. To describe the morphological and ultrastructural features of these rootlets we used a set of methods including histological mounts and sections, TEM, SEM, antibody staining with CLSM.

Results. In the particular research were described specialized rootlets of rhizocephalan barnacles infiltrating nervous ganglia of the host. The tips of these rootlets were modified into "Goblet-shaped organs". The shape and ultrastructure of these organs differed a lot from the common trophic rootlets. Cell organization indicated high level of biosynthesis and transmembrane transport.

However, we have found one more site of the direct contact between parasite and host's nervous system: trophic rootlets of the parasite were enlaced by a network of hosts neurons.

Conclusion. We suggest that both sites of direct contact between hosts nervous system and rhizocephalan parasite play a key role in the host-parasite interactions.

WILDP9

NEW INSIGHTS INTO THE ORIGIN OF THE ORTHONECTIDS` PARASITIC PLASMODIUM (BILATERIA: ORTHONECTIDA)

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Background. In the northern hemisphere, diverse groups of marine invertebrates - molluscs, nemerteans, turbellarians, echinoderms, ascidians - are infected by peculiar metazoan endoparasites, orthonectids. Orthonectids are highly derived annelids that have undergone secondary reduction. Orthonectids have acquired an unusual for bilaterians adaptation to parasitism – so-called parasitic plasmodium. The plasmodium consists of a shapeless mass of protoplasm and develops inside the host body from an infectious larva. The plasmodium penetrates host tissues and can cause collateral damage. Sexual generation emerges from the plasmodium and leaves the host for copulation. The origin of the orthonectids` plasmodium remains controversial – it is either an enlarged cytoplasmic portion of a parasitised host cell or an independent organism, the parasitic generation of the orthonectids` life cycle.

Objectives. Solving the puzzle of the plasmodium origin requires clarifying the plasmodium structure and determining if any orthonectid genes are expressed in the plasmodium.
Material and Methods. We worked with two orthonectids from the Barents Sea, *Intoshia linei* and *Intoshia variabili*. They parasitise ribbon worms *Lineus ruber* and flatworms *Graffiellus croceus*, respectively. Hosts infected by orthonectids` plasmodia were processed for TEM and confocal microscopy examination. We detected and annotated plasmodium-specific genes by analysing stage-specific RNA-seq data.
Results. The orthonectids` plasmodium is a multinucleated parasitic body separated from host tissues by two plasma membranes. Its cytoplasm is visually distinguished from the surrounding host cells and contains organelles and numerous nuclei typical for other orthonectids` stages. Hundreds of orthonectids` proteins are expressed only at the parasitic stage. Plasmodium-specific proteins are involved in the defence against host immunity, host-parasite communication, host nutrients uptake, development and growth inside the host. Most of the revealed proteins are known effectors of other endoparasites.
Conclusion. Obtained results indicate orthonectids` plasmodium is an independent organism of a parasitic origin.

Funding source: Russian Science Foundation grant № 19-74-10013, Russian Foundation for Basic Research grant № 19-04-00218

WILDP10

EFFECT OF *Strigea robusta* (DIGENEA: STRIGEIDAE) METACERCARIAE ON DEVELOPMENT OF BROWN FROG TADPOLES

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Background. Anomaly P is a polymorphic syndrome affecting some populations of European water frogs of the genus *Pelophylax*. It was discovered by French writer and biologist Jean Rostand in the late 1940s and studied by him during next 20 years. Novel records of the anomaly P “hotspots” with heavy forms had led to discovery of the cause. We have shown in experiments that anomaly P developed in water frog tadpoles after infestation of trematode *Strigea robusta*. This effect turned out to be stage- and dose-dependent. However, syntopic brown frogs (genus *Rana*) had no anomalies. This possible specificity of the action is of great interest, and the aim of this work was to test the effect of *S. robusta* on tadpoles of brown frogs in the laboratory experiments.
Material and Methods. We used tadpoles of two brown frog species: *Rana arvalis* and *R. temporaria*. The tadpoles were exposed to five doses of *Strigea robusta* cercariae: 0 (control), 8 (low), 16 (low), 32 (medium) and 48 (high). Fourteen tadpoles in each group were tested. In an additional experiment, 14 tadpoles of *R. arvalis* were exposed to 8 cercariae on early and late stages of limb development. In total, 182 tadpoles were involved in the experiments. After exposure, the tadpoles were kept in 60 L aquariums.
Results. The survival rate of tadpoles varied from 36 to 93%; 57% on average for *R. arvalis* and 77% for *R. temporaria*. Despite the presence of cysts in developing tadpoles, none of the experimental specimens showed anomalies. Thus, the specificity of the effect, previously assumed on the observations in nature, was confirmed in experiments.

Funding source: The research was supported by the Russian Science Foundation grant № 21-74-00079, https://rscf.ru/en/project/21-74-00079/

WILDP11

PHYLOGEOGRAPHY OF GENUS *Paranoplocephala* (CESTODA) FROM RODENTS OF BOREAL ZONE OF EURASIA

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Background. Cestodes from genus *Paranoplocephala* (Anoplocephalidae) are widespread over the area of Holarctic in different species of rodent. To-date, three valid species of the genus *Paranoplocephala* were

registered in Eurasia: *P. omphalodes* (Hermann, 1783), *P. kalelai* (Tenora, Haukisalmi & Henttonen, 1985) Tenora, Murai & Vaucher, 1986, and *P. jarrelli* Haukisalmi, Henttonen & Hardmann, 2006. The present study is focused on the comparative phylogeography of these species from boreal zone of Eurasia using *cox*1 and *nad*1 genes partial sequence.

Material and Methods. The studied cestodes were collected from rodents inhabit area from Karelia Republic to Magadan Oblast. Also, all sequences from GeneBank database for genus *Paranoplocephala* were used. The *cox*1 and *nad*1 genes were amplified using the primers described by Haukisalmi *et al.* (2004) and Littlewood *et al.* (2008), respectively. Phylogenetic reconstruction was performed using the Bayesian inference approaches with MrBayes v.3.2.1. Popart 1.7 software was used to calculate and visualize the median-joining network (Bandelt *et al*. 1999).

Results. For *P. omphalodes* we may note relatively low level of genetic diversity if compare to another two studied species. The majority numbers of haplotypes were registered on the territory of Europe. One, the most frequently found, haplotype was registered in enormous area from Italy to Siberia. For *P. kalelai* we have identified two clearly determined clades found from Fennoskandia to Magadan Oblast. For *P. jarrelli* we have registered relatevy high level of genetic diversity. Three main claides could be found: 1) from area of the Republic of Buryatia, 2) from area in the Republic of Buryatia and Alaska region, and 3) with Holarctic distribution.

Conclusion. Based on molecular data, we may conclude that all studied species from genus *Paranoplocephala* are characterized by different history of dispersal during the Pleistocene-Holocene that is apparently related to dispersal of their hosts.

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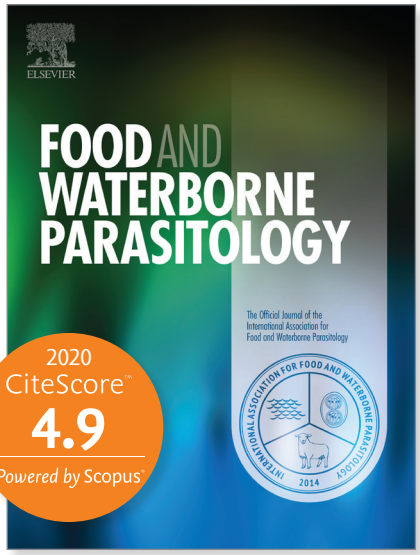

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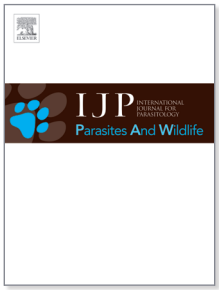

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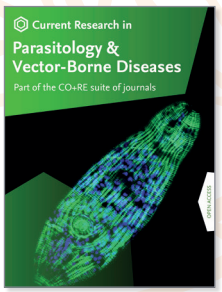

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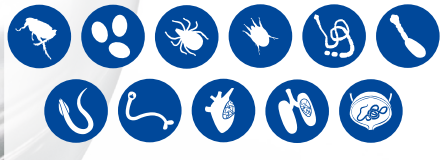

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