ApicoWplexa

5.-7. Oct. 2022

Bern, Switzerland


apicoWplexa

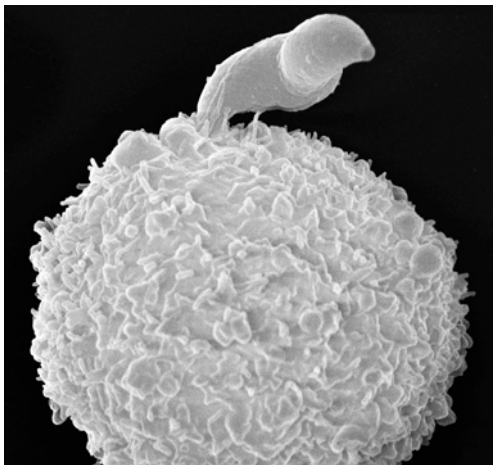
ApicoWplexa 2022

6th International Meeting on Apicomplexan Parasites in Farm Animals

PROCEEDINGS

October 5-7, 2022
Kongresszentrum Kreuz
Bern
Switzerland





Scientific committee

• Alexandre Leitao, University of Lisbon
• Andrew Hemphill, University of Bern
• Brian Cooke, James Cook University, Cairns
• Dominique Soldati-Favre, University of Geneva
• Emanuel Heitlinger, Humboldt University of Berlin
• Eskild Petersen, Aarhus University
• Gereon Schares, Friedrich Loeffler Institute
• Jitender P. Dubey, USDA
• J. Stone Doggett, Oregon Health & Science University, Portland
• Jonathan Charles Howard, Instituto Gulbenkian de Ciência, Oeiras
• Kerry Louise Woods, University of Bern
• Luis-Miguel Ortega-Mora, Complutense University of Madrid
• Rafael Calero Bernal, Complutense University of Madrid
• Virginia Marugan-Hernandez, Royal Veterinary College London
• Walter Basso, University of Bern
• Wesley C. Van Voorhis, University of Washington

Local organizers

• Alexandre Leitao, University of Lisbon
• Andrew Hemphill, University of Bern
• Barbara Gautschi, University of Bern
• Barbara Bracher, University of Bern
• Dennis Ihmhof, University of Bern
• Kai Hänggeli, University of Bern
• Katharina Gerber, University of Bern
• Luis-Miguel Ortega-Mora, Complutense University of Madrid
• Norbert Müller, University of Bern
• Pamela Schumacher, University of Bern
• Vreni Balmer, University of Bern

Welcome to Apicowplexa 2022!

It is our great pleasure to welcome you to the 6th International Meeting on Apicomplexan Parasites in Farm Animals here in Bern.

After three years of pandemic scares and not being allowed to meet in person, we can finally connect again in real life. Getting to know people, networking, and exchanging knowledge and experiences simply works better in-person than in a virtual environment, and we are happy that you decided to join!

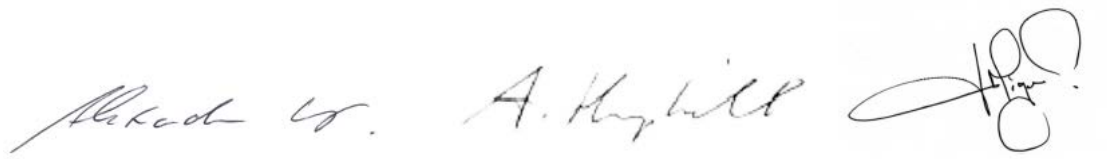
This meeting marks the 10th birthday of the ApicoWplexa network. After the highly successful inauguration meeting in Lisbon in 2012, exciting and enjoyable follow-up conferences were held in Kusadasi in 2013 organized by Tulin Karagenç and her colleagues, in Edinburgh in 2015 hosted by Frank Katzer and his team, at the beautiful site of El Escorial in 2017 organized by Luis Ortega and his group, and in Berlin in 2019, perfectly set up by Gereon Schares and Franz Conraths.

Over 140 delegates from all over the world have registered for this year's meeting, including 10 keynote speakers. 107 abstracts were submitted, covering a wide range of interests within the field of apicomplexans that affect livestock, including One-Health, molecular and cell biology, host-parasite interactions, immunology, diagnosis, epidemiology, drug development, in vitro and in vivo models, and vaccines and control of infection. To select 30 of these abstracts for oral presentations has been a real challenge.

This meeting was made possible through a collaborative effort of keynote speakers, the members of the scientific committee and the local organizers and last, but not least, thanks to the support provided by our sponsors and partners.

We wish you an interesting and stimulating meeting and hope that you will enjoy these days here in Bern!

On behalf of the organizers



Alexandre Leitao Andrew Hemphill Luis-Miguel Ortega-Mora

Programme

Wednesday, October 5, 2022

15:00- 17:00 **Registration (Kongresszentrum Kreuz)**

17:00 **Opening Session**

Chairs: **Alexandre Leitao, Andrew Hemphill, Luis-Miguel Ortega-Mora**

17:00-17:15 **Welcome to ApicoWplexa**

17:15-17:45 **Keynote: Jonathan Howard**, Instituto Gulbenkian de Ciência Oeiras, Portugal.
K-1 Between Scylla and Charybdis; how *Toxoplasma gondii* maintains avirulence

17:45-18:15 **Keynote: Gereon Schares,** Federal Research Institute for Animal Health,
K-2 Greifswald-Insel Riems, Germany
Toxoplasma gondii genotypes in Europe–a lot we know–a lot we do not know

18:30 **Get together, welcome reception**

Thursday, October 6, 2022

08:30 **Session 1: One-Health**

Chairs: **Radu Blaga, Clare M. Hamilton**

08:30-09:00 **Keynote: Eskild Petersen**, University of Aarhus, Denmark
K-3 A One-Health approach to disease surveillance in humans

09:00-09:30 **Keynote: Wesley C. Van Voorhis,** University of Washington, Seattle, USA
K-4 BKIs for treatment of apicomplexan diseases: Aligning the holes in the Swiss cheese for optimal safety, efficacy, and PK

09:30-09:45 **Filip Damek**, INRAE, Ecole Nationale Vétérinaire d'Alfort, Laboratoire de
O-1 Santé Animale, BIPAR, Maisons-Alfort, France
Systematic review and modelling of the age-dependent prevalence of *Toxoplasma gondii* in livestock, wild-life and felids in Europe

09:45-10:00 **Marieke Opsteegh,** RIVM, The Netherlands
O-2 *In vitro* assay to determine inactivation of *Toxoplasma gondii* in meat samples

10:00-10:15 **Paul Murdoch Bartley**, Moredun Research Institute, UK

O-3	Identification of *Cryptosporidium* sp. in livestock, domestic animals, wildlife and water source samples from seven farms in Wales.
10:15-10:30 **O-4**	**Patrick Scherrer,** Vetsuisse Faculty, University of Bern *Toxoplasma gondii* infection in the Eurasian beaver (*Castor fiber*) in Switzerland
10:30-11:00	**Coffee break**

Session 2: Epidemiology /diagnostic tools

Chairs: **Gastón More, Solange M Gennari**

11:00-11:30 **K-5**	**Keynote: Emanuel Heitlinger**, Humboldt University Berlin, Germany Improved quantification and discrimination of *Eimeria* species promotes research on evolutionary ecology and epidemiology
11:30-11:45 **O-6**	**Dadin Prando Moore**, Facultad de Ciencias Agrarias, Universitad Nacional de Mar del Plata, Balcarce, Argentina. Neosporosis in Argentina: past, present and future perspectives
11:45-12:00 **O-7**	**Johan Hiroto Standar,** Moredun Research Institute, UK Do we see changes in the genetic diversity of *Cryptosporidium parvum* genotypes during the calving season within a dairy herd?
12:00-12:15 **O-8**	**Laurence Malandrin,** INRAE, Oniris, France Asymptomatic carriers of equine piroplasmosis: prevalence differences between 4 French regions and low genetic diversity of *Theileria equi* and *Babesia caballi*
12:15-12:30 **O-9**	**Luca Villa,** Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, Lodi, Italy *Neospora caninum* antibodies in tank bulk milk from dairy cattle farms in Italy: spatial analysis and effects on reproductive and productive performances
12:30-14:30	**Lunch / Poster session 1**

Session 3: Immunology / vaccines

Chairs: **Esther Collantes-Fernández, Dadin Prando Moore**

14:30-15:00 **K-6**	**Keynote: Virginia Marugan-Hernandez**, Royal Veterinary College, London, UK Vaccines for coccidian: are we there yet?

15:00-15:15 **Juan Mosqueda,** Autonomous University of Queretaro, Mexico
O-10 The Spherical Body Protein 4 from *Babesia bigemina* is a novel gene, contains conserved B-cell epitopes and induces cross-reactive, neutralizing antibodies in *Babesia ovata*

15:15-15:30 **Po-Yu Liu,** Royal Veterinary College, London, UK
O-11 Multi-omics analysis reveals regime shifts in the gastrointestinal ecosystem in chickens following anticoccidial vaccination and *Eimeria tenella* challenge

15:30-15:45 **Dennis Imhof,** Institute of Parasitology, Vetsuisse Faculty, University of Bern, CH
O-12 Immunization with a multivalent *Listeria monocytogenes* vaccine leads to a strong reduction of vertical transmission and cerebral parasite burden in pregnant and non-pregnant mice infected with *Neospora caninum*

15:45-16:00 J**ose Manuel Jaramillo-Ortiz,** Royal Veterinary College, London, UK
O-13 Yeast-vectored oral immunisation in commercial layer chickens against *Eimeria tenella*

16:00-16:30 **Coffee break**

Session 4: Molecular and cellular biology

Chairs: **Norert Müller, Inês L.S. Delgado**

16:30-17:00 **Keynote: Dominique Soldati-Favre,** Department of Microbiology and Molecular
K-7 Medicine, Faculty of Medicine, University of Geneva, CH
Nanoscale imaging of the conoid and functional dissection of its dynamics in Apicomplexa

17:00-17:15 **Sebastian Shaw,** University of Pennsylania, USA
O-14 Highly efficient genetic crosses in *Cryptosporidium*

17:15-17:30 **David Arranz-Solis,** University of California, Davis, USA and Complutense
O-15 University of Madrid
Toxoplasma Late Embryogenesis Abundant proteins are important for oocyst resistance to environmental stresses

17:30-17:45 **Elizabeth Attree,** Royal Veterinary College, London, UK
O-16 Advancing understanding of the spatial proteome of *Eimeria tenella* sporozoites using hyperLOPIT

17:45-18:00 **Chandra Ramakrishnan,** Institute of Parasitology, Vetsuisse Faculty, University
O-17 of Zürich, CH
Besnoitia besnoiti genomics and transcriptomics reveal key molecular players in the intermediate and definitive host

From 19:00 Congress Dinner at Kornhauskeller

Friday, October 7, 2022

Session 5: Treatment and control

Chairs: **Kayode K. Ojo, Gema Alvarez-Garcia**

09:00–09:30 **K-8** **Keynote: Joseph Stone Doggett,** Oregon Health & Science University, Portland, OR, USA
Endochin-like quinolone prodrug, ELQ-422, a lead candidate for toxoplasmosis with broad activity against apicomplexan pathogens

09:30-09:45 **O-18** **Deborah Maus,** Robert Koch Institute, Berlin, Germany
The mode of action of *Toxoplasma gondii* tissue cyst inhibitors

09:45-10:00 **O-19** **Christopher D. Houston**, University of Vermont, College of Medicine, USA
Using a mutator *Cryptosporidium parvum* strain to enable studies of anticryptosporidial mechanism-of-action and drug resistance

10:00-10:15 **O-20** **Roberto Sánchez-Sánchez,** SALUVET, Complutense University of Madrid, Spain
Treatment with the novel bumped kinase inhibitor BKI-1748 confers protection against congenital toxoplasmosis in sheep.

10:15-11:00 **Coffee break**

Session 6: Host-parasite interactions

Chairs: **Pilar Horcajo, Carlos Hermosilla**

11:00-11:30 **K-9** **Keynote: Kerry Louise Woods**, Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, CH
Modulation of host FOXO1 activity in *Theileria*-transformed cells

11:30-11:45 **O-22** **Anand Srivastava,** National Institute of Animal Biotechnology (NIAB), India
Identification of CDK-cyclin pair(s) in *Theileria annulata*

11:45-12:00 **O-23** **Andrea Largo de la Torre**, Complutense University of Madrid, Spain
Archetypal type II and III *Toxoplasma gondii* oocysts induce different immune responses and clinical outcomes in experimentally infected piglets

12:00-12:15 **O-24** **Andrea Gaspare Valenti**, Vetsuisse Faculty, University of Bern, Switzerland
Genome-wide CRISPR/Cas9 screens for the identification of essential host factors for *Toxoplasma gondii* infection

12:15-12:30 **O-25** **N. Bishara Marzook**, The Francis Crick Institute, United Kingdom
A microscopy-based CRISPR screen reveals the essential host genome for *Cryptosporidium* parvum growth and development in vitro

12:30-12:45 **Jean-Michel Répérant,** ANSES, France

O-26 *Eimeria* of chicken: microgametes start their life with a tail before turning into flagella

12:45-14:30 **Lunch and Poster session 2**

Session 7: *In vitro* and *in vivo* models
Chairs: **Chandra Ramakrishnan, Walter Basso**

14:30-15:00 **Keynote: Rafael Calero Bernal**, SALUVET, Complutense University of Madrid, Spain
K-10 *In vitro* and *in vivo* models for the evaluation of *Toxoplasma gondii* strain virulence

15:00-15:15 **Anna Sophia Feix**, Institute of Parasitology, University of Veterinary Medicine, Vienna, Austria
O-27 *In vitro* inhibition of *Cystoisospora suis* sexual stage specific proteins

15:15-15:30 **Julie Tottey,** INRAE, Université de Tours, Nouzilly, France
O-28 Impact of physicochemical parameters of the digestive tract on *Cryptosporidium parvum* infection

15:30-15:45 **Yanina P. Hecker,** Complutense University of Madrid, Spain; INTA-CONICET, Balcarce, Argentina
O-29 Heifers inoculated with *Neospora caninum* live tachyzoites at prepubertal age reactivate their infection during gestation

15:45-16:00 **Julio Benavides,** SANPATRUM, Animal Health Department. University of Leon
O-30 Infection outcomes after challenge of sheep at mid-pregnancy with *Toxoplasma gondii* isolates showing different phenotypic traits

16:00-16:30 **Coffee break**

16:30-17:30 **Awards for junior scientists: best talk, best poster**

Round-up and goodbye

Poster session 1

Thursday October 6, 12:45-14:30

P-1 *Neospora caninum* natural infection in Tunisian rams: serological study and molecular identification of infection in semen, **Yosra Amdouni**

P-2 GENOTYPING OF *Toxoplasma gondii* FROM FREE-RANGE CHICKENS OF THE AMAZON REGION CONFIRMS ITS WIDE GENETIC DIVERSITY IN SOUTH AMERICA, **Solange Maria Gennari**

P-3 Pathogen-hopping to identify high-value drug leads against *Babesia spp.,* **Peter R. Hyson**

P-4 Genetic diversity of *Toxoplasma gondii* in the endangered Iberian lynx (Lynx pardinus), **Rafael Calero-Bernal**

P-5 Anti-*Toxoplasma gondii* antibodies in European residents within the last 20 years: A systematic review and meta-analysis, **Rafael Calero-Bernal**

P-6 Transcriptional changes in *Besnoitia besnoiti* infected primary bovine aorta fibroblasts identify relevant fibrosis-associated pathways, **Gema Álvarez-García**

P-7 *In vitro* safety and efficacy of new bumped kinase inhibitors against *Besnoitia besnoiti* tachyzoites, **Gema Álvarez-García**

P-8 In search of *Toxoplasma gondii* oocyst-specific proteins with source-attributing usefulness: key steps and limitations, **Gema Álvarez-García**

P-9 Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in sheep flocks from the Humid Pampa region, Argentina, **Yanina Paola Hecker**

P-10 Insights on Host Response to *Cryptosporidium parvum* Infection. **Mariko Dale**

P-11 Switzerland-wide *Neospora caninum* seroprevalence in cattle and identification of risk factors for infection. **Diana S. Gliga**

P-12 Counting parasites with DNA - quantification of coccidia can be high-throughput, precise, open-ended and species-specific, **Susana Carolina Martins Ferreira**

P-13 RNA-seq identifies parasite genes potentially associated with virulence in *Theileria annulata* transformed macrophages, **Khawla Elati**

P-14 Secreted *Theileria annulata* effector protein Ta9 binds and activates proto-oncogenic macrophage Hck., **Shahin Tajeri**

P15 Short-term culture adaptation of *Toxoplasma gondii* types II and III modifies isolate virulence in mice. **Andrea Largo De la torre**

P-16 Disseminated sarcocystosis in a domestic pig (Sus scrofa): case report of a neglected parasitic infection. **Selena Rubiola**

P-17 Serodiagnosis of *Toxoplasma gondii* infection in small ruminants from the Czech Republic by commercial and in-house ELISAs. **Jirina Markova**

P-18 Investigation of *Sarcocystis spp.* found in sheep and horses from Lithuania, **Petras Prakas**

P-19 Dynamic remodeling of the bovine placenta proteome upon infection with high and low virulence *Neospora caninum* isolates, **Pilar Horcajo**

P-20 *Eimeria* infections in small ruminants from mountain areas of northern Portugal, **Filipa Teixeira Rodrigues**

P-21 *In vitro* assessment of dinuclear thiolato-bridged arene ruthenium complex-sulfadoxine hybrid drug against *Toxoplasma gondii,* **Ghalia Boubaker**

P-22 An *in vitro* model to evaluate anticoccidial compounds, **Sara Arias-Maroto**

P-23 Detection of selected parasites in minced meat and meat products from retail using molecular methods, **Barbora Zalewska**

P-24 Development, inter-laboratory SOP evaluation and application of a molecular method for detection of *Toxoplasma gondii* oocysts in ready-to-eat leafy-green salads in a multicentre study in Europe, **Barbora Zalewska**

P-25 Development of *in vitro Cryptosporidium parvum* models using bovine intestinal organoids, **Mathilde Svensen Varegg**

P-26 Characterization of the translationally controlled tumor protein gene (tctp) in *Babesia bovis* and evaluation of its participation in the establishment of infection, **Juan Mosqueda**

P-27 Selection of conserved surface antigens of *Eimeria sp.* through a bioinformatic approach, **Virginia Marugan-Hernandez**

P-28 Cultivation of *Theileria annulata* transformed cells in serum-free media, **Khawla Elati**

P-29 Comparative view on the asexual cycle of *Besnoitia besnoiti* and *Toxoplasma gondii in vitro.* **Catherine Graue**

P-30 Identification of *Sarcocystis* species present in diaphragm muscles from wild boars (*Suis scrofa*) in Switzerland, **Gastón More**

P-31 Coccidia infection in Italian intensive swine breeding: epidemiological update and analysis of risk factors, **Alessia Libera Gazzonis**

P-32 “Shedding light” on *Eimeria spp*. Dense Granules: fluorescent reporters as reliable biomarkers for subcellular localization studies. **Gonzalo Sanchez-Arsuaga**

P-33 Response to *Toxoplasma gondii in vitro* infection of macrophages and neutrophils from vaccinated sheep, **Daniel Gutiérrez-Exposito**

P-34 Comparison of paromomycin efficiency on the *Cryptosporidium parvum* development by standard methods and impedance spectroscopy, **Anne-Charlotte Leniere**

P-35 Characterization of intestinal mononuclear phagocyte subsets of young lamb at homeostasis by single cell RNA-Seq and during *Cryptosporidium parvum* infection by flow, **Sonia Lacroix-Lamande**

P-36 *Eimeria* Vaccine Development Through Graph Based Machine Learning, **Roman Baptista**

P-37 Are *Toxoplasma gondii* infected water voles (*Arvicola amphibius s.l.*) easy prey for cats? **Miguel Pardo Gil**

P-38 *Toxoplasma gondii* and *Neospora caninum* seroprevalence among cattle in Ukraine, **Maryna Galat**

P-48 *In vitro* anticoccidial effect determination of naringenin and dehydrated grapefruit peel (*Citrus x paradisi*). **Maria Cristina Guerrero Molina**

Poster session 2

Friday October 7, 12:45-14:30

P-39 Tropism and persistence of *Toxoplasma gondii:* from pork carcass to dry sausage, **Filip Dámek**

P-40 Meat processing as part of a quantitative microbial risk assessment for *Toxoplasma gondii* infections in Europe. **Marieke Opsteegh**

P-41 Diagnosis of *Sarcocystis spp.* in pigs and wild boars from Argentina, **Gastón More**

P-42 Evaluation of risk of infection by *Toxoplasma gondii* from retail meat in São Paulo city, **Hilda FJ Pena**

P-43 Detailed anatomical distribution of *Toxoplasma gondii* in tissues of infected pigs, **Filip Dámek**

P-44 Comparison of *Toxoplasma gondii* distribution in tissues of experimentally infected pigs, **Filip Dámek**

P-45 Molecular detection of different *Sarcocystis* spp. in cattle carcasses affected by bovine eosinophilic myositis, including a putative new species**, Selene Rubiola**

P-46 Detection of viable *Toxoplasma gondii* in game meat, **Clare M. Hamilton**

P-47 Efficacy of paromomycin against calves cryptosporidiosis, **Joze Staric**

P-49 Identification of the phosphodiesterase inhibitor beminafil as a potential anti-cryptosporidial drug, **Jubilee Ajiboye**

P-50 *Toxoplasma gondii* MOB1 protein at the crossroad of cytokinesis and tissue homeostasis, **Inês L. S. Delgado**

P-51 Quantification of integrated selection markers in *Toxoplasma gondii* knockouts. **Kai Pascal Alexander Hänggeli**

P-52 Elucidating the cellular and molecular mechanisms of stage progression in cyst-forming Apicomplexa, **Valerio Anelli**

P-53 A novel preconoidal complex identified in a sCas9 phenotypic screen is essential for motility in *T. gondii,* **Elena Jimenez-Ruiz**

P-54 Global proteomic analysis of different in vitro stages of *Cystoisospora suis*, **Teresa Cruz-Bustos**

P-55 Imaging the ultrastructures of *Toxoplasma gondii* by expansion microscopy, **Romuald Haase**

P-56 Structure-based functional investigation of the glideosome associated connector of *Toxoplasma gondii,* **Gloria Meng-Hsuan Lin**

P-57 A synergistic approach identifies host factors essential for the survival of intracellular Apicomplexa, **Marina Maurizio**

P-58 BLT-1 blocks apicomplexan parasite replication *in vitro,* **Liliana M. R. Silva**

P-59 Deciphering the pathogen-host interaction at the placenta in the bovine neosporosis, **Iván Pastor-Fernandez**

P-60 *Toxoplasma gondii*-driven host cell DNA damage in primary human cells through the effector protein HCE1/TEEGR, **Zahady Velasquez**

P-61 The antigen recognition portion of African buffalo class I MHC is extremely polymorphic consistent with a complex pathogen challenge environment and the 3' region suggest distinct haplotype configurations, **Isaiha Obara**

P-62 Effect of the deletion of NcBpk1 or NcRop2 on the virulence of *Neospora caninum,* **Rafael Amieva**

P-63 A new highly-performant competitive ELISA for the detection of *Besnoiti besnoiti* antibodies in cattle, **Laura Olagnon**

P-64 First description of an immunogenic recombinant protein for *Besnoitia besnoiti* antibody detection in cattle, **Laura Olagnon**

P-65 Seroprevalence of *Neospora caninum* in cattle in the Madeira Island (Portugal), **Helga Waap**

P-66 Exploring serological and hematological markers for diagnosis and monitoring of *Besnoitia besnoiti* in a naturally infected herd in the early phases of infection, **Alessia Libera Gazzonis**

P-67 *Toxoplasma gondii* genetic diversity in the South Region of Brazil and study of its population structure in the country, **Hilda F. J. Pena**

P-68 Genotyping *Toxoplasma gondii*: whole genome sequences of isolates across Europe reveal diversity within European type II strains, **Pavlo Maksimov**

P-69 Detection of anti-*Toxoplasma gondii* antibodies in meat juice from retail meat cuts, **Hilda F. J. Pena**

P-70 First identification of *Sarcocystis spp*. in synanthropic and wild rodents from Argentina, **Gastón Moré**

P-71 Exposure of Galapagos birds to *Toxoplasma gondii* suggests different transmission pathways, **Juan Daniel Mosquera**

P-72 *Neospora caninum, Sarcocystis spp*. and *Toxoplasma gondii* infections and their relationship with milk production in goats from Argentina, **Gastón Moré**

P-73 *Toxoplasma gondii*-abortion outbreak in dairy sheep from Argentina, **Yanina P. Hecker**

P-74 Detection of *Toxoplasma gondii* specific antibodies in pigs using an oral fluid-based commercial ELISA: advantages and limitations, **Johanna Kauter**

P-75 Difficulties in the control of bovine neosporosis in Terceira Island, Azores, Portugal, **Pedro Faria**

P-76 Typing of European *Toxoplasma gondii* type II strains by a novel Ion AmpliSeq-based technique, **Maike Joeres**

P-77 Evaluation of *Eimeria spp.* parasites as surrogates for the study of *Toxoplasma gondii* oocyst inactivation, **Laure Augendre**

P-78 *In vitro* model to screen plant extracts and evaluate anticoccidial activities of innovative compounds, **Sonia Lacroix-Lamandé**

Abstracts of Keynotes

K-1

Between Scylla and Charybdis; how *Toxoplasma gondii* maintains avirulence

Jonathan Charles Howard

Instituto Gulbenkian de Ciencia, Oeiras, Portugal
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Persistent parasites have to avoid speedy elimination by the immune system, but they also have to avoid killing the host before they can be transmitted. *Toxoplasma gondii* is a master of the art. We are beginning to discover how it does this, at least in the mouse. Mice defend themselves against *T. gondii* infection using a family of immune-inducible GTPases, IRG proteins, that seem to be dedicated to a surprising extent to resistance against *T. gondii*. At least that's how we have been thinking of them. IRG proteins bind to the parasitophorous vacuole membrane and disrupt it, killing the parasite, an action that is life-saving for the mouse. However, we have now shown that each PVM is decorated with one or more specific high affinity receptors for individual IRG proteins, and the best bet is that these receptors are secreted by the parasite, forcing us to consider that it may after all be the parasite that is pulling the strings.

K-2

Toxoplasma gondii genotypes in Europe – a lot we know – a lot we do not know

Gereon R.M. Schares[1], Maike Joeres[1], Garance Cardron[1], Pavlo Maksimov[1], Pablo Angulo-Lara[2], Rafael Calero-Bernal[2], Mercedes Fernández-Escobar[2], Břetislav Koudela[3,4], Paolo Vatta[5], Caccio Simone[5], Luis Ortega-Mora[2], Pikka Jokelainen[6]

1: Federal Research Institute for Animal Health, Institute of Epidemiology, Greifswald-Insel Riems; 2: SALUVET, Animal Health Department, Faculty of Veterinary Sciences, Complutense University of Madrid, Spain; 3: Central European Institute of Technology (CEITEC), University of Veterinary Sciences Brno, Czech Republic; 4: Faculty of Veterinary Medicine, University of Veterinary Sciences Brno, Czech Republic; 5: Italian National Institute of Health, Rome, Italy; 6: Statens Serum Institut, Copenhagen, Denmark
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It is well established that *Toxoplasma gondii* has a largely clonal population structure in Europe, which is dominated by clonal types II and III. There is evidence that representatives of these European *T. gondii* clonal lineages are, to a limited extent, genetically diverse. However, it remains unclear what caused this genetic variability and its possible epidemiological implications. A number of previous reports indicated a putative involvement of unusual genotypes in human cases of ocular and cerebral toxoplasmosis in particular European countries. However, detailed knowledge on the existence of non-clonal *T. gondii* genotypes in Europe is lacking and many European geographical regions and life-cycle compartments (e.g. wildlife, environment) are understudied. Further studies, using harmonised genotyping tools with a high resolution are necessary.
Aims of TOXOSOURCES Work Package 5, funded by the One Health European Joint Programme:

- The detailed characterization of as many whole genome sequences of European T. gondii as possible
- Establishment of a new Next Generation Sequencing-based typing tool along with the harmonization of microsatellite typing, the present reference for genotyping and fingerprinting T. gondii
- Initiation of a number of local as well as European wide studies to add further knowledge on the genotypes circulating in Europe

The main outcome of TOXOSOURCES Work Package 5 is going to be summarized and still existing gaps of knowledge discussed.

Funding: 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 No 773830: One Health European Joint Programme.

K-3

A One Health approach to disease surveillance in humans

Eskild Petersen

Professor emeritus, Institute of Clinical Medicne, Faculty of Health Science, University of Aarhus, Denmark; Chair ESCMID (European Society for Clinical Microbiology and Infectious Diseases) Emerging Infections Task Force, Basel, Switzerland
eskild.petersen@gmail.com

Sixty percent of new infections in humans over the past seventy years have a zoonotic origin and many well-known emerging new infections in humans have a zoonotic reservoir. This applies to parasites, bacteria and virus. Examples include pandemic H1N1 influenza (2009), SARS-CoV-1 and 2, *Coxiella burnetii*, avian influenza H7N9, Ebola virus and HIV has a zoonotic origin.
Surveillance for new emerging pathogens need to be strengthened not least in the light of SARS-CoV-2 and monkeypox. The obvious points of surveillance are situations with intimate contact between many animals and humans, for instance animal farms like pigs, goats, poultry and the situation in mink in Denmark in the autumn of 2020 is another example. Such surveillance should be based on technologies able to identify new, previously unknown pathogens for instance novel coronavirus.
For well-known zoonotic infections of human importance, infections like *Brucella* spp., *Toxoplasma gondii* and *Cryptosporidia* spp. among others surveillance must be focused on the transmission routes aiming at reducing animal-to-human transmission. The production of pathogen free food and drinking water must be the ultimate goal and the presentation will focus on whether this is possible.

K-4

BKIs for treatment of apicomplexan diseases: Aligning the holes in the Swiss cheese for optimal safety, efficacy, and PK

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Bumped-kinase inhibitors (BKIs) have been shown to treat a number of apicomplexan animal and human diseases, including cryptosporidiosis, toxoplasmosis, neosporosis, sarcocystosis, and cystoisospororosis. A lead that has the safety properties consistent with treatment of both animal and human diseases is highly desirable. Recently we have focused on 5-aminopyrazole-4-carboxamide (AC) compounds, as they have less hERG inhibition (cardiotoxicity for humans) and have superior efficacy and safety profiles. We will report the investigation of 3 AC leads, BKI-1770, -1841, and -1708. All three showed superior efficacy in the *C. parvum* newborn calf model, in both diarrhea abrogation and parasitological reduction. BKI-1770 was found to enlarge the epiphyseal growth plate of young mice and calves at doses only slightly higher than efficacious doses. BKI-1841 and BKI-1708 did not demonstrate bone toxicity in 3-4-week-old mice. BKI-1841 and BKI-1770 caused reversible dropped fetlocks and plantigrade stance in calves at or near the lowest therapeutic dose. We hypothesized these compounds might have CNS effects. We studied mice treated with the 3 leads for 5 days in the locomotor activity box test after the final dose, where mouse movement is tracked by lasers. Both BKI-1770 and -1841 treatment led to decreased movement (increased resting time, reduced distance traveled) in this model. Treatment with BKI-1708 did not show these effects, but slightly increased motion was observed. Thus, BKI-1770 and -1841 studies have been halted on account of toxicity. Subsequent studies with BKI-1708 have demonstrated a significant safety index between the systemic concentration attained in mice and calves during minimal cryptosporidiosis efficacious dosing and the concentrations associated with adverse reactions in mice, rats, and dogs. BKI-1708 is converted in vivo by CYP450 enzymes to a hemiaminal metabolite that retains anti-parasitic activity, and the metabolite is slowly cleared, leading to sustained anti-parasitic effect. Because of this persistence of the active metabolite, daily and even single dose therapy may be possible with BKI-1708. Thus BKI-1708 has become our pre-clinical lead, for its superior safety profile and the possibility of fewer doses for efficacy.

K-5

Improved quantification and discrimination of *Eimeria* species promotes research on their evolutionary ecology and epidemiology

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Coccidian parasites are neglected in evolutionary ecology and epidemiology. One reason for this might be that these disciplines are conceptually “lumping” protists together with bacteria and even viruses as “microparasites”. In addition, seemingly trivial difficulties in distinguishing and quantifying Coccidia make them less attractive than macroparasites for field biologists. We therefore developed methods for the quantification of *Eimeria* and tested them in house mice. We show that the amount of parasite DNA in fecal samples can be biologically meaningful, even if it does not agree well with complementary oocyst counts. DNA-based parasite intensity, for example, correlates better with the hosts’ weight loss in *E. ferrisi* infections of house mice. Parasite DNA can be quantified at relatively high throughput using PCR (qPCR), but amplification needs a high specificity and does not distinguish parasite species. Therefore, we tested whether counting sequence variants for a relatively universal primer pair has the potential to be both more specific and more open-ended. We show that: 1) amplicon sequencing distinguishes between three closely related *Eimeria* species, 2) discovers (off-target) taxa for which the amplification was not preconceived, and 3) allows precise species-specific quantification. Based on this, we use amplicon sequencing to study the evolutionary ecology and epidemiology of *Eimeria* species hoping to increase awareness for the peculiarities of apicomplexan parasites in these disciplines. I will give examples of how the house mouse, as both an “accidental farm animal” and biomedical model species, allows us to study the evolution and transmission of Coccidia in semi-natural and agricultural settings.

K-6

Vaccines for coccidian: are we there yet?

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With the Covid19 pandemic we have been spectators of the quickest development of a vaccine and release to the market. These were exceptional circumstances that counted with some critical points: funding, integration of the whole development process and a direct cooperation between Industry and Academia. We have also seen the recent approval of the first malaria vaccine. If we look at our specific field, vaccines against apicomplexan protozoa of veterinary importance, where can we consider we are?

Looking in particular to the coccidian group, there are live vaccines commercially available for *Eimeria* and *Toxoplasma*, but these date from few decades ago. In terms of vaccine research, live attenuated-vaccines under investigation have shown best results in efficacy by far. However, issues related with safety by a potential reverting to virulence or registration of GMOs as vaccines could be challenges for their commercialisation. Attempts to develop new generation recombinant vaccines have led to the discovery and evaluation of multiple antigens. The rationale behind antigen discovery has followed different approaches such as the presence of antibodies in natural infections; parasite stage specificity; important roles in key biological processes (e.g. host-cell invasion, formation of the parasitophorous vacuole); bioinformatic pipelines based on protein features prediction (e.g. signal peptides, transmembrane domains, MHC predicted binding regions), population-based genetic fingerprinting, etc... There are stories of success for some antigens, which are still on path for potential commercialisation. Other did not achieved good results, others that seemed promising just disappeared forever when specific funding finished.

Looking at the host, in most of the cases, animals develop protective immunity after one or further expositions to the parasite, therefore, understanding the immune mechanisms activated after a primary and a secondary infection would be paramount to define the type of responses that we should generate with a vaccine. As intracellular parasites, protective immunity is generally linked to a Th1 cell-mediated adaptive immunity response. In consequence, recombinant vaccines based on peptides identified as T cell epitopes – antigens directly involved in stimulating adaptive immune responses by binding MHCs – will potentially confer immunity against parasite re-infection. Additionally, selection of delivery system and adjuvants helping to stimulate Th1 responses would be a preferred choice.

It is generally agreed that a recombinant vaccine would require a pool of different antigens due to the complexity of these parasites, therefore, there is still scope for research in antigen discovery that could enhance the levels of protection achieved by the current candidates. Integrated approaches including commercial companies since initial steps could speed up the vaccine development process and increase the chances to reach the market.

K-7

Nanoscale imaging of the conoid and functional dissection of its dynamics in Apicomplexa

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Members of the Apicomplexa phylum are unified by an apical complex tailored for motility and host cell invasion. It includes regulated secretory organelles and a conoid attached to the apical polar ring from which subpellicular microtubules emerge. In coccidia, the conoid is composed of a cone of spiraling tubulin fibers, two preconoidal rings and two intraconoidal microtubules. The conoid extrudes through the apical polar ring in motile parasites. Advances in proteomics, expansion microscopy and cryo-electron tomography provide a more comprehensive view of the spatial and temporal resolution of proteins belonging to the conoid subcomponents. In combination with the phenotyping of targeted mutants, the biogenesis, turnover, dynamic and function of the conoid begin to be elucidated.

K-8

Endochin-like quinolone prodrug, ELQ-422, a lead candidate for toxoplasmosis with broad activity against apicomplexan pathogens

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Endochin-like quinolones are cytochrome bc_1 complex inhibitors that are highly effective *in vitro* and in mouse models of acute and chronic toxoplasmosis. ELQ-316 and the ELQ-316 prodrug, ELQ-422, are superior to current drugs for toxoplasmosis across multiple measures of efficacy. ELQ-316 is 50 to 500 times more potent, *in vitro,* than clinically used drugs, and is 10 times more effective at reducing systemic parasite burden than atovaquone when given orally for 5 days. In a highly virulent survival model of toxoplasmosis, oral administration of ELQ-422 resulted in greater survival than pyrimethamine, atovaquone and the antimalarial ELQ-331. Recently, we have determined that ELQ-422 is effective in a congenital mouse model of toxoplasmosis and prevents reactivation of *T. gondii* cysts when administered once every 3 weeks intramuscularly. In addition to promising efficacy and pharmacokinetic properties, ELQ-422, did not result in observable toxicity in long duration efficacy models or in high dose rat toxicity studies. The efficacy of the active compound, ELQ-316, also extends to other apicomplexan pathogens, *Plasmodium* species, *Babesia* species*, Theileria, Besnoitia* and *Neospora caninum.* Based on these results, ELQ-422, is a lead preclinical candidate for veterinary and human infections caused by apicomplexan pathogens.

K-9

Modulation of host FOXO1 activity in *Theileria*-transformed cells

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Theileria are tick-borne apicomplexan parasites that modify the phenotype of their host cell to a remarkable extent. Within a few days of infection, parasitized leukocytes acquire a transformed phenotype comprising uncontrolled proliferation, resistance to apoptosis, immortality and increased invasiveness. RNA sequencing allowed us to identify extensive gene expression changes in primary macrophages following infection with *Theileria*. These include an upregulation of genes involved in DNA replication, cell cycle and translation, and a downregulation of genes involved in adhesion and innate immune response. Intriguingly, we noticed that expression of the transcription factor Forkhead box protein O1 (FOXO1) is increased following infection, and immunofluorescence analysis confirmed that FOXO1 is localized in the nucleus of infected cells. FOXO transcription factors regulate many cellular processes including nutrient metabolism, DNA damage response, autophagy, cell cycle progression and oxidative stress response. To try to understand how *Theileria* modulates FOXO1 activity, we used a comparative bioinformatics approach to identify *Theileria*-encoded effector proteins that are exported into the host nucleus. To facilitate this we performed Oxford Nanopore and Illumina whole genome sequencing on seven *T. annulata* clones isolated from different geographical regions. We developed a bioinformatic workflow to analyse features such as selective pressure (dN/dS ratio) and protein disorderedness to identify and validate novel *Theileria* effector proteins. One of the identified proteins, TaC12_008960, is highly enriched in the host nucleus where it binds to FOXO1. We are exploring the functional significance of this interaction and testing what effect TaC12_008960 nuclear expression has on FOXO1 activity.

K-10

In vitro and *in vivo* models for the evaluation of *Toxoplasma gondii* strain virulence

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Toxoplasma gondii genetic and phenotypic diversity has been proposed to be responsible for the variation in clinical outcomes, mainly related to reproductive failure and ocular and neurological signs. Different *T. gondii* genetic haplogroups showed strong phenotypic differences in laboratory mice infections, which provide a suitable model for mimicking acute and chronic infections. In addition, it has been observed that degrees of virulence might be related to the physiological status of the host and its genetic background. Currently, mortality rate (lethality) in outbred laboratory mice is the most significant phenotypic marker, which has been well defined for the three archetypal clonal types (I, II and III) of *T. gondii*; nevertheless, such a trait seems to be insufficient to discriminate between different degrees of virulence of field isolates. Many other non-lethal parameters, observed in both *in vivo* and *in vitro* experimental models, have been suggested as highly informative, yielding promising discriminatory power. An overview of *in vivo*, *ex vivo*, and *in vitro* models applied in literature for the evaluation of *T. gondii* phenotypic traits is summarized. While genetic characterization has been normalized to some extent, neither phenotypic characterization nor even the definition of virulence have been harmonised. Recalling the importance of the bioethical aspects, the present talk also proposes a baseline (minimum required information) for the phenotypic characterization of *T. gondii* virulence and intends to highlight the needs for consistent methods when a panel of *T. gondii* isolates is evaluated.

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Abstracts of oral presentations

One-Health

O-1

Systematic review and modelling of the age-dependent prevalence of *Toxoplasma gondii* in livestock, wildlife and felids in Europe

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Toxoplasma gondii is a zoonotic parasite of importance to both human and animal health. The parasite has various transmission routes, and meat of infected animals appears to be a major source of infection in Europe. We aimed to develop an age-dependent model for *T. gondii* prevalence in a selection of its key animal host species in Europe.
A systematic literature review, containing 226 eligible publications, was followed by a meta-analysis using a Bayesian model, including relevant covariates, to create an age-dependent model for *T. gondii* prevalence in more than 50 animal species.
Overall estimated seroprevalence ranged from 2.0 % in indoor-kept lagomorphs to 78.5 % in outdoor-kept sheep. Prevalence estimates at slaughter age varied between European regions and types of detection method applied. Using indirect detection methods, *T. gondii* seroprevalence estimates were the highest in Eastern Europe, whilst they were the lowest in Northern and Western Europe.
The estimates from the model provide a unique overview and valuable input for source attribution approaches aiming to estimate the relative contribution of different sources of *T. gondii* human infection. The data, with emphasis on regional and age-related *T. gondii* prevalence estimates, will be used as input data in a multi-country quantitative microbiological risk assessment within the TOXOSOURCES project. The work will contribute to the development of effective One Health prevention strategies.

O-2

In vitro assay to determine inactivation of *Toxoplasma gondii* in meat samples

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Consumption of raw and undercooked meat is considered as an important source of *Toxoplasma gondii* infections in Europe. However, most non-heated meat products are processed by salting and addition of additives. NaCl affects *T. gondii* viability, but published experiments show variable results and are not always performed in line with industrial processing. Currently, mouse bioassay is the standard for determining *T. gondii* viability, but the use of experimental animals limits possibilities for large scale testing. It was our aim to develop an in vitro method to substitute the bioassay and to determine the effect of salting on *T. gondii* viability.
Two sheep were orally inoculated with 6.5×10^4 oocysts, and infection confirmed by the detection of anti-*T. gondii* antibodies in combination with qPCR on tissue samples collected 10 weeks post inoculation. Grinded meat samples of 50 g were prepared from heart, diaphragm, four meat cuts (thick flank, strip loin, topside and silverside), and a pooled sample made from those meat cuts. The pooled meat samples were either kept untreated (positive control), frozen (negative control) or supplemented with 0.6%, 0.9%, 1.2% or 2.7% NaCl. All samples were digested in pepsin-HCl solution. For each, 1 ml of digest was inoculated in duplicate onto monolayers of RK13 (a rabbit kidney cell line). Cells were maintained for 4 weeks, replacing 50% of the cell culture medium two times per week. Parasite growth was monitored by assessing the Cq-values in *T. gondii* qPCR for the cell-culture supernatant in intervals of one week. Decreasing Cq-values (i.e. a positive ΔCq) indicated multiplying parasites. To confirm viability of parasites in untreated meat samples, 1 ml of each digest for the individual meat cuts, heart and diaphragm were inoculated in duplicate in IFNγ KO mice. Mice were euthanized when developing signs of toxoplasmosis or kept for six weeks, and tested serologically and by qPCR.
Both sheep developed an antibody response and tissue samples contained similar concentrations of *T. gondii* DNA. For all untreated meat samples positive ΔCq values were detected in cell culture. These results indicated viability of parasites in individual meat samples; this was in line with mouse bioassay, with the exception of a negative mouse bioassay for the heart of the second sheep.
Samples supplemented with 0.6%-1.2% NaCl showed positive ΔCq values over time. The frozen sample and sample supplemented with 2.7% NaCl remained qPCR positive but with high Cq-values which indicated no growth.
In conclusion, the in vitro method has successfully been used to detect viable *T. gondii* in tissues of experimentally infected sheep, and a clear difference in *T. gondii* viability was observed between the samples supplemented with 2.7% NaCl and those with 1.2% NaCl or less. In future, more NaCl concentrations or additives will be tested to obtain better insight in the risk of *T. gondii* infection via non-heated processed meat products.

O-3

Identification of *Cryptosporidium* sp. in livestock, domestic animals, wildlife and water source samples from seven farms in Wales.

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Cryptosporidiosis is a zoonotic disease known to affect humans and domestic livestock species. Infection in humans is predominantly caused by either *Cryptosporidium parvum* or *C. hominis* and is considered the second most common cause of infant human diarrhoeal disease and death in Asia and Africa. In the UK, there are usually 4000 – 7000 cases of human cryptosporidiosis reported annually, though fortunately deaths are very infrequent. Cryptosporidiosis is also one of the most commonly diagnosed causes of neonatal ovine enteritis / mortality in the UK.

Faecal samples (n=187) were collected from neonatal lambs aged <1 week - 8 weeks and adult sheep from farms (n=7) across Wales. Faecal samples (n=17) were also collected from other domestic/wildlife species (cattle, cat, dog, rabbit and pheasant). Each sample was weighed and diluted 6.5x in water. Following dilution, samples ≤1.5ml went directly to DNA extraction, for all other samples up to 15ml of diluted faeces was processed through saturated salt floatation to concentrate *Cryptosporidium* oocysts. Repeated water samples (n=34 total) were collected from boreholes and water sources on farms (n=4). Each water sample (2-10L) was concentrated through a membrane filtration and IMS prior to final elution.

All samples underwent 10x rounds of freeze / thawing prior to DNA extraction (Macherey-Nagel Nucleospin Tissue Kit). *Cryptosporidium* DNA was detected using a nmPCR for both *Cryptosporidium* sp. and *C. parvum* 18S DNA (in triplicate). All *C. parvum* PCR positive samples were also analysed using a nested *gp60* PCR, Amplicons from all 18S and *gp60* positive samples were purified and sent for sequence analysis.

For the sheep, 50/187 (26.7%) samples tested positive for *Cryptosporidium* sp. DNA, with positive samples being identified on all seven farms. *Cryptosporidium parvum* DNA was identified in 40/50 (80%) positive samples, while 7/50 (14%) and 3/50 (6%) were positive for *C. xiaoi* and *C. ubiquitum* respectively.

For the domestic/wildlife species 6/17 (35.3%) were positive for *C. parvum*, (4x calf, 1x rabbit and 1x dog), while 2/17 (11.8%) samples (2x dog) were positive for *C. canis*.

For the water samples 24/34 (70.6%) of samples tested positive for *Cryptosporidium* sp. DNA, with positive water samples being identified from all four farms. Sequence analysis identified 16/24 *C. andersoni*, 5/24 *C. parvum* and one samples for each *C. ubiquitum, C. canis* and *C. proventriculi*, interestingly 23/34 samples were also positive by *C. parvum* PCR.

The *gp60* data showed 31/50 sheep, 2/6 calves and 11/24 water all had the IIaA15G2R1 genotype, while IIaA13G2R1 and IIaA16G3R1 genotypes were seen in one calf and two water samples respectively.

Our results show that *C. parvum* was commonly detected on all of the farms, in livestock, domestic animals and water samples. Mixed *Cryptosporidium* species were frequently observed in water samples and IIaA15G2R1 is the most common genotype identified.

O-4

Toxoplasma gondii infection in the Eurasian beaver (*Castor fiber*) in Switzerland

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Toxoplasma gondii is a coccidian parasite which is able to infect practically all warm-blooded animals, and causes one of the most widespread zoonoses worldwide – toxoplasmosis. Definitive hosts are felids that can shed oocysts, which are resistant to harsh environmental conditions and can be found in soil and water. The Eurasian Beaver (*Castor fiber*) is a widespread rodent along Swiss rivers and streams. In recent years *Toxoplasma* infections have readily been detected in beavers subject to post-mortem examination. With a semi-aquatic lifestyle and a strictly herbivorous diet, infection via oocyst ingestion is the main route of infection. Mass die-offs in California sea otters, which have a similar way of living, have been linked to sewage-contaminated coastal waters. In Switzerland beavers share close habitats with other wildlife, livestock and domestic animals and can function as somewhat of a sentinel species for environmental contamination. Prevalence of infection in Swiss beavers is currently unknown and so is the clinical relevance. The parasite has a high genetic variability and some *T. gondii* genotypes were associated with a higher virulence in mice and more severe disease manifestation in humans. The aim of this study was to assess the epidemiological role of the beaver as an intermediate host by determining seroprevalence, parasite occurrence within tissues, and to identify which *T. gondii*-genotypes occur in the Swiss beaver population and might circulate in the sylvatic and domestic fauna. For the seroprevalence study, 247 serum samples collected from dead beavers between 2002 and 2022 were tested by ELISA. A total of 114/247 (46.2%) samples tested positive for *T. gondii* antibodies. Besides, DNA was extracted from fresh brain samples of a subset of animals (*n*=41), and tested for *T. gondii* DNA by real-time PCR. *T. gondii* DNA was detected in 23/41 (56.1%) samples. An adapted multilocus PCR-RFLP approach, including 11 markers, was used for further genotyping. Complete allele patterns were obtained from 22 samples and revealed genotypes ToxoDB #1 (2/22) and ToxoDB #3 (19/22), which belong to the archetype II and its closely related type II variant form. One sample revealed an allele pattern that was not previously described. In this novel genotype all markers corresponded to type II except marker alt. SAG2, which is a type I allele.
Further investigations are still ongoing to better understand the potential effects of different genotypes on the clinical and histopathological disease presentation. However, a preliminary conclusion is that the high seroprevalence indicates frequent contact between beavers and the parasite. Detection of *Toxoplasma* DNA in brains and the following genotyping, which was carried out for the first time in Swiss beavers, show that both intermediate virulent genotypes commonly found in Europe, ToxoDB #1 and #3, as well as new genotypes occur.

Epidemiology / diagnostic tools

O-6

Neosporosis in Argentina: past, present and future perspectives

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Neosporosis, caused by the protozoan *Neospora caninum*, was first diagnosed in Argentinean cattle in the ´90. Having a national bovine stock is approximately 51 million heads, cattle industry is social and economically relevant in Argentina. Severe economic losses have been estimated in 33 and 12 million US dollars annually in dairy and beef cattle, respectively. Nowadays approx. 9% of bovine abortions in the Buenos Aires province are caused by this parasitic disease. The first isolation of *N. caninum* from oocysts in the feces of a naturally infected dog was performed in Argentina in 2001. Another 3 isolates (Nc-Argentina LP1, LP2 and Nc-Axis) were obtained from cattle and chital (*Axis axis*), respectively. Many seroepidemiological and fetal studies revealed a high distribution of *Neospora*-infections not only in dairy but also in beef cattle. Prevalence may vary from 5 to 80% in dairy cattle and from 2 to 50% in beef cattle without antecedents of reproductive losses. Although many attempts were done to obtain successful experimental vaccines to prevent *Neospora*-abortions and transmission, prevention and control strategies are, nowadays, based on the identification at the herd level of seropositive cattle and culled. Embryo transfer has been successfully used in avoiding *Neospora*-vertical transmission. Also, reduction of both seroprevalence and *Neospora* -related abortions have been achieved in dairy farms by the use of selective breeding strategies. *Neospora*-infections have been also reported in goat, sheep, red deer, water buffaloes and foxes. Moreover *Neospora*-related reproductive losses in sheep and red deer were recently reported and could be more frequent que previously though. Furthermore, *Neospora*-abortions have been registered in sheep, water buffaloes and chital. Even though the disease can be easily confirmed by practitioners with the help of diagnostic laboratories, control of neosporosis is not optimal. The development of new strategies including new antiprotozoal drugs and vaccines is needed.

O-7

Do we see changes in the genetic diversity of *Cryptosporidium parvum* genotypes during the calving season within a dairy herd?

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Bovine cryptosporidiosis is a common cause of calf enteritis across the United Kingdom, which is mainly caused by an infection with *Cryptosporidium parvum*. In addition, this infection can be passed onto humans and is overall a cause of damage to both the economy and health. However, a lack of vaccination methods and a limited option of treatments, highlight the importance of this infection.
A longitudinal study on *C. parvum* infection was conducted on a dairy farm in Midlothian to examine genotypic changes during the calving season in order to identify potential sources of infection. Faecal samples were collected before and throughout calving with samples being grouped based on collection location. The pens contained either adult cattle, new-born calves or older calves. We conducted oocyst concentration steps on all samples before DNA extraction. *Cryptosporidium* speciation was done using an 18S nPCR and sequencing. All *C. parvum* positive samples were genotyped using a *gp60* gene PCR and a panel of variable-nucleotide tandem-repeat (VNTR) markers.
Current data, based on the *gp60* locus, shows that there is one genotype (IIaA15G2R1) present in the calf samples and all but 3 of the *C. parvum* positive adult samples, regardless of timing or location. 2 of the adult samples were IIaA15R1 and 1 was IIaA14G2R1. The IIaA15G2R1 *gp60* genotype is the most common *C. parvum* genotype in the United Kingdom. Analysis using the VNTR markers shows that all the IIaA15G2R1 positive samples have the 4-14-5-8-18-37-16 genotype, suggesting a clonal population. In previous years, a IIaA19G2R1 genotype was predominant in the calves on this farm, while more *C. parvum* diversity was seen in adult cattle. This current lack of *C. parvum* genotypic diversity on the farm may indicate that the IIaA15G2R1 genotype has a selective advantage in infecting calves.
Cleaning or sterilisation procedures by the farmer seem to have no impact on the *C. parvum* genotype. Deep littering of the pens (adding fresh straw on top of the existing straw) has no effect on the genotype calves become infected with. Deep cleaning procedures including removal and replacement of straw and disinfection via hydrogen peroxide has no effect either as new-born calves housed into cleaned pens still get infected by the same IIaA15G2R1 genotype almost immediately following birth. The same effect can also be seen when using pens that have not been used for several years. New-born calves housed into these pens were found to be infected with the IIaA15G2R1 genotype on the same day they were moved in. This all suggests that the source of infection is not from the pens that the calves are moved into, but that they become infected either while they are kept with their mothers or from the farmers handling the calves. However, once the infection has established itself in a pen, calf to calf transmission is the predominant transmission route and a full cleaning procedure must be performed to clear/reduce oocyst contaminations.

O-8

Asymptomatic carriers of equine piroplasmosis: prevalence differences between 4 French regions and low genetic diversity of *Theileria equi* and *Babesia caballi*

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Equine piroplasmosis is a tick-borne disease of equids, and is caused by two protozoan parasites, *Theileria equi* (formerly *Babesia equi*) and *Babesia caballi*. In France, equine piroplasmosis is endemic but the prevalence of carrier horses and the genetic diversity of both parasites have never been assessed.

Blood samples were collected between 2019 and 2022 from horses without any symptoms of piroplasmosis. The equids were presented in each of the 4 French Veterinary Schools in France located in Maisons-Alfort (North central, 106 equids), Nantes (West central, 165 equids), Lyon (South East, 151 equids) and Toulouse (South West, 75 equids). Each school was considered as a focal point with horses located in their surroundings, without overlapping localizations. The purpose of the study was explained to each participant, and the signed agreement of the owner was obtained for the recruitment of his animal. Data on the characteristics of the sampled animals were collected through questionnaires completed by the owner on location during sample collection. The infected status of equids was determined by nested PCR specific for each parasite, targeting the 18S rRNA gene, and genotyping was performed by sequencing partial 18S rRNA from most positive samples.

As a whole, 40.5% of the equids were asymptomatic carriers, with a majority of *T. equi* carriers (38.8%) and few *B. caballi* carriers (3.9%), most of them (58%) being also *T. equi* carriers. For *T. equi*, these values were 2.4 times higher than the serological prevalence values described in France in previous studies based on the CFT test (15.9%). Most of the *B. caballi* carriers were identified in the South of France, around Toulouse and Lyon (89.5%), none in Nantes and 10.5% around Paris. For *B. caballi*, the carrier prevalence (3.9%) was twice as low as the previously described seroprevalence.

The piroplasmosis infection prevalence varied greatly between the four veterinary schools with an increasing North to South gradient: 16% around Paris, 30.3% around Nantes, 53.6% around Lyon and 72.9% around Toulouse. More studies are needed, especially on the tick vectors, as they probably explain this gradient of prevalence.

Genotyping was performed by sequencing the amplified portion of the 18S rRNA. Sequences from 193 isolates were obtained for *T. equi*, and from 18 *B. caballi* isolates. Blastn and phylogenetic analysis revealed that 98% of *T. equi* sequences belonged to the genotype E, previously described in several European countries. The remaining isolates belonged to the A genotype, described in many countries around the world. All 18 *B. caballi* sequences clustered in the A genotype.

O-9

Neospora caninum antibodies in tank bulk milk from dairy cattle farms in Italy: spatial analysis and effects on reproductive and productive performances

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Among the available diagnostic techniques, antibody detection in bulk tank milk (BTM) represents a useful tool to estimate and monitor *Neospora caninum* herd prevalence.
To evaluate the prevalence of *N. caninum* and the effect of parasite infection on herd performances, BTM samples collected from 586 dairy herds located in one of the largest dairy production areas in Italy (Lombardy) were analyzed by an indirect ELISA (Innovative Diagnostics, France). Univariate generalized linear models (GLMs) were developed. A purely spatial analysis scanning for clusters with high or low rates for *N. caninum* using the Bernoulli model (SaTScan vers. 10.1) was performed. A maximum entropy approach (MaxEnt vers. 3.4.4) was used to estimate the probability of distribution of the pathogen based on occurrence records together with 19 environmental variables (BIO01-BIO19) obtained from WorldClim.
All sampled farms bred Italian Holstein Friesian under the intensive production system (mean consistency= 351.6 animals; medium age= 3.2 years old). 180 herds resulted positive to *N. caninum* antibodies (Prevalence=30.7%). Smaller farms (< 500 animals) were at a higher risk of infection if compared to bigger ones (> 500 animals) (P=33.8% and P=19.2%; OR=2.15, p-value<0.005); instead, the age did not result a risk factor (medium age=3.3 in positive and 3.2 in negative farms).
The geographical distribution of *Neospora*-positive farms with the highest level of probability (P>0.70) covers the central and the western sectors of the Po valley. Two significant clusters (p-value<0.001) were shown with the most likely cluster of the positive farms with prevalence of 50% and relative risk (RR) of 2.1 and the low risk cluster with no infection cases and expected value of 20.6%. For *N. caninum* positive farms the climate variables with highest gain when used in isolation resulted annual mean temperature followed by precipitation of wettest month, mean temperature of warmest quarter, max temperature of warmest month, precipitation of coldest quarter, temperature seasonality.
Concerning reproductive and productive performances, days open (172.4 vs. 159.2) were higher in positive than in negative farms (p-value<0.001); herd annual (1453591.2 vs. 2231278.5 liters), herd daily (3982.4 vs. 6113.1 liters), and head daily (23.2 vs. 28.5 liters) milk production were lower in positive farms if compared to negative ones (p-value<0.001). Instead, considering milk quality parameters, somatic cell counts were higher (p-value<0.01) in positive than negative farms (257601.8 vs. 238220.9 cells/ml).
Neosporosis is widely distributed in Italian dairy herds and an impact of the parasite on herd performances could be hypothesized. Even if the role of *N. caninum* in alterations of reproductive and productive parameters should be further explored, veterinarians and farmers should be aware of neosporosis, and control plans should be adopted.

Immunology / vaccines

O-10

The Spherical Body Protein 4 from *Babesia bigemina* is a novel gene, contains conserved B-cell epitopes and induces cross-reactive, neutralizing antibodies in *Babesia ovata*.

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Bovine babesiosis is a tick-transmitted disease caused by intraerythrocytic protozoan parasites of the genus *Babesia*. *B. bigemina* and *B. bovis* are the main causative agents of bovine babesiosis in the Americas and *B. ovata* affects cattle in Asia. All *Babesia* species secrete proteins stored in organelles of the apical complex, which are involved in all steps of the invasion process of vertebrate host cells. Unlike other apicomplexans, instead of dense granules, *Babesia* parasites have large, round intracellular organelles called spherical bodies. The evidence suggests that proteins from these organelles are released during the red blood cell invasion process, where the spherical body proteins (SBP) play an important role in cytoskeleton reorganization. To date, there are four spherical body proteins characterized in *B. bovis*: SBP1, SBP2, SBP3 and SBP4. Of these four, SBP4 is more conserved among the different isolates. In this study we describe the gen that codifies for SBP4 in *Babesia bigemina*. By using the *B. bovis* SBP4 sequence, a BLAST analysis was made in the genome of *B. bigemina*. An ORF was identified, specific primers were designed, and the full gene was amplified, cloned, and sequenced. This gene is transcribed and expressed in erythrocytic stages of *B. bigemina* as determined by RT-PCR and western blot analysis respectively and codifies for a 277 amino acids-long polypeptide, including a signal peptide, with a predicted molecular weight of 29.36 KDa. Four peptides with predicted B-cell epitopes were identified to be conserved in 19 different isolates from Brazil, Mexico, Argentina, Israel, USA and Australia. Antibodies against these conserved peptides reduced parasite invasion *in vitro*: 57%, 44%, 42% and 38% for peptides 1, 2, 3 and 4, respectively, compared with the pre-immunization sera ($p<0.05$). Moreover, sera from cattle chronically infected with *B. bigemina* contain antibodies that recognized the individual peptides, as determined by indirect ELISA. Importantly, when cattle were immunized with recombinant *B. bigemina* SBP4, their sera identified *B. bigemina* and *B. ovata* merozoites by confocal microscopy and Western Blot and were able to neutralize parasite multiplication *in vitro* in both species. All these results support the notion that SPB4 is a vaccine candidate against babesiosis caused by *B. bigemina* and *B. ovata*.

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O-11

Multi-omics analysis reveals regime shifts in the gastrointestinal ecosystem in chickens following anticoccidial vaccination and *Eimeria tenella* challenge

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Coccidiosis caused by protozoans of the genus *Eimeria* significantly impacts the poultry industry, commonly manifesting as poor growth and feed conversion with diarrhoea, bloody droppings, and mortality in severe cases. Dysbiosis of gut microbiota is aggravated by coccidiosis and is a cofactor in necrotic enteritis caused by *Clostridium perfringens*. The gut microbiota supports nutrient absorption, immune maturation and protection against pathogens but coccidiosis compromises intestinal ecosystems and raises the risk of comorbidities and diseases. Anticoccidial vaccines protect against subsequent *Eimeria* challenge, but little is known about chicken gastrointestinal phenotypes and gut microbiota changes induced by either infection or vaccination.
Cobb500 broiler chickens were given 15000 E.tenella (Houghton strain) oocysts at day 21 after hatch. Caeca were collected at 6 days post-infection (dpi) and 10 dpi. We assessed metabolomic profiles of chicken caecal tissue alongside a 16S rRNA sequencing survey of microbiota during a vaccine trial using an experimental yeast-based coccidiosis vaccine. Gut pathology (lesion scores) and *Eimeria tenella* parasite load correlated with changes in both host metabolome and gut microbiota composition. There was higher microbiota richness in infected chickens with notable increases in potential pathogenic species including *Proteus mirabilis*, *Clostridium innocuum*, and *Clostridium paraputrificum*. In contrast, the lactic acid-producing *Bifidobacterium*, known as a probiotic, was elevated in vaccinated-challenged chickens suggesting that the yeast-based vaccine could contribute added value by improving gastrointestinal health. A multi-omics factor analysis (MOFA) machine learning model was used to systematically integrate data on gut microbiota and host metabolome, revealing distinct MOFA model profiles among healthy, infected and recovered chickens. Vitamin B metabolism was activated in uninfected healthy and recovered chickens and associated with short-chain fatty acid-producing bacteria, such as *Caproicibacter fermentans* and *Ruminococcoides bili*. On the other hand, essential amino acid metabolisms and cell membrane lipid metabolisms were enriched in infected and vaccinated chickens and metabolites related to nerve cell and cell membrane structures (e.g., sphingomyelin) were notably found in vaccinated chickens.
In summary, the chicken gastrointestinal ecosystem was differentiated into distinct phenotypes corresponding to vaccination and infection status. The MOFA model uncovered latent biomarkers that have potential for diagnosis, treatment, and recovery of coccidiosis which can be targeted to monitor the efficacy of anticoccidial vaccines.

O-12

Immunization with a multivalent *Listeria monocytogenes* vaccine leads to a strong reduction of vertical transmission and cerebral parasite burden in pregnant and non-pregnant mice infected with *Neospora caninum*

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Neospora caninum is an apicomplexan parasite causing abortion and stillbirth in cattle due to vertical transmission of the parasite. Even though neosporosis is a huge veterinary health problem, there is still no vaccine or treatment on the market for the prevention of the disease. The attenuated *Listeria monocytogenes* vaccine vector Lm3Dx was previously generated by deleting the virulence genes *actA*, *inlA* and *inlB* to target the vaccine vector to antigen-presenting cells and to prevent systemic spread of the bacteria. After safety parameters of the *Listeria* vector were assessed *in vitro* as well as *in vivo*, the single mutant strain Lm3Dx_NcSAG1 was tested for its efficacy in a pregnant neosporosis mouse model, with initially promising results. To further improve the efficacy of the *Listeria*-based vaccine formulation, polyvalent vaccine vectors were generated, by inserting multiple immunodominant *N. caninum* antigens such as *sag1*, *gra7* and *rop2* inserted into the vector Lm3Dx resulting in the following mutant vaccine strains: the single mutant Lm3Dx_NcSAG1, the double mutants Lm3Dx_NcSAG1_NcGRA7 and Lm3Dx_NcSAG1_ROP2, and the triple mutant Lm3Dx_NcSAG1_NcGRA7_NcROP2. Female BALB/c mice were randomly allocated into six experimental groups and inoculated three times at two-week intervals with $1x10^7$ CFU of the appropriated vaccine strain by intramuscular injection. The third immunization took place shortly after mating. Seven days post-mating, mice were challenged with a sublethal dose of the highly virulent *N. caninum* strain NcSpain-7 by subcutaneous infection. Non-pregnant mice, dams and their offspring were observed daily until day 25 post-partum, before all mice were euthanized. Immunization with the single mutant Lm3Dx_NcSAG1 and with the triple mutant Lm3Dx_NcSAG1_NcGRA7_NcROP2 resulted in a postnatal pup survival rate of 70%, while in the groups that were inoculated with the two double mutants (Lm3Dx_NcSAG1_NcGRA7 and Lm3Dx_NcSAG1_NcROP2) only 50% and 58% of pups survived respectively. In the positive control group, which was inoculated with the empty vector Lm3Dx, 7% of pups remained alive at the end of the study and almost all pups survived in the negative control group. Dams that were immunized thrice either with the double mutants or the triple mutant vaccine showed a significant decrease in cerebral parasite load compared to the positive control group. In non-pregnant mice, significant reductions of cerebral parasite burden were achieved by inoculating animals either with the double mutant Lm3Dx_NcSAG1_NcGRA7 or with the triple mutant Lm3Dx_NcSAG1_NcGRA7_NcROP2.
Overall, we were able to strongly reduce the vertical transmission as well as the offspring mortality by vaccinating infected mice with the triple mutant. Additionally, significant reductions in cerebral infections could be achieved in non-pregnant mice and dams when immunizing with Lm3Dx_NcSAG1_NcGRA7_NcROP2.

O-13

Yeast-vectored oral immunisation in commercial layer chickens against *Eimeria tenella*

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Coccidiosis is caused by the apicomplexan *Eimeria* spp. and affects poultry production worldwide. *Eimeria tenella*, which infects chicken caecal epithelial cells, is one of the most widespread, pathogenic and economically important *Eimeria* species. Current controls include chemoprophylaxis and the administration of live parasite vaccines, although the widespread occurrence of drug resistance and challenges in vaccine production remain limiting. Novel alternatives for anticoccidial vaccination include use of the yeast *Saccharomyces cerevisiae* expression system for vaccine antigen production and delivery. Yeasts such as *S. cerevisiae* are routinely included in chicken diets as a source of protein and an immunostimulant. We have previously developed and evaluated a *S. cerevisiae*-based vaccine system for oral antigen delivery to chickens, incorporating *E. tenella* Apical Membrane Antigen 1 (EtAMA-1), Immune Mapped Protein 1 (EtIMP-1) and a single Microneme Protein 3 (EtMIC3) repeat. In this study, two different yeast expression systems were used in a chicken vaccination trial: surface display (pYD1) and cytoplasmic inducible (pYES). Hy-Line Brown layer chickens (n=33-34 /group) were orally vaccinated every 3-5 days from day 7 of age with a formulation of three pYD1 lines, each expressing one of the three antigens, or three pYES lines, each expressing one of the same antigens. Groups left unvaccinated or vaccinated using *S. cerevisiae* carrying the empty pYD1 and pYES plasmids were used as controls. Fourteen days after final vaccination, all groups were orally challenged with a high *E. tenella* dose (4,000 oocyst/animal). An additional unvaccinated, unchallenged group was included for comparison. No significant reduction in parasite replication was observed in vaccinated chickens where high intra-group variation was observed. However, a significant reduction in lesion score was demonstrated in both yeast vaccinated groups (Kruskal-Wallis plus Dunn's multiple comparisons test, $p<0.05$). These preliminary data provide relevant information about new candidate yeast-vectored vaccines with refined antigen expression.

Molecular and cellular biology

O-14

Highly efficient genetic crosses in *Cryptosporidium*

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The single celled protozoan parasite *Cryptosporidium* is one of the main causes of diarrheal disease and a major driver of early childhood mortality. *Cryptosporidium* has a single host life cycle whereby both asexual and sexual stages are found in the epithelial cells of the small intestine of a single individual. These haploid parasites are transmitted by a meiotic spore, the oocyst, and inheritance follows canonical mendelian rules. These characteristics make it possible to use forward genetics to unravel the biology of *Cryptosporidium* through genetic crossing. We devised a novel drug resistance cassette based on a single nucleotide mutation in the phenylalanyl tRNA synthetase gene, and demonstrated robust selection of transgenic parasites using luminescent and fluorescent reporters. Importantly, this selection marker can be used in cis and trans, allowing for the generation of multi-locus mutants. We have conducted crosses within and between species to evaluate the boundaries of sexual recombination in *Cryptosporidium*. We crossed *C. parvum* parasites that carry this new selection marker with a *C. parvum* strain marked with the previously established neomycin resistance gene. Co-infection of mice readily produced double drug resistant parasites revealing highly efficient sexual recombination. Flow cytometry, PCR analyses, and whole genome sequencing demonstrated that we could isolate large numbers of recombinant progeny and cleanly exclude the parental lines. Co-infection of mice with *C. parvum* and *C. tyzzeri* did not produce recombinant progeny, indicating that there is a strong interspecies boundary. The development of a highly efficient model for genetic crosses in *Cryptosporidium* will allow us to investigate the genetic basis of phenotypic variation between different strains and we have developed a pipeline for phenotypic and genetic analys

O-15

Toxoplasma Late Embryogenesis Abundant proteins are important for oocyst resistance to environmental stresses

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Toxoplasma gondii oocysts are highly infectious and remain stable in the environment for many years, being resistant to most inactivation procedures. Although the oocyst wall provides an important physical barrier that protects the parasites within oocysts from many chemical stressors, the genetic basis for their resistance to temperature changes, desiccation or high salinity (environmental stressors) has not been studied. Here we show that a cluster of four genes encoding Late Embryogenesis Abundant (LEA)-related proteins are required to provide *Toxoplasma* sporozoites resistance to environmental stresses. LEAs are intrinsically disordered proteins that become structured under stress conditions, playing a role in the protection of cells to changes of water availability, and have been described to provide resistance to environmental stresses in a variety of organisms, including plants, bacteria and invertebrates. *Toxoplasma* LEA-like genes exhibit the characteristic features of intrinsically disordered proteins, explaining some of their properties. Our *in vitro* biochemical experiments using recombinant LEA proteins show that they have cryoprotective effects on *Toxoplasma* lactate dehydrogenase, and the induced expression in *E. coli* results in increased bacterial resistance to freezing. In addition, we knocked out all four *Toxoplasma* LEA genes *en bloc*, since they are all located in a cluster (LEAc) on chromosome XII, by using the CRISPR/Cas9 technology in a cat-compatible ME49 strain, and subsequently assessed the oocyst sensitivity to environmental stresses. We observed that LEAc knockout oocysts were significantly more susceptible to high salinity, freezing and desiccation compared to those from wild-type parasites. In summary, we provide the first detailed description in any protozoan parasite of genes related to LEA proteins and their impact on stress tolerance *in vivo*. Future studies using knockout strains for each of the LEA genes alone or in combination are now warranted to elucidate which LEAs are needed for the resistance effect.

O-16

Advancing understanding of the spatial proteome of *Eimeria tenella* sporozoites using hyperLOPIT

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Eimeria spp. parasites are causative agents of the enteric disease coccidiosis in chickens which, based on recent estimates, costs the global poultry industry ~£10.4 billion annually. *Eimeria tenella* is one of ten obligate intracellular apicomplexan parasites of the genus *Eimeria* that can infect chickens. It is highly pathogenic and commonly found to be among the most prevalent species circulating within flocks. Despite recent advances in genome sequencing and assembly, 46% of *E. tenella* proteins are currently annotated as hypothetical with little indication to their functions. Predicting subcellular localisations of proteins provides an important step in inference of function and allows for better understanding of parasite biology.

Using the high throughput spatial proteomics protocol hyperLOPIT, we have employed a Bayesian based machine learning model to resolve protein subcellular localisations based on protein expression profiles generated from equilibrium density ultracentrifugation. We have identified 1,489 proteins and assigned subcellular localisations to 722 at ≥0.99 confidence. New localisation assignments for a significant number of *E. tenella* sporozoite proteins will greatly improve the research community's understanding of the parasite and its close relatives, including 70 proteins newly assigned to the rhoptry, 48 of which are currently annotated as hypothetical.

Gene ontology analysis revealed functional enrichment of novel proteins in subcellular niches consistent with the known function of the niche, for example three out of four of the newly localised apicoplast proteins are enriched for the biological processes fatty acid/lipid biosynthesis and metabolism and haem biosynthesis. Isoelectric points of proteins have also exhibited significant differences among subcellular niches. Significant differences have been observed in the amino acid compositions of signal peptides in proteins destined for different subcellular niches. Statistically significant co-expression correlations were also observed for proteins localised to several niches, supporting the hypothesis of compartment-correlated transcriptional control. A proteogenomic approach was employed, where alternative models possessing important functional features such as signal peptide and transmembrane domains were identified that were absent in current reference annotations, further improving annotation of the *E. tenella* genome.

O-17

Besnoitia besnoiti genomics and transcriptomics reveal key molecular players in the intermediate and definitive host

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Bovine besnoitiosis is an emergent disease caused by the apicomplexan parasite *Besnoitia besnoiti.* Transmission takes place via close contact or horizontally via haematophagous insects. Despite several attempts to discover the definitive host, none has so far been identified.

Our analysis of the *B. besnoiti* genome reveals genes orthologous to those in the related coccidian *Toxoplasma gondii* that are important for sexual reproduction and oocyst formation. The intactness of these genes and their regulatory elements indicates that the development in the definitive host is still functional. Upon inhibition of histone deacetylase, we observe an upregulation of merozoite and gamete genes corroborating our genomic data and showing the presence of an active reproduction in a definitive host.

We have performed transcriptomics on the fast-replicating tachyzoites, that mediate acute infection, and bradyzoites that are contained in the chronic tissue cysts of *B. besnoiti*. We show that canonical marker genes of *T. gondii* tachyzoites and bradyzoites are similarly regulated in *B. besnoiti*. The latter displays a higher number of stage-specific genes compared to *T. gondii* indicating a higher enrichment of specific stages can be achieved with *B. besnoiti*. Thus, *B. besnoiti* may be suitable as a model organism to study tissue cyst biology in cyst-forming coccidia. However, to date despite many efforts from several research groups, there is no *in vitro* culturing system for *B. besnoiti* tissue cysts that would allow systematic and high through-put molecular analysis. To counter this important limitation, we have recently developed a novel cell culture system that shows promising results.

Taken together, this work provides the basis to address fundamental questions in the biology of *B. besnoiti* and other related cyst-forming coccidia, but also opens up the path to investigate prevention and treatment strategies of chronically infected cattle.

Treatment and control

O-18

The mode of action of *Toxoplasma gondii* tissue cyst inhibitors

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Toxoplasma gondii forms persisting bradyzoites that reside within cysts in brain and muscle tissues and are responsible for transmission and remission of toxoplasmosis. Available medical treatment options are only effective against the pathogenic tachyzoites but fail to eradicate the chronic stages of *T. gondii*.

To address this shortcoming, we screened a set of 253 myxobacterial compounds and 400 compounds from the MMV PathogenBox against tachyzoites and pan-resistant in vitro tissue cysts. We have identified 51 inhibitors that are effective against tachyzoites and 33 against bradyzoites. Interestingly, only 19 compounds inhibit growth of both forms of the parasite underlining their divergent biology.

To investigate the mode of action of these substances we developed an untargeted metabolomics approach using HILIC-UHPLC-MS. We detect a complex metabolome encompassing both known and unsuspected metabolites in *T. gondii*. Based on these data we compare the response of the parasite to our screening hits with those to established metabolic inhibitors.

Several tested compounds effective against T. gondii, like derivates of Aurachin, ELQ-400 and Buparvaquone, share commonly regulated metabolites with the bc1-complex inhibitor Atovaquone, while the majority elicited unreported metabolic signatures. The myxobacterial compound Chlorotonil uniquely affects a N-acetyl-sugar nucleotide that is metabolized by the Golgi apparatus and ultrastructural analysis confirms marked defects in the trans-golgi Chlorotonil treated parasites.

In conclusion, we identified the bc1-complex as a viable target in *T. gondii* bradyzoites and identified a range of bradyzoidal compounds whose modes of action remain to be determined in detail.

O-19

Using a mutator *Cryptosporidium parvum* strain to enable studies of anticryptosporidial mechanism-of-action and drug resistance

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Cryptosporidium species are an important public health burden for humans and an economic burden for the livestock industry. These intestinal Apicomplexa cause life-threatening diarrhea in children, immunocompromised people, and young cattle. The single anticryptosporidial approved for humans, nitazoxanide, is only modestly effective for children and equivalent to a placebo for the immunocompromised, the two groups at greatest risk. And the only approved treatment for bovine cryptosporidiosis, halofuginone, is effective for prevention but has uncertain efficacy for treating established infection. We and others have identified numerous anticryptosporidial drug leads over the last ~12 years. Unfortunately, the mechanisms-of-action (MOAs) for most drug leads identified using cell-based methods remain unknown and methods for Cryptosporidium drug target identification are limited. The piperazine-based compound MMV665917 is one such promising lead series that has proven efficacy in multiple mouse models, dairy calves, and gnotobiotic piglets. While testing an unrelated compound in dairy calves, we observed resistance due to single point mutations in the target but using calves to generate resistance to multiple compounds is impractical. Instead, we are developing methods that use a C. parvum mutator stain (CpMut) to select drug-resistant C. parvum in mice to enable drug target identification and resistance studies. We used CRISPR to engineer CpMut by introducing two amino acid mutations (A300D and A302E) into the DNApol2 (cgd6_4410) proofreading domain. Passaging CpMut in mice for six months demonstrated an 11-fold increase in spontaneous mutations. We then exposed CpMut to episodic high doses of MMV665917 in mice, and phenotypic resistance developed following four doses. Purified oocysts were 57-fold resistant to MMV665917 compared to unselected CpMut parasites. Susceptibility to MMV665917 analogs was also reduced, while the parasites remained susceptible to unrelated anticryptosporidials. Genomic sequencing revealed 111 SNPs specific to the MMV665917-resistant parasite line, but the presence of excess mutations in the mutator line confounds identification of the resistance conferring SNPs. We are now: 1) formally comparing the rate that resistance can be selected in mice using CpMUT and wildtype *C. parvum*; 2) repeating selection of MMV665917 drug resistant parasites to identify mutations common to multiple experiments; and 3) backcrossing MMV665917 resistant parasites with wildtype parasites to map resistance-linked mutations. We expect to gain insight into MMV665917's MOA while simultaneously developing a generally applicable method to study anticryptosporidial MOA and evolution of drug resistance.

O-20

Treatment with the novel bumped kinase inhibitor BKI-1748 confers protection against congenital toxoplasmosis in sheep

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Congenital Toxoplasma gondii infection in humans and in some mammal species, such as small ruminants, is a well-known cause of abortion and fetal malformations. First-line antifolate therapy has a high rate of adverse effects, such as hematologic toxicity and allergic reactions, that compromise their use for prophylactic and therapeutic treatments. Therefore, it is of great importance to identify novel dual-use candidates that would be well-tolerated in humans and animals. Calcium-dependent protein kinase 1 (CDPK1) represents a promising drug target. Bumped kinase inhibitors (BKIs) specifically inhibit the activity of CDPK1. In previous studies using a congenital model of ovine toxoplasmosis, treatment with BKI-1294 conferred a reduction of 71% in abortions and 53% in the vertical transmission of the parasite. However, BKI-1294 has some drawbacks, such as the inhibition of hERG (human ether-a-go-go related gene), that exclude its use in humans. Recently, the newly developed BKI-1748 displayed excellent safety and efficacy features in vitro (parasitostatic effect) and in mice models of T. gondii infection. The aim of this work was to investigate the efficacy of BKI-1748 in a congenital model of ovine toxoplasmosis. Eighteen pregnant sheep were distributed in three experimental groups. Group 1 (G1, n=7) and group 2 (G2, n=8) were dosed orally with 1,000 T. gondii sporulated oocysts at 90 days of gestation (dg). Animals from group 3 (G3, n=3) were mock dosed with PBS at 90 dg. Beginning 48 hours post infection, BKI-1748 was administered orally to G1 at 15 mg/kg, 10 doses every other day. Maximum plasma drug concentrations (C_{max}) of 2-5 µM were reached at 12 hours after each BKI-1748 dose. In addition, BKI-1748 was found in fetal plasma samples (C_{max} of 0.5 µM at 20 hours after treatment). Rectal temperatures were significantly lower in G1 than in G2 between days 5 and 10 pi while in G3 they were in the physiological range throughout the study. All sheep from G2 aborted. By contrast, all sheep from G1 and G3 gave birth to healthy lambs. While all sheep from G2 showed seroconversion, none of the sheep from G1 and G3 seroconverted. Finally, parasite DNA was not detected in cotyledons nor target tissues from the lambs in G1. Therefore, results obtained here demonstrate that BKI-1748 confers protection against congenital toxoplasmosis in sheep. Future studies would be important to investigate the efficacy of this compound using different dose regimes and/or different infection doses before testing its efficacy in field trials.

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Host-parasite interactions

O-22

Identification of CDK-cyclin pair(s) in *Theileria annulata*

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The Cyclin Dependent Kinase (CDK)-Cyclin pair formation is essential for nuclear cell division, DNA synthesis, transcription and differentiation of the cell. The activities of CDKs are regulated by their cyclin partners. The functionality of CDK-cyclin pair(s) has been exhaustively studied in higher eukaryotes and yeast. A few CDK-cyclin(s) have been identified and characterized in *P. falciparum* and *Toxoplasma gondii*. However, no CDK-cyclin pairs have been identified in the case of *Theileria annulata* parasite. In this study, we predicted that *T. annulata* encodes four cyclins (TA11790, TA07820, TA11045, and TA09750) and eight CDKs to regulate cellular processes. However, only three *T. annulata* CDKs (TA09000, TA06730, and TA07000) were predicted to have a role in cell cycle/transcription. These three CDKs and four cyclins were further analysed for their interaction using yeast two hybrid screening method. Our studies suggest that TA07820 specifically interacts with TA09000, while TA11045 interacts with two CDKs, namely TA07000 and TA09000. Further, two cyclins, namely TA11790 and TA09750, interact with CDKs TA09000 and TA06730. The co-localization studies further confirmed interaction of these CDK-cyclin(s). Overall, we identified specific CDK-cyclin pair(s) in *T. annulata* in this study.

O-23

Archetypal type II and III *Toxoplasma gondii* oocysts induce different immune responses and clinical outcomes in experimentally infected piglets

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Livestock animals, such as swine, are an important source of *T. gondii* in the human population. Currently, there is limited knowledge regarding the potential influence that the *T. gondii* genotype might exert on establishing infection in swine. Herein, we investigated the role of 2 *T. gondii* isolates, type II and III, representative of the genotypes circulating in Europe, in the immune response and infection dynamics in piglets. Recently obtained oocysts (10^3) from the *T. gondii* field isolates TgShSp1 (type II, ToxoDB genotype #3) and TgShSp24 (type III, ToxoDB #2) were used for oral infection. Thirteen 50-day-old female piglets of the Landrace-Large White crossbreed were randomly allocated into three different groups: Group 1 (G1, n=5), inoculated with TgShSp1; Group 2 (G2, n=5), inoculated with TgShSp24; and Group 3 (G3, n=3), a noninfected control group. Clinical signs were monitored daily until 42 days postinfection (dpi) when piglets were euthanized. Blood samples were collected weekly to examine the cellular immune response (cytokine profile) in parasite-stimulated peripheral blood and specific IgG, IgG1 and IgG2 responses in sera. Parasite distribution and burden were evaluated in target tissues (brain, heart, tongue, diaphragm, semimembranosus, triceps, longissimus dorsi, masseter and intercostal muscles) using a mouse bioassay and quantitative RT–PCR (qPCR).
Apathy and a moderate decrease in feed consumption were observed in G1 and G2 piglets between 5 and 8 dpi, coinciding with fever (>40 °C). G2 piglets exhibited higher temperatures for a longer duration than G1 animals. Using a mouse bioassay and qPCR, the detection frequency was observed to be higher in G2 vs. G1, and the highest burdens in target tissues were found in G2. Seroconversion was detected at 14 dpi in both infected groups, but higher antibody levels were observed in G2 piglets. Cytokine analyses in stimulated blood revealed the production of IL-8, IL-1β and IFN-ɤ from 7 dpi in both infected groups. Moreover, IL-12 was produced from 7 dpi in G1 and from 14 dpi in G2. Levels of IL-8 were higher in G2, but IL-1β, IL-12 and IFN-ɤ were higher in G1 at 14 dpi. No detectable levels of GM-CSF, IL-4, IL-6, IL-10, TGF-β1, or TNF-α were observed throughout the experiment. This cytokine profile reveals a predominant proinflammatory response that could be involved in limiting *T. gondii* infection in piglets, although it is more efficient against a TgShSp1 type II-driven infection.
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O-24

Genome-wide CRISPR/Cas9 screens for the identification of essential host factors for *Toxoplasma gondii* infection

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Toxoplasma gondii is an apicomplexan parasite infecting virtually all warm-blooded animals, including birds. Roughly one fourth of the human population is chronically infected with this parasite. While mostly asymptomatic, chronic toxoplasmosis can exacerbate as a life-threatening acute condition in immunocompromised adults such as AIDS and chemotherapy patients. From a veterinary point of view, Toxoplasma is also a parasite of significant economic burden, causing losses in livestock production mainly due to abortion in small ruminants. Currently, no treatments available are sufficient to eliminate quiescent tissue cysts and eradicate the parasite from its host. Whilst to date most of the research on the molecular basis of the host-parasite interaction is focused on the parasite itself, little is known regarding the host factors that Toxoplasma requires for infection. Notably, obligate intracellular parasites largely rely on the host cell, e.g. for the acquisition of essential metabolites and the release of toxic waste products. Here, we are currently testing FACS-based genome-wide CRISPR knockout screens with the aim of identifying host factors that Toxoplasma gondii needs for invasion, intracellular development and egress from the host. Some of these may also be required by other parasites of the phylum Apicomplexa. If successful, our results would broaden our knowledge on host-pathogen interactions and open new possibilities to develop therapeutic approaches to treat toxoplasmosis, specifically targeting the host instead of the parasite.

O-25

A microscopy-based CRISPR screen reveals the essential host genome for *Cryptosporidium* parvum growth and development in vitro

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Cryptosporidium is a leading cause of diarrheal disease in newborn humans and commercial livestock. As an obligate intracellular parasite, *Cryptosporidium* is reliant on its host cell for the supply of nutrients and shelter during replication. To better understand this dependency, we devised a microscopy-based genome-wide CRISPR-Cas9 screen to examine the effect of the loss of every protein-coding human gene during a 48-hour *Cryptosporidium* infection. Because our experimental readout was microscopy-based, we were able to simultaneously measure multiple parameters of infection including: 1) the number and size of parasitophorous vacuoles, 2) the viability of the host cells, 3) the presence of host actin beneath the parasite (known as the *Cryptosporidium* 'actin pedestal'), and 4) the development of parasite sexual stages.

Firstly, general correlations could be drawn from this dataset, such as a positive relationship between host cell viability and sexual stage development, as well as one between parasitophorous vacuole size and the presence of actin pedestals. On a more granular level, surprising insights into the basic cell biology of *Cryptosporidium* asexual and sexual development in epithelial cells were revealed by this arrayed knockout screen. Specific metabolic dependencies for parasite development were identified and the influence of key host signalling pathways on *Cryptosporidium* infection have prompted a re-examination of this parasite's invasion process.

O-26

Eimeria of chicken: microgametes start their life with a tail before turning into flagella

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Coccidia belonging to the genus Eimeria are the most frequent parasites encountered in poultry (Blake et al., 2020). Their monoxenous life cycle may be divided into three major stages: schizogony, gamogony and sporogony. During gamogony, flagellated microgametes are produced and are supposed to fertilize macrogametes, although the process has never been observed. These microgametes have been described by several authors, always with two or three flagella, depending on the species of Eimeria (Scholtyseck et al., 1972). Some years ago, we described a new form of the microgamete, observed in Eimeria acervulina of the chicken, and Eimeria meleagrimitis in the turkey, which has a tail instead of two flagella (Répérant et al., 2015). We observed that these microgametes were liberated from microgamonts at the same time as flagellated microgametes. Therefore, our aims were to identify the evolution of this identified form and to understand its link with flagellated microgametes. Thus, birds were inoculation by oral gavage with different doses of infectious sporulated oocysts and were humanely euthanized at different times to observe under the microscope the parasitic elements of scraped content of the mucosa. Images and videos were captured for biometric measurements and developmental stage identification. Our observations showed that these tailed microgametes, that we named eel-like microgametes, can evolve to flagellated forms, suggesting that they may be the first stage of this organism. We have videos of an eel-like microgamete turning into a bi-flagellated form, and of another one with incomplete transformation, with two flagella emerging at the end of the tail. The lengths of the eel-like and flagellated forms suggest that the tail is in fact an envelope containing the two flagella. However, as these two forms co-exist, there might be an interest for them in the process of fertilization, as previously suggested, favouring the eel-like forms in liquid contents and the flagellated forms in semi-solid forms. Our study provides the first report of the evolution of premature form of microgametes into flagellated microgametes, which is an important element to better understand the developmental stages of Eimeria life cycle.

Blake, D.P., Knox, J., Dehaeck, B., Rathinam T., Ravipati V., et al. (2020). Re-calculating the cost of coccidiosis in chickens. Veterinary Research, 51, 115
Scholtyseck, E., Mehlhorn, H., & Hammond, D. (1972). Electron microscope studies of microgametogenesis in Coccidia and related groups. Z. Parasitenkd, 38, 95-131
Répérant J.-M., Thomas-Hénaff M., Benoit C., Le Bihannic P., Eterradossi N. & Jestin V. (2015) Atypical forms observed among microgametes of two species of avian Eimeria. 3rd International Meeting on Apicomplexan Parasites in Farm Animals, ApiCowplexa, 30 June – 3 July 2015, Edinburgh

In vitro and *in vivo* models

O-27

In vitro inhibition of *Cystoisospora suis* sexual stage specific proteins

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Coccidia display a characteristic life cycle, where the parasites switch between asexual and sexual development, resulting in an environmental stage, the oocyst. As *Cystoisospora suis* causes severe economic losses in the pig-producing industry worldwide, interest in possible transmission-blocking strategies is rising. During gamogony substantial changes occur at the cellular and subcellular level, leading to cell fusion of micro- and microgametes and the development of a zygote that forms a protective outer layer for environmental survival as an oocyst as transmissible stage. Previous studies in *C. suis* already identified changes in transcription profiles during different time points in the parasite's development and identified proteins with potential roles in the sexual development of this parasite. In a follow-up study, selected candidates were evaluated as targets for antibody-based blocking of parasite development *in vitro*. Hapless protein 1 (HAP2) plays important roles in signal transduction and gamete fusion during the fertilization process, and oocyst wall forming protein 1 (OWP1) is a homologue of oocyst wall-forming proteins of other apicomplexan parasites. To determine inhibition of sexual stage development and oocyst wall formation by specific antibodies *in vitro*, we adapted a previously developed host cell free culture system for treatment of merozoites with egg yolk-derived chicken antibodies. Purified merozoites, were therefore treated with 2µg/ml of either anti-OWP1-serum or anti-HAP2-serum and the development of new life stages was monitored daily. We could show that an inhibition of HAP2 with an anti-HAP2-serum can interrupt the development of microgametes and oocysts. Furthermore, the expression of OWP1, expressed in macrogametes, can be inhibited by anti-OWP1-serum. This results in mitigating oocyst wall formation during the parasite's development *in vitro*, and consequently significantly reduced oocyst formation. Especially OWP1 appears to be a good target for transmission blocking-strategies, as it is highly conserved, and its biological function in formation of the oocyst wall is retained in all Coccidia. In summary, up to 100 % inhibition of the development of sexual stages and formation of oocysts with purified chicken IgY sera against recombinant HAP2 and especially OWP1 could be demonstrated for *C. suis*. We conclude that the investigated sexual stage-specific proteins constitute targets for *in vivo* intervention strategies to interrupt parasite development and parasite transmission to susceptible hosts.

O-28

Impact of physicochemical parameters of the digestive tract on *Cryptosporidium parvum* infection

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Cryptosporidium parvum is responsible for a zoonotic disease affecting both human health and livestock. The parasite infects its host through the oral route and develops in ileal epithelial cells, leading to acute and sometimes lethal diarrhoea. The severity of cryptosporidiosis is closely related to the immune status of its host, young ruminants, infants, and immunocompromised individuals being more susceptible. Other non-immune-related factors, such as digestive physicochemical properties, can also vary widely with age but their contribution to infection establishment in young individuals has never been investigated.

In vitro digestion models have been designed and validated to study the fate of orally ingested substances while closely mimicking the physiological processes occurring during digestion. Among the available systems, the dynamic and multicompartmental TNO gastrointestinal model (TIM-1) is currently the most complete simulator of the human upper gastrointestinal tract. The TIM-1 was used for a comparative study of *C. parvum* infection under adult and young children (from 6 months to 2 years) digestive conditions.

The TIM-1 was programmed to reproduce both physicochemical digestive conditions upon simulated ingestion of a glass of mineral water, which was experimentally contaminated with *C. parvum* oocysts. The parasite excystation kinetics throughout the course of the *in vitro* digestion was determined by flow cytometry analysis. Parasite invasion ability was assessed after reinoculation of sporozoites collected from the TIM-1 onto HCT-8 cells. A luciferase reporter gene assay was also used to follow sporozoite activity throughout the digestive process.

Our data show that the parasite excystation rate is almost maximal in the duodenal compartment, one hour after the beginning of digestion in the TIM-1. However, a higher number of parasites reaches the distal ileal compartment while protected in their oocyst shell upon simulation of child compared to adult digestive conditions. This suggests that a lower amount of sporozoites is released in the small intestine in children, nevertheless, the luciferase activity expressed by these free stages in the ileal compartment is significantly higher in children compared to adults. Invasion assay performed on HCT-8 cells suggests that only sporozoites collected in the ileum after three hours of digestion are able to invade host cells.

Our study is the first one exploring the impact of different digestive conditions on *Cryptosporidium* using a sophisticated gastrointestinal model. A global transcriptome analysis by RNA-Seq is also being performed on samples collected during in vitro digestion to identify parasite genes that are differentially expressed under child and adult digestive conditions.

O-29

Heifers inoculated with *Neospora caninum* live tachyzoites at prepubertal age reactivate their infection during gestation

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Neospora caninum is recognized for causing cattle abortion, provoking severe economic losses in the livestock industry worldwide. Field observations suggested that naturally exposed cattle develop protective immune mechanisms against abortions in a subsequent *Neospora*-related outbreak. Furthermore, endogenous transplacental transmission is more likely to occur in cattle than postnatal infection. It has been reported that *N. caninum* vertical transmission could be prevented when cows are experimentally inoculated with live tachyzoites before mating and then challenged during their gestation. Recently, our group showed that inoculation with live tachyzoites of the NC-Argentina LP1 local isolate in 6-month-old female calves elicited a specific cellular immune response with antibody levels that decreased at day 120 post-infection. Nevertheless, whether these infected animals could reactivate the infection and transmit the parasite during their reproductive life on the farm was not studied. In addition, it would be interesting to know if the memory immune response generated at a young age in these animals could protect against a heterologous challenge. The aim of the present study was to evaluate the reactivation and foetal infection in pregnant heifers inoculated with live *N. caninum* tachyzoites before puberty. Fifteen 30-month-old pregnant heifers were allocated into four groups: animals inoculated with live tachyzoites of NC-Argentina LP1 isolate before puberty and challenged with live tachyzoites of NC-1 strain at 210 days of gestation (DG) (Group A); animals mock inoculated before puberty and challenged with NC-1 strain at 210 DG (Group B), animals inoculated before puberty but not subsequently challenged (Group C); and noninfected and nonchallenged animals (Group D). The results of the present study clearly show that animals inoculated before puberty had parasitic reactivation, as *Neospora*-DNA was detected in their PBMCs, and an increase in the specific antibody titres from the 7th month of gestation onwards was observed. In addition, in 3 and 2 calves from Groups A and C, respectively, congenital infection was confirmed. The results of the present study show for the first time that the inoculation of live tachyzoites of *N. caninum* in prepubertal female calves is not effective in preventing the reactivation of the parasite during pregnancy, showing that the infected animals were unable to eliminate the parasite at their young age. In addition, although prepubertal infection elicited a specific immune response against *N. caninum*, this response was not sufficient to prevent congenital infection after heterologous challenge. Therefore, we provide evidence that the use of live *N. caninum* tachyzoites in young animals as a strategy to induce protection is neither safe nor effective.

O-30

Infection outcomes after challenge of sheep at mid-pregnancy with *Toxoplasma gondii* isolates showing different phenotypic traits

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The genetic diversity of *Toxoplasma gondii* has been associated to virulence variations in *in vitro* and murine models. However, whether these variations could be extrapolated to small ruminants, remains largely unexplored. This study *investigates* how infection by three *T. gondii* isolates that showed different phenotypic traits *in vitro* (growth rate [GR] in ovine trophoblast cells and cell infection rate [cIR]) in ovine macrophages) and in outbred mice (mortality rate [MR]) models might influence the outcome of toxoplasmosis in pregnant sheep. The isolates traits were as follows: (i) TgShSp1 (ToxoDB#3; low GR; low cIR; 0% MR), (ii) TgShSp16 (ToxoDB#3; low GR; moderate cIR; 21% MR) and (iii) TgShSp24 (ToxoDB#2; very high GR; high cIR; 18% MR).
Seventy-three primiparous sheep were distributed in four groups according to the isolate used in the challenge and were either orally dosed with 10 sporulated *T. gondii* oocysts or mock-challenged at day 90 of gestation. The four groups were as follows: G1 infected with TgShSp1 (n=20), G2 infected with TgShSp16 (n=20), G3 infected with TgShSp24 (n=19) and G4 of non-infected control sheep (n=14). After infection, five sheep of each infected group (G1, G2 and G3) were euthanized at 14 and 28 days post infection (dpi). Five sheep from the control group (G4) were also euthanized as scheduled above. The remaining animals from each group were left until early-late abortion or delivery occurred. In sum, rectal temperature, occurrence of abortions, serological anti-*T. gondii* specific antibodies, and histopathological lesions in placenta and foetuses, as well as tissue distribution of *T. gondii* were studied.
All infected sheep showed hyperthermia between six to nine dpi, although the rise in temperature was 1 day later in G1 when compared to G2 and G3 (P<0.05). Early abortions (before 14 dpi) were more frequent in G3 (6/19; 32%) than G1 or G2 (4/20; 20%) but the difference was not significant. After the occurrence of acute abortions and serial culling, the remaining sheep (6 in G1, 6 in G2 and 3 in G3) suffered late abortions (1 in G1 and 1 in G2) or stillbirths (3 in G1) or lambed live but weak offspring (2 in G1, 5 in G2 and 3 in G3). All sheep seroconverted after infection although ewes of G2 and G3 showed specific serological antibodies before (14 dpi) than sheep of G1 (19 dpi) (P<0.05). Sheep in G4 gave birth to normal lambs.
Histological lesions in placentomes were only detected at 28 dpi in all groups, although G1 showed more lesions than G2 and G3 (P<0.05). At 14 dpi, *T. gondii*-DNA was only detected in G1 (3/5 ewes) in the 9% of the placentomes. However, at 28 dpi the detection was higher in G1 (5/5 ewes), G2 (2/5 ewes) and G3 (5/5 ewes) although the percentage of DNA-positive placentomes was significantly different (96%, 8% and 63%, respectively). Regarding foetuses, no lesions neither DNA-parasite were detected in those ones from ewes culled at 14 dpi. Significant differences were found at 28 dpi, both regarding the histological lesions (p=0.002) found in 87.5%, 0% and 60% of their foetuses (G1, G2 and G3, respectively) and *T. gondii*-DNA identification in at least one of the tested organs (p=0.003): 88%, 20% and 90%, respectively. Regarding lambed or lately aborted foetuses/stillbirths, 73%, 55% and 66% of G1, G2 and G3, respectively, showed microscopic lesions (p>0.1) whereas *T. gondii*-DNA was evidenced in the 100%, 63% and 100% of them (p=0.015), respectively.
Infection outcome (abortions, stillbirths, and weak lambs) caused by the challenge with the 3 different *T. gondii* isolates in a pregnant sheep model were similar. However, there were differences on the lesions and parasites distribution, suggesting that the phenotypic variation of the isolates could influence the dynamic of infection. The mechanisms behind these differences, such as the host immune response or the parasite burden reached in each location, must be investigated in other to further clarify the pathogenesis of this disease.

Abstracts Poster Session 1 (1-38)

P-1

Neospora caninum natural infection in Tunisian rams: serological study and molecular identification of infection in semen

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The aim of this study was to estimate the seroprevalence, molecular prevalence and perform a molecular identification of *Neospora caninum* in semen of Tunisian rams.

A total of 92 blood samples were collected from four farms located in four Tunisian governorates (Jendouba, Kairouan, Zaghouan and Ben Arous) and samples were screened with a commercial ELISA kit for *N. caninum* antibodies. For the same rams, semen samples were collected and tested for presence of *N. caninum* ITS1 gene using PCR. Five amplicons were randomly selected for sequencing. A phylogenetic tree was constructed to compare the partial sequences of the ITS1 gene with sequences deposited in GenBank.

The seroprevalence of *N. caninum* infection was 25% (23/92), and PCR revealed that the molecular infection prevalence in semen was 11.95% (11/92). Kappa test showed an average agreement between seroprevalence and parasite prevalence in semen ($\kappa = 0.44$). The highest molecular prevalence was for rams that accomplished more than two mating seasons (21.0 ± 12.1%) compared to those performed less than two mating seasons and yearling individuals (4.0 ± 5.5%) ($P = 0.01$). There were no differences in *N. caninum* molecular prevalence according to either breed or locality. Comparison of the partial sequences of the ITS1 gene revealed 99–100% similarity with those deposited in GenBank.

To the best of our knowledge, this is the first detection and molecular identification of *N. caninum* in semen from rams in North Africa. Our findings indicate that *N. caninum* infection rate was high in rams.

P-2

GENOTYPING OF *Toxoplasma gondii* FROM FREE-RANGE CHICKENS OF THE AMAZON REGION CONFIRMS ITS WIDE GENETIC DIVERSITY IN SOUTH AMERICA

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There is great interest in the molecular investigation of *Toxoplasma gondii* genotypes from animals and humans, especially in the Amazon region, where there are studies showing that isolates from this region can cause a diverse and severe clinical picture in humans. In the present study, isolates of *T. gondii* from free-range chickens were obtained and these were genotyped to study the diversity of this coccidia. Adult free-range chickens were acquired from 29 municipalities located in the Brazilian Amazon region, including states of Pará (n=6 municipalities and the Marajó island), Amazonas (n=6), Roraima (n=2), Acre (n =7 and 2 highways), Amapá (n=8) and from 5 municipalities in the Amazon region located in the borders with Peru (n=2), Bolivia (n=1), Guyana (n=1) and Venezuela (n=1). Samples of heart, brain, and blood from 401 chickens were obtained. Anti-*T. gondii* serum antibodies were detected in 273 (68.08%) chickens using Modified Agglutination Test (MAT ≥ 5), and bioassays in mice were performed on 243 samples (heart and brain homogenates) of birds (titers < 5 to 10 = 60 bioassays and titers ≥ 20 = 183 bioassays). A total of 116 isolates were obtained from chickens with titers ≥ 20. From those isolates, 97 (83.62%) led to acute sickness of all infected mice, 9 (7.76%) led to acute sickness to part of the infected mice and 10 (8.62%) infected mice did not get acutely ill. Complete PCR-RFLP genotyping (11 markers) was obtained from all isolates, revealing 43 genotypes, of which 20 have been described for the first time. The new genotypes were found in birds from Brazil, Bolivia, Peru, and Guyana. The most abundant genotype was ToxoDB PCR-RFLP #7, with 40 isolates, distributed in four Brazilian states in this study, and had already been described in North, Northeast, Center West and South regions of Brazil, which suggests that it is a possible successful Brazilian clonal lineage. The archetypal clonal Types I, II and III genotypes were not observed, but the prevalent Brazilian lineage Types BrII and BrIII were present. From the 10 isolates that infected mice did not get acutely ill, nine had allele III at CS3 marker. To increase the resolution of genotyping, microsatellite (MS) analysis was used, with complete genotyping (15 markers) of 111 isolates. Examining the 8 MS typing markers, 34 types were identified, however, observing the set of 15 markers, a total of 82 genotypes were revealed among the 111 examined and 25 isolates seem to represent clones, that is, they are identical, not independent samples. The study confirms previous results of a wide variety of *T. gondii* genotypes in Brazil and emphasizes the importance of the Amazon region to this diversity.

P-3

Pathogen-hopping to identify high-value drug leads against *Babesia spp*

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Babesia species are tick-borne, malaria-like apicomplexan parasites that infect and lyse red blood cells of vertebrate animals including humans and livestock. In healthy humans babesiosis can be asymptomatic or a mild, flu-like illness, but in immunocompromised hosts, especially those with asplenia, infection can be overwhelming and result in critical illness and death. Bovine babesiosis is characterized by fever, anemia, hemoglobinuria and high mortality and poses a significant threat of economic loss. Incidence and endemic range of human babesiosis are rising, likely due to climate change. Current first-line therapeutics for human babesiosis include azithromycin and atovaquone or clindamycin and quinine, all of which are antimicrobials developed for other indications. While these treatments may be adequate in immunocompetent hosts, in the immunocompromised infections can be severe, relapsing, and even incurable despite use of multi-drug regimens. In such patients, resistance to available agents has also been documented. It may be that less-than-optimal drug efficacy against *Babesia* stems from the fact that the dosing of these compounds is not designed to achieve and maintain adequate serum concentrations capable of inhibiting parasite growth. From a broader perspective, twenty years of emphasis on straightforward compound repurposing in attempt to foster drug development for small therapeutic markets has not yielded effective treatments. An alternative approach is to identify compounds with antibabesial activity that are in development *for related parasites*. We have combined this approach with cell-based methods and animal models to identify anticryptosporidial drug leads, some of which have progressed to late stages of development. Drug repurposing in this vein, so-called “pathogen-hopping”, allows for speedy and cost-effective identification of high-value drug leads, while still enabling optimization with respect to potency, pharmacokinetics, and pharmacodynamics for the specific indication. We now hope to leverage our existing anticryptosporidial leads to identify lead compounds for developing improved drugs for babesiosis. To this end we are deploying a SYBR green fluorescence-based assay to screen our existing anticryptosporidial lead series and publicly available antimicrobial collections, including the MMV Malaria and Pathogen Boxes. While there are multiple species within the genus *Babesia,* with *microti* and *divergens* the major human pathogens and *bovis* and *bigemina* the main pathogens in cattle, we are beginning with *B. duncani* because it is the only human pathogen that can be continually cultured in vitro. We believe that the pathogen-hopping approach which we have used successfully for drug discovery against *Cryptosporidium* has promise as an efficient means of improving treatments for babesiosis and thereby addressing an emerging infectious disease of interest in both humans and in animal husbandry.

P-4

Genetic diversity of *Toxoplasma gondii* in the endangered Iberian lynx (Lynx pardinus)

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The Iberian lynx (*Lynx pardinus*) is the most endangered felid species in the world. Previous studies focused on the detection of anti-*Toxoplasma gondii* antibodies in this species but to date there was no attempt to detect nor isolate viable parasites from lynxes' tissues. We aimed to identify the *T. gondii* genotypes infecting the Iberian lynx, and its interrelation with genetic variants circulating in other sympatric hosts. Thus, parasite isolation (by mouse bioassay and cell culture) and genetic characterization (by PCR-RFLP, microsatellites [MS] typing, and PCR-sequencing methods) was attempted in muscle tissues of wild and captive Iberian lynxes from the four main distribution areas of this species in Spain. Mouse bioassay was conducted in muscle tissues (heart and/or tongue) from 39 animals, and viable *T. gondii* strains were isolated from 5 lynxes, and named as TgLynxSp1-5 isolates. ToxoDB genotypes #3 (80%, 4/5) and #2 (20%, 1/5) were identified by the PCR-RFLP protocol. Moreover, MS typing allowed to confirm these findings, and provided additional subtyping information with the identification of 3 genetic variants among the 4 type II strains. Genetic characterization of isolates was completed with the PCR-sequencing of *ROP18* and *ROP5* virulence factors and the predictive virulence molecular marker *CS3*. All identified allelic profiles corresponded to those expected in non-virulent strains. Additionally, *T. gondii* DNA was searched in the muscle tissues of 143 animals, and direct MS typing was possible in samples yielding qPCR amplifications of Ct<32 (n=5). Thus, *T. gondii* DNA was detected in 18.2% (26/143) of animals and direct MS typing identified an additional genetic variant of a type II strain not detected among the isolates studied. Therefore, a larger genetic diversity of *T. gondii* strains seems to be present in the Iberian lynx population compared with domestic compartments. Current observations in the Iberian lynx apparently reflects the same predominance of type II and type III strains described to date in the literature infecting domestic animals in the Iberian Peninsula.
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P-5

Anti-*Toxoplasma gondii* antibodies in European residents within the last 20 years: A systematic review and meta-analysis

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Toxoplasmosis is a worldwide distributed zoonosis of major impact in animal and public health. Until date there was no systematic revision focused on human *Toxoplasma gondii* infection seroprevalence from a European perspective. The present review aimed at comprehensively assess the seroprevalence and reported risk factors favouring the infection in the European residents in the period 2000-2020. Medical subject headings terms (toxoplasmosis, seroprevalence, human, Europe) were used to search for specific bibliography in electronic databases (PubMed, Science Direct, Scopus and WoS). Finally, 136 studies from 30 countries met inclusion criteria (serosurveys [no case-control studies nor reviews], years 2000 to 2020, continental Europe, any language), and data related to study area, target population, method of analysis and risk factors were retrieved from studies dealing with anti-*T. gondii* IgG and IgM data. The overall seroprevalences of anti-*T. gondii* IgG observed in European residents was 32.1% (95% CI 29.0-35.2), with a great variability between countries. Subgroup analysis identified different pooled prevalence data depending on geographic area (North, 20.1%; West, 38.5%; East, 39.7%; South, 27.5%; $p < 0.0001$), target population (general population, 38.6%; pregnant women, 28.3%; other, 31.1%: $p = 0.0147$), and serological assays (11 methods used; $p = 0.0059$). A high heterogeneity ($I^2 = 100\%$, $p < 0.001$; $Q = 3.5e+05$, d.f. = 135, $p < 0.001$) and degree of publication bias (Egger's test = 6.14, $p < 0.001$) was observed in the studies considered. Among 136 studies, 88 (64.7%) also showed anti-*T. gondii* IgM seroprevalence results, and the values (0.0% to 32.7%) showed a great variability between studies, areas, target populations and diagnostic methods. The detection of IgM antibodies in 57 of the 88 studies corresponded to pregnant woman or newborn children. In addition, 8 main risk factors have been identified in Europe, and "consumption of raw (or undercooked) meat", "work in contact with soil", and "consumption of unwashed vegetables" were identified as the most frequently associated risk factors of *T. gondii* infection. The results evidenced that a noticeable segment of population presents IgG antibodies. Therefore, exposition to *T. gondii* seems to be frequent and should be considered especially among people at risk. However, under the view of scattered and fragmented available data, a normalized system for *Toxoplasma*-infection surveillance system in European countries is missing. In addition, an extra effort searching for an updated pan-European risk-factor analysis focusing on detailed source attribution should be implemented in such dynamic and evolving society since culinary customs (and preferences) are rapidly changing.
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P-6

Transcriptional changes in *Besnoitia besnoiti* infected primary bovine aorta fibroblasts identify relevant fibrosis-associated pathways

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Bovine besnoitiosis is caused by the intracellular cyst-forming apicomplexan parasite *Besnoitia besnoiti*. This parasitic disease impairs reproductive parameters since infected bulls might develop orchitis that may end up with sterility. Endothelial activation, inflammation and fibrosis are predominant lesions in the testis of sterile bulls during acute and chronic besnoitiosis. Moreover, fibroblasts are target cells during chronic besnoitiosis and are well known key players in the fibrotic process and immune regulators during infection.

The purpose of the study was to dissect the underlay molecular mechanisms that promote a drift towards fibrosis rather than regeneration during the disease progression by dual transcriptomic profiling of primary bovine aorta fibroblasts and *B. besnoiti* tachyzoites.

Transcriptomic analyses by means of RNA-Seq were carried out at two times post-infection (pi), representative of invasion (12h pi) and intracellular proliferation (32h pi). Next, we investigated the expression of relevant differentially expressed genes (DEGs) in the scrotal skin of sterile naturally infected bulls in which fibrosis was a predominant lesion.

Differential expression analysis between infected and non-infected bovine fibroblasts identified a higher number of DEGs at 12h pi compared to 32h pi. Functional classification of DEGs showed that most of the genes were associated with the fibrogenesis process, such as cytokines (IL-7, IL-34), cell adhesion molecules (platelet and endothelial cell adhesion molecule 1), extracellular matrix components (collagen type VII and XXVII), mitogen-activated protein kinases (MAPKs) (MAPKs 3, 7, 10) mediators of angiogenesis (vascular endothelial growth factors A, C) and cell proliferation and tumor-like biologic behaviours (fibroblast growth factor 1, -FGF-1-), transforming growth factor β1, -TGFβ-1-, FosB proto-oncogene AP-1 transcription factor subunit, -FosB- and plasminogen activator urokinase receptor-PLAUR-). Pathway enrichment analysis supported the predominance of fibrosis regulation, at both time points. MAPKs and cancer signalling pathways were modulated upon infection at 12h and 32h pi. However, TNF and hippo signalling pathways were only modulated at 12h pi whilst malaria, regulation of actin cytoskeleton and focal adhesion signalling pathways were only regulated at 32h pi. Interestingly, some relevant profibrotic DEGs identified in the transcriptomic data (FGF-1, FosB, PLAUR and TGFβ-1) were also significantly regulated in the scrotal skin of naturally infected bulls with a chronic infection. The present study provides new insights into the disease progression. Fibrosis is triggered early upon infection and results are compatible with signaling of fibroblast activation and a profibrotic myofibroblast phenotype. The identified profibrotic DEGs and pathways might be potential drug targets.

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P-7

In vitro safety and efficacy of new bumped kinase inhibitors against *Besnoitia besnoiti* tachyzoites

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Besnoitia besnoiti is a tissue cyst-forming apicomplexan parasite responsible for bovine besnoitiosis, a chronic and debilitating disease that causes systemic and skin manifestations in cattle and sterility in bulls. Unfortunately, neither drugs nor licensed vaccines are currently available. In the search of therapeutic candidates, previous *in vitro* studies demonstrated the efficacy of the bumped kinase inhibitors (BKIs) named 1294, 1517 and 1533 against *B. besnoiti in vitro.*

The aim of this work was to investigate the safety and efficacy of eight recently synthesized BKIs named 1673, 1708, 1748, 1757, 1841, 1913, 1914 and 1916 against *B. besnoiti* tachyzoites. Initially, a previously standardized *in vitro* drug screening system was adapted to 96-well plates. All experiments were performed three times in quadruplicate and BKI 1294 and drug solvent (DMSO) were included as positive and negative controls, respectively. Cytotoxicity of BKIs was assessed in Marc-145 cells and all candidates proved to be safe and no alterations in cell morphology were detected. Invasion and proliferation inhibition were checked at 5 µM concentration by immunofluorescence assay (IFAT) and BKIs 1708, 1748 and 1841 were selected for further work based on the best results in terms of safety and percentages of invasion and proliferation inhibition higher than 90%. Next, the lowest IC_{99} values corresponded to 1708 (1.01 µM) and 1748 (1.22 µM) that were determined by qPCR in 3-day proliferation assays. The effect of long-term treatment efficacy with 1708 and 1748 compounds (for 6, 24 and 48 h) at IC_{99} was also investigated up to 10 days post-treatment by qPCR and IFAT. Tachyzoites were still viable and were able to re-infect the host cells at 8 days post-treatment thus, suggesting a parasitostatic effect of these drugs. In summary, BKIs 1708 and 1748 may be effective drug candidates to control *B. besnoiti* infection and further experiments *in vivo* should corroborate these results.

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P-8

In search of *Toxoplasma gondii* oocyst-specific proteins with source-attributing usefulness: key steps and limitations

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Toxoplasma gondii is a zoonotic apicomplexan parasite that can infect any warm-blooded animal. The environmental transmission route of *T. gondii* infection, through the ingestion of oocysts that contaminate fresh produce, water, and soil, is now considered more relevant than previously thought. However, serological discrimination between oocysts- and tissue cysts-driven infections is still a challenge.
Proteins predicted to be specific to oocyst wall (n=7), sporozoites (n=17), and bradyzoites (n=2), as well as hypothetical proteins (n=8), were screened through a restricted workflow. In this regard, sera samples from prepubertal sows (n=25) experimentally infected *per os* with 400 oocysts or 10 tissue cysts from type II and III isolates, bled 1 week prior to infection up to 6 weeks post-infection (wpi) (panel 1, n=158), and sera samples from piglets non-infected (n=3) and experimentally infected (n=10) *per os* with 1,000 oocysts from type II and III isolates, bled prior to infection up to 42 days post-infection (dpi) (panel 2, n=103), were characterized through three in-house and three commercial serological tests. Then, the proteins were screened by Western Blot (WB) with selected reference sera from three and two pigs infected with oocysts or tissue cysts, respectively, up to 21 dpi (panel 1), and one non-infected and two infected pigs with oocysts up to 42 dpi (panel 2). Proteins that induced seroconversion and presumably discriminated between oocyst- and tissue cyst-driven infections were used to develop an ELISA test and after that, all samples from both sera panels were analyzed by protein based-WBs and the newly developed protein based-ELISAs. Additionally, proteins with reported promising results in the literature were analyzed by WB using whole panels of sera, independently of the screening results.
After the screening process, most of the proteins, including OWP1, OWP8 and SporoSAG, that showed promising results in previous studies, were discarded. Only CCp5A, TgERP, SR1 and SR1-LH2, all predicted sporozoite specific proteins, passed the screening. However, when all samples from both sera panels were analyzed by the protein based-WBs and -ELISAs, some pigs tested positive prior to infection and just few ones seroconverted. Neither CCp5A nor TgERP could discriminate between oocyst- and tissue cyst-driven infections. An increase in anti-SR1 and -SR1-LH2 specific IgGs was observed in pigs infected with tissue cysts, but no significant differences among groups were detected. A restrictive workflow was employed, and no proper oocyst-specific marker was found. Relevant limitations hampered an accurate interpretation of results: high individual variability, influence of parasite dose, isolate and host age, proteins recognition prior to infection, lack of proteins stage specificity and low proteins immunogenicity. There is further on-going work.

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P-9

Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in sheep flocks from the Humid Pampa region, Argentina

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Sheep industry has an important socio-economic impact in Argentina. The productivity of the sheep flocks depends on their reproductive efficiency. *Toxoplasma gondii* and *Neospora caninum* are obligate intracellular protozoan parasites that infect a wide range of intermediate hosts and cause reproductive failure. *Toxoplasma gondii* is one of the most frequent causes of reproductive failure in sheep worldwide and ovine abortions are often associated with this parasite. Historically, *N. caninum* has been considered a major cause of abortion mainly in cattle, however recent evidence points out its importance as causal agent of reproductive failure in sheep. Although the presence of both protozoan parasites has been confirmed in Argentinean flocks, information about *T. gondii* and *N. caninum* occurrence in the different productive systems is scarce. The aim of this study was to evaluate the seroprevalence of *T. gondii* and *N. caninum* in 1928 ovine sera (1,143 and 785 samples from 8 dairy and 9 meat flocks, respectively) in 17 flocks from the Humid Pampa region, using an indirect fluorescence antibody test. *Toxoplasma gondii* global seroprevalence was 47.7% (95% CI, 45.5-49.9%), being higher in animals older than 1.5 years (OR = 1.716; CI = 1.414-2.078; $P<0.0001$). Regarding *N. caninum*, a global prevalence of 15.9% was found (95% CI, 14.34-17.61%), and seropositivity was more frequent in animals younger than 1.5 years (OR =1.291; CI =1.004-1.657; $P=0.0496$). There was an association between the serological status for both protozoa and the type of production system, being higher the prevalence to *T. gondii* (OR = 1.969; CI = 1.634-2.369; $P<0.0001$) and *N. caninum* (OR =1.524; CI =1.174-1.979; $P<0.0001$) in dairy than meat flocks. Of the total of analysed serum, 1,143 samples came from 8 dairy flocks and antibodies to *T. gondii* or *N. caninum* were detected in 54.5 % (95% CI, 51.6-57.4%) and 18.2 % (95% CI, 15.9-20.4%), respectively. In contrast, 37.8% (95% CI, 34.4-41.2%) and 12.7% (95% CI, 10.4-15.1%) of the samples from the nine meat flocks were positive for *T. gondii* and *N. caninum*, respectively (n=785). Association between age and seropositivity was observed for both *T. gondii* and *N. caninum* in both production systems ($P<0.05$). This association could be related to the main transmission routes of these protozoa, explaining the higher seroprevalence of *T. gondii* in older sheep associated with the horizontal transmission, and the higher levels of seropositivity to *N. caninum* in younger animals associated with vertical transmission. In addition, intensive management conditions in dairy farms could explain the higher seroprevalence to both protozoa compared with meat flocks. The results of the present study confirm that *T. gondii* and *N. caninum* are widely distributed in dairy and meat sheep flocks in the Humid Pampa region in Argentina.

P-10

Insights on Host Response to *Cryptosporidium parvum* Infection

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Diarrhoeal diseases are responsible for 10% of child deaths worldwide, making them the second leading cause of child mortality. The zoonotic parasite *Cryptosporidium parvum* is a major contributor to high diarrhea-related mortality rate. Nitazoxanide is currently the only FDA-approved drug that shortens the duration of illness in immunocompetent patient, but unfortunately does not better than a placebo in young children. The life cycle occurs in a single host and includes the asexual and sexual stages. For the fecal-oral transmitted parasite, the host cells of the intestinal epithelium are crucial for development and replication. Currently, little is known about the host response to infection. We analyzed the transcriptome to study the dynamics in host cells related to infection. Several time points were chosen after infection throughout the life cycle of the parasite. Interestingly, we were able to observe dramatic changes on the transcriptional level over time. These results could form the basis for a better understanding of the host response that contributes to the severe intestinal pathology caused by the parasite.

P-11

Switzerland-wide *Neospora caninum* seroprevalence in cattle and identification of risk factors for infection

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Neospora caninum is an important cause of abortion in cattle worldwide. Infection in cattle occurs horizontally by ingestion of oocysts shed by canids or vertically, from an infected dam to the fetus, and may result in abortions, stillbirths, or birth of seropositive offspring. The control of bovine neosporosis is difficult and costly. Current representative data on the seroprevalence of *N. caninum* infection in cattle in Switzerland is missing. The objectives of this study were to estimate the nationwide seroprevalence of *N. caninum* infections in Swiss cattle and to assess risk factors for infection with this parasite. To determine the seroprevalence, we conducted a cross-sectional study with cattle farms randomly selected and stratified according to population size. This resulted in 758 serum samples from female cattle from 159 farms distributed over all Swiss cantons. The serum samples were tested for antibodies against *N. caninum* using a commercial ELISA. In case of inconclusive results, samples were retested by an in-house immunoblot technique. To collect farm parameters relevant to *N. caninum* transmission and prevention, farm owners were mailed in February 2022 a questionnaire which addressed topics putatively related to *N. caninum* infection such as farm management (e.g., water source, feed type and storage), history of abortion, and presence of dogs on farm. In June 2022 farmers that did not respond previously were sent a reminder. By ELISA and immunoblot, 4.4% (33/758) of cattle sera yielded positive results. At the farm level, 16.4% (26/159) of the sampled farms had at least one seropositive animal. To date, 53% of the farms summitted the questionnaire. A univariate analysis by logistic regression was conducted on the preliminary questionnaire data. Odds for farm seropositivity were 4.5 times higher when restocking was done by buying-in compared to closed (home) breeding. The multivariate analysis on risk factors will be completed before submission of the full manuscript. The seroprevalence of 4.4% obtained in the current study was surprisingly low compared to older studies. However, the vast majority of those studies included cattle with a history of abortion. Only one other study was conducted on a sample representative for the Swiss dairy cattle population of 1994 and found a seroprevalence of 11.5%. Thus, *N. caninum* seroprevalence appears to have dropped over the last three decades. One possible reason is that since 1995 neosporosis is a reportable disease in Switzerland. This may have increased disease awareness and has probably aided individual farmers in controlling the infection in their herds. The type of cattle restocking was the most important factor for *N. caninum* seropositivity identified. Through our seroprevalence data and the upcoming complete risk factor analysis we expect to gain a comprehensive epidemiological understanding of the current situation of bovine neosporosis in Switzerland and identify future research needs.

P-12

Counting parasites with DNA - quantification of coccidia can be high-throughput, precise, open-ended and species-specific

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Quantifying parasites is a common goal in parasitological studies. We have previously shown that the amount of parasite DNA in faecal samples can be a biologically meaningful measure, even if it does not agree well with complementary counts of transmission stages (oocysts). Parasite DNA can be quantified at relatively high throughput using qPCR, but amplification needs a high specificity and does not distinguish parasite species. Counting of amplicon sequence variants (ASVs) from high-throughput marker gene sequencing using a relatively universal primer pair has the potential to distinguish between closely related co-infecting taxa and uncover the community diversity, thus being both more specific and more open-ended.

We here compare qPCR to single and multiple amplicon sequencing to quantify *Eimeria* in experimentally infected mice. We show that using amplicon sequencing ASV abundance 1) allows *Eimeria* quantification providing that adequate normalisation is performed; 2) unveils previously unknown Eimeria diversity; 3) discovers (off-target) taxa for which the amplification was not preconceived. We compare these characteristics between single amplicon and multiple amplicon (targeting several marker genes and regions) sequencing approaches. We conclude that single amplicon sequencing is more sensitive in detecting Eimeria, particularly when parasite DNA is low but less specific than multi amplicon. We conclude that amplicon sequencing provides underused potential for the quantification of parasites in faecal material at high throughput.

P-13

RNA-seq identifies parasite genes potentially associated with virulence in *Theileria annulata* transformed macrophages

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Theileria annulata is an apicomplexan parasite transmitted by *Hyalomma* ticks with a significant impact on livestock production. Vaccination of cattle using attenuated cultured schizonts was shown to be an effective control option. Evaluation of attenuation is typically empirical by performing infection experiments in cattle, which can be costly and laborious. As a means to further increase our understanding of the attenuation process and identify potential attenuation markers, we conducted a comparative transcriptome analysis of a virulent (passage 26) and attenuated (passage 296) *T. annulata* Tunisian cell line (named Beja or CL2 and the attenuation was validated by Darghouth et al., 1996; 2008) using RNAseq followed by validation of the RNAseq results by quantitative real time PCR (qRT-PCR) to identify genes associated with attenuation. The gene expression level analysis revealed that of a total of 3,538 expressed genes in the cultures, 59 and 41 were uniquely expressed in the low and high passage, respectively, whereas 3,438 genes were expressed in both passages. A total of 214 genes were differentially expressed where 149 genes were up-regulated and 65 were down-regulated. The functional annotation of differentially expressed genes (DEGs) using the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis showed that they are involved in various metabolic pathways such Carbon metabolism, Oxidative phosphorylation, protein processing in endoplasmic reticulum, Phagosome and biosynthesis of secondary metabolites. From the top 37 DEGs, four proteins were predicted to contain a signal peptide and ten possess at least one transmembrane domain, suggesting an involvement in host-parasite interaction. The results of RNAseq were confirmed by qRT-PCR. Significant DEGs could be potential markers for attenuation and require further confirmation.

P-14

Secreted *Theileria annulata* effector protein Ta9 binds and activates proto-oncogenic macrophage Hck.

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In tropical theileriosis of cattle, the major driver of pathology is the transformation of host macrophages by the intracellular apicomplexan parasite Theileria annulata. In Theileria-transformed leukocytes several oncogene-associated signaling pathways, including Activator Protein 1 (AP-1) and NF-□B, are constitutively activated in a parasite-dependent manner. We have previously shown that the proto-oncogene haematopoetic cell kinase (Hck) of the Src family of non-receptor tyrosine kinases is constitutively active in T. parva-transformed B cells and contributes to AP-1-driven transcription (1). Ta9 is a T. annulata secreted protein that we have shown capable of stimulating AP-1-driven transcription (2) raising the possibility that Ta9 might do so by augmenting Hck signaling. Using two independent anti-Hck antibodies we now demonstrate co-localization of Ta9 with the active form of Hck in/on cytoplasmic vesicle-like structures. Overexpression of full-length GFP-tagged Ta9 in mouse 3T3 cells results in the appearance of numerous spike-like membrane protrusions ('hairy phenotype'), induction of a slower migrating Hck band in Western blots and Ta9-Hck co-localization. These results are consistent with Ta9 binding to and activating Hck and treatment of T. annulata-infected macrophages with a selective Hck inhibitor (A419219) negatively impacted on the parasite-dependent transformed phenotype, as estimated by cell proliferation and soft agar colony formation assays.

1. Baumgartner et al. 2003, Blood.
2. Unlu et al. 2018, PLOS ONE.

P-15

Short-term culture adaptation of *Toxoplasma gondii* types II and III modifies isolate virulence in mice.

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Toxoplasma gondii is the causative agent of toxoplasmosis in animals, including humans. Most of the *T. gondii* research has been carried out using isolates that have been maintained under laboratory conditions (mice to mice or cell culture) for long periods of time. Previous studies showed that *in vitro* adaptation of type II and III *T. gondii* isolates is rapid with significant phenotypic changes after only 20-30 passages that results in a significant increase on parasite growth rate and a decrease on cyst formation and tachyzoite to bradyzoite conversion capabilities. Here, we examined the influence of culture adaptation at short-term on virulence in mice of four type II isolates: TgShSp1 (Genotype ToxoDB#3), TgShSp2 (#1), TgShSp3 (#3) and TgShSp16 (#3); and two type III (#2) isolates: TgShSp24 and TgPigSp1. Two groups of mice (n=10) per isolate were intraperitoneally inoculated with the tachyzoite lethal dose 50 determined at 10 passages (*p*10) and the same dose after 40 passages in cell culture (*p*50). Potential changes in the virulence degree between *p*10 *vs p*50 were evaluated by cumulative mortality and morbidity, and other parameters such as parasite load in brain and lung. Results showed events of exacerbation on the virulence as i) mice infected with TgShSp2 *p*50 and TgShSp3 *p*50 exhibited higher cumulative morbidity than mice from *p*10 groups, with the appearance of mortality due to neurological signs and high parasite loads in brain, ii) mice infected with TgShSp24 *p*50 succumbed earlier to infection than mice inoculated with TgShSp24 *p*10 (100% at *p*50 *vs* 30% at *p*10), mostly associated with the appearance of severe respiratory distress, high parasite burdens in lungs and absence of tissue cysts in brain, iii) all mice infected with TgPigSp1 *p*50 or *p*10 succumbed to infection, but clinical signs and mortality appeared earlier in mice infected with TgPigSp1 *p*50. In addition, attenuation events have been observed: i) mice infected with TgShSp16 *p*50 displayed the absence of mortality at *p*50 (50% at *p*10 *vs* 0% at *p*50) and low parasite burdens in brain, and ii) mice infected with TgShSp1 *p*50 showed the lowest parasite burdens and cysts loads in brain, suggesting a better control of the infection. This study demonstrates the rapid adaptation of *T. gondii* isolates by maintenance in cell culture (after 40 passages) that affect/influence virulence in mice. Present findings suggest potential divergence of the phenotypic traits in the laboratory-adapted *T. gondii* isolates comparing to the field isolates and open new discussion about the use of laboratory strains for inferring keys of parasite virulence in *in vivo*.

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P-16

Disseminated sarcocystosis in a domestic pig (Sus scrofa): case report of a neglected parasitic infection

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Sarcocystis is a genus of apicomplexan protozoa with a worldwide distribution, including more than 200 species infecting mammals, birds, reptiles and possibly fishes. Among these, wild and domestic pigs can act as intermediate hosts for two species, *Sarcocystis suihominis* and *Sarcocystis miescheriana*, using humans and canids, respectively, as definitive hosts. Despite the zoonotic risk for consumers of raw or undercooked pork meat, data on the occurrence of *Sarcocystis* spp. in wild boars in Italy are limited to one recent study, while the presence of *Sarcocystis* spp. in domestic pigs hasn't been investigated so far. Here we report the first case of disseminated macroscopic sarcocystosis in a domestic pig in Italy.
In June 2022, multifocal white lesions were detected throughout the carcass of a Large White x Landrace four years old sow, during the post mortem meat inspection. Due to the extensive involvement of different muscles, including diaphragm, oesophagus, tongue, masseter, neck, shoulders and back muscles and skeletal muscles of the fore and hind limbs, the carcass was condemned and samples were taken for further investigation. Samples containing visible lesions were subjected to histological and molecular examination. A subset of samples was placed in 10% buffered formalin and processed using the conventional histological method; sections of 4 µm thick were stained with hematoxylin and eosin and observed using a light microscope. Meanwhile, 10 lesions were isolated, DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilding, Germany) and analyzed by PCR targeting the *cox1* gene; PCR products were sequenced to achieve species identification. The same molecular protocol was applied on 10 muscle samples without lesions.
Muscle samples showed well demarcated, oval or elongated lesions, up to 1 cm in length and 3 mm in diameter, histologically identified as *Sarcocystis* macrocysts. The majority of the cysts were calcified and surrounded by a slight non suppurative inflammatory reaction. The partial amplification and sequencing of the *cox1* mtDNA gene revealed the presence of *Sarcocystis miescheriana* DNA in all sampled lesions, while healthy muscle samples tested negative.
Our study reports the first molecularly confirmed case of *Sarcocystis miescheriana* infection in a domestic pig in Italy; besides, herein the presence of disseminated macroscopic lesions due to sarcocystosis in a pig resulting in carcass condemnation is reported for the first time. The present study points out the need to increase the data related to the occurrence and the prevalence of *Sarcocystis* spp. in wild and domestic pigs, highlighting, on one hand, the zoonotic potential of *Sarcocystis suihominis*, and on the other hand, the possible economic losses related to carcass condemnation due to macroscopic *Sarcocystis* spp. lesions.

P-17

Serodiagnosis of *Toxoplasma gondii* infection in small ruminants from the Czech Republic by commercial and in-house ELISAs

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Toxoplasma gondii infection is widespread in humans, livestock, and wildlife across the world. Moreover, *T. gondii* is a major cause of reproductive failure in small ruminant flocks and serological diagnosis can help to inform about the possible occurrence of disease on farms and also to determine the level of risk for transmission of pathogens into the food chain. In this study, a total of 808 sera of small ruminants (418 sheep and 390 goats) from 41 farms and 10 regions in the Czech Republic were examined for the presence of *T. gondii*-specific antibodies. These samples were obtained during 2019 from animals older than one year. Two independent ELISAs were selected, the commercially available ID Screen® Toxoplasmosis Indirect Multi-species (IDvet) and an *in-house* ELISA based on lyophilized tachyzoites named TgSALUVET ELISA 2.0. Antibodies directed against *T. gondii* were detected in animals from all tested farms. A total of 457 (56.6%) sheep and goat sera were positive by ID Screen ELISA: 289 sheep (69.1%) and 168 goats (43.1%). When TgSALUVET ELISA was employed, 396 (49.0%) sheep and goat sera were positive: 243 sheep (58.1%) and 153 goats (39.2%). The possible cross-reactivity against *Neospora caninum* was also investigated by NcSALUVET ELISA 2.0 and the results were verified by Western Blot (WB). Eight out of 808 animal sera were positive by NcSALUVET ELISA 2.0 and WB analysis confirmed five seropositive animals (0.6%), two goats and three sheep. Results suggest a low seroprevalence of *N. caninum* infection. However, a possible cross-reaction with *N. caninum* antibodies was detected in four sera tested by both ID Screen and TgSALUVET ELISA, whereas only two samples were positive by TgSALUVET ELISA 2.0 when a cut-off selected to increase specificity was employed (RIPC>32). Further analysis by *T. gondii* WB is required to confirm potential co-infections. According to the results obtained, the seroprevalence of *T. gondii* in sheep and goats from our study corresponds to the average seroprevalence measured at the European level. Nevertheless, small ruminants exposed to *T. gondii* parasite may be a potential source of infection for humans, especially when consuming raw or undercooked meat, or other products from infected animals.

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P-18

Investigation of *Sarcocystis spp.* found in sheep and horses from Lithuania

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Members of the genus *Sarcocystis* are common worldwide distributed parasites having two-host prey predator life cycle. Sarcocysts are formed in extra-intestinal tissues of intermediate hosts, mainly in muscles; while sporocysts develop in the small intestine of definitive host and are excreted into the environment together with faeces. Acute *Sarcocystis* infections and macroscopic sarcocysts in carcasses cause significant losses in the animal husbandry industry. The study aimed to identify Sarcocystis spp. in sheep and horses, as well as to evaluate the distribution of detected species in different types of water bodies in Lithuania.

In 2017-2022, 59 neck muscle samples of horses, 276 diaphragm, oesophagus, and heart muscle samples of 118 sheep and 150 water samples throughout Lithuania from lakes, rivers, ponds, wetlands, and coast of the Baltic Sea were collected.

By methylene-blue staining, sarcocysts were observed in 25.4% of neck muscles of horses. The parasite load was very low, ranging from 1 to 25 cyst per 1g of muscle (mean =2.1). Due to the low infection density, sarcocysts were isolated from muscles of only four animals. Sarcocysts were 660 × 120 µm (450-880 × 35-120) in size, having finger-like protrusions, which were 4-6 µm in length. Banana-shaped bradyzoites measured 10-16 × 2-4 µm. Ten sarcocysts isolated from four animals were subjected to 18S rRNA, 28S rRNA and cox1 analysis and were identified as S. bertrami.

Microcysts detected in sheep muscles by means of light microscopy and sequencing of cox1 were identified as S. arieticanis or S. tenella. Based on peptic digestion and species-specific PCR, all examined animals were positive for Sarcocystis spp., and the detection rates of separate species in diaphragm, oesophagus, and heart samples ranged from 82.1% to 100%. The prevalence of S. tenella was significantly higher in the diaphragm than oesophagus ($\chi^2 = 24.32$, $p < 0.001$), whilst differences in prevalence of S. arieticanis in three muscle types were insignificant ($\chi^2 = 2.67$, $p = 0.26$).

Based on species-specific PCR targeting cox1, 94.7%, 89.3%, 61.3% and 26.7% water samples were positive for S. tenella + S. arieticanis, S. tenella, S. arieticanis and S. bertrami, respectively. In Lithuania, intermediate hosts of S. bertrami are exclusively horses, while intermediate hosts of S. tenella and S. arieticanis can be sheep and mouflon. However, the mouflon population is scarce, and its distribution is limited only to the central part of the country. In general, detection rates of Sarcocystis spp. from sheep and horses in water samples was similar to percentage of infection detected in muscles of intermediate hosts.

In conclusion, *Sarcocystis* species found in muscles of horses and sheep are transmitted via canids, which are abundant predators in Lithuania. Also, the molecular method for the detection of identified Sarcocystis species in water samples is suggested.

P-19

Dynamic remodeling of the bovine placenta proteome upon infection with high and low virulence *Neospora caninum* isolates

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Neospora caninum features an extraordinary ability to cross the bovine placenta and infect the offspring through the transplacental route, which ensures the maintenance of the infection within herds. This ability determines the pathogenesis of *N. caninum*-induced abortion, whose occurrence is influenced by the parasite competence to replicate and cross the placenta. Previously, and using a pregnant bovine model at mid-term, we have compared the infection dynamics between the high (Nc-Spain7) and a low virulence (Nc-Spain1H) isolates at 10- and 20-days post-infection (dpi). This study revealed that the high virulence isolate was already present in the placenta at 10 dpi (1/5 heifers) and broadly present in the placenta at 20 dpi (5/5 heifers), when it had triggered the fetal death in the 40% of the animals (2/5 heifers). By contrast, the low virulence isolate was scarcely detected in placenta at 20 dpi (1/5 heifers) with an apparent absence of negative consequences for pregnancy. Here, in an attempt to reveal the early events implicated in abortion (Nc-Spain7) or parasite control (Nc-Spain1H), caruncles and cotyledons collected at 10 dpi were analysed using label-free LC-MS/MS shotgun proteomic approaches. When comparing both isolates, the number of differentially abundant proteins (DAP) was higher in caruncles (714 DAP) than in cotyledons (269 DAP). In caruncles infected with the high virulence isolate, biological processes related to mounting an immune response (leukocyte migration, and proinflammatory cytokine production) were over-represented. This suggests that the Nc-Spain7 isolate induces an early pro-inflammatory response that not only fails to control the infection, but also may contribute to lesion development and foetal death. We also observed a regulation of the phosphatidylinositol-mediated signalling, which was associated with cell migration, and vesicle-mediated transport. This agrees with previous proteomic findings observed in caruncles at 20 dpi, where the Nc-Spain7 isolate triggered an exacerbated immune response compared to the Nc-Spain1H. By contrast, cotyledons did not display clear evidence of immune response stimulation after infection with the high virulence isolate, and cell death and apoptosis were over-represented processes. In conclusion, these and previous results indicate that infection with the high virulence Nc-Spain7 isolate induces an earlier pro-inflammatory immune response in caruncles that is unable to control parasite multiplication and that could contribute to the occurrence of abortion.

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P-20

Eimeria infections in small ruminants from mountain areas of northern Portugal

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Infection of sheep and goats with coccidia of genus Eimeria has a worldwide distribution.
The breeding of small ruminants in mountain regions of Portugal is based on a traditional production system, on extensive/semi-extensive grazing and direct use of existing natural resources.
This study aimed to determine the gastrointestinal Eimeria prevalence and intensity of infection of small ruminants in the Marão, Aboboreira and Montemuro mountains, in northern Portugal.
A total of nine flocks (sheep, goats or mixed), with different numbers of animals (from 10 to 380) and different types of production (i.e. dairy and meat) were enrolled for this study, Faecal samples, from a minimum of 10 animals to 10% of the herd, were collected directly from the rectum in different occasions, from autumn 2018 to summer 2021 (seven occasions for sheep and eight for goats). Coprological qualitative analyses were made by flotation, and the number of oocysts per gram of faeces (opg) was determined by means of the modified McMaster method with an analytical sensitivity of 100 opg.
A Chi-squared test was used to compare the prevalence between species, while the impact of the different variables (sex, specie, herd size, mountain and sampling period) on infection intensity was examined using an ANOVA test. Differences were determined to be statically significant at $p < 0.05$.
The overall prevalence of coccidial infection varied from 73.6% in the autumn of 2020 to 96.2% in the summer of 2021.There was no relationship between prevalence and the different sampling periods ($p>0.05$). The overall prevalence of infection in goats (94.2%) was higher than in sheep (78.6%), with significant difference ($p< 0.05$) between species.
The mean oocyst shedding (opg values) was significantly higher ($p<0.05$) in goats (ranging from 351.0 to 1530.6) than in sheep (ranging from 237.0 to 601.1).
There was no relationship between oocyst elimination intensity and the sampling periods ($p>0.05$). However, there was an overall substantial decrease in opg values in the samples collected in 2020 (summer and autumn). According to the Portuguese Institute for Sea and Atmosphere, the summer of 2020 was considered very hot and very dry, and the year of 2020 was one of the hottest in the last 90 years.
No important clinical signs of coccidiosis were seen in the examined sheep or goats. Most samples collected were consisted of well-formed faeces, and severe diarrhoea was not reported.
The present study identified that coccidial infection is prevalent in mountain areas in northern Portugal and that animals can shed considerable amounts of Eimeria oocysts. This highlights a need for further research to better understand the potential economic impacts of Eimeria infection in small ruminant herds in mountain regions in Portugal, and also if more effective control strategies are necessary.

P-21

In vitro assessment of dinuclear thiolato-bridged arene ruthenium complex—sulfadoxine hybrid drug against *Toxoplasma gondii*

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In this study, we created a hybrid drug namely EP2-115, consisting of a dinuclear ruthenium complex (EP1-084) conjugated to sulfadoxine. In vitro efficacy assessments showed that EP2-115 was highly active in inhibiting proliferation of T. gondii, with a half-maximal inhibitory concentration (IC50) of around 125 nM. Viability of HFF was not affected at 2.5 µM. The individual components of this complex were largely inactive: neither EP1-084 nor sulfadoxide alone affected T. gondii proliferation when applied at 1 µM, nor did they notably induce cytotoxicity in HFF when applied at 2.5 µM. Thus, conjugation of two largely inactive compounds resulted in a conjugate with high anti-parasitic specificity and low cytotoxicity against host cells. However, when applied at 5 µM and 10 µM, Ep2-115 reduced HFF to 63% and 31%, respectively, treatments with EP1-084 alone at 10 µM eliminated HFF viability (0%), while sulfadoxine did not impair HFF viability at all.
EP2-115 at 2 µM was applied to spleen cell cultures from naïve mice. In contrast to HFF monolayers, the viability and proliferation of stimulated T- and B- cells was reduced to less than 30% compared to non-treated splenocytes. To assess a potential impact of EP2-115 in vivo, mice were treated with EP2-115 during 3 days at 5mg/kg/day, either perorally by gavage or by intraperitoneal injection. The mice did not show any signs of adverse effects of the treatments. Following the isolation of splenocytes, neither the viability nor the proliferation of stimulated B- and T- lymphocytes was impaired, indicating that in vivo treatment did not affect splenocytes.
Using a zebrafish (Danio rerio) embryo acute toxicity test, EP2-115 interfered in embryo development in a dose dependent manner. A positive impact score (SI) was assigned for 0.2 and 2 µM (SI = 2.5 and SI = 2), indicating no impact on zebrafish embryo development), while a negative score was noted for 20 µM (SI = -16.5).
To check whether EP2-115 could interfere with the stability of the mitochondrial membrane potential, we carried-out a Tetra-Methyl Rhodamine Ethyl ester (TMRE) uptake assay by treating T. gondii infected HFF with EP2-115 (1 µM, 30 min), followed by parasite isolation and TMRE measurements. EP2-115 did not affect TMRE uptake, neither in T. gondii nor in non-infected HFF, indicating that EP2-115 treatment did not affect the mitochondrial membrane potential. This in contrast to the control carbonylcyanide-p-trifluoromethoxyphenylhydrazone (FCCP) used as uncoupler of oxidative phosphorylation. This is in accordance to TEM investigations where no ultrastructural changes in parasite mitochondria were detected after 6 and 12 h, and alterations in the mitochondrial ultrastructure were only at later timepoints (24 and 48 h).
In conclusion, according to this in vitro assessment study, EP2-115 represents a potential candidate for future in vivo evaluation using available murine models for *T. gondii.*

P-22

An *in vitro* model to evaluate anticoccidial compounds

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Eimeria tenella is one of the most important species responsible for chicken coccidiosis, an enteric disease characterized by malabsorption, diarrhoea, and hemorrhage that has a significant impact on chicken meat and egg production worldwide, causing losses of more than £10 billion per year. Live vaccines and, in particular, the administration of anticoccidial medicines through feed or drinking water are used to control the disease. Nonetheless, resistance to current anticoccidial compounds as well as the potential prohibition of ionophore antibiotics in some parts of the world has led to the evaluation of novel alternative compounds. *Eimeria spp*. sensitivity to anticoccidial drugs has been classically investigated through in vivo assays using large numbers of chickens. The implementation of in vitro models for anticoccidial pre-screening as an alternative to preliminary in vivo tests can lead to the replacement and reduction of an important number of chickens used in research of these anticoccidial compounds, as well as reduce the time and cost of such tests. We have recently developed an in vitro model to evaluate inhibition of compounds in invasion and intracellular growth of *E. tenella* sporozoites using the immortalised Madin-Darby Bovine Kidney (MBDK) epithelial cell line, that serve as host cell for Eimeria sporozoites (released from oocysts shed by chickens), which will invade and replicate once. Infected monolayers will be recovered and quantitative PCR (qPCR) methods will be applied to calculate the number of compound-treated parasites capable of invading and replicating in comparison to the untreated controls.
With the aim to increase throughput and streamline the use of this in vitro system, the model has been miniaturised to simultaneously analyse a higher number of traditional drugs in a more cost-effective way. In addition, the effects of a series of universal solvents (dimethyl sulfoxide (DMSO), ethanol, methanol, and acetone) on sporozoite invasion and growth have been evaluated to recommend or discard their use to reconstitute new potential anticoccidials aimed to be tested in the in vitro model. The miniaturisation into a 96-well plate format perfectly mimicked the invasion and replication observed before in the 24-well plate format and allowed reducing the number of chickens used for the generation of parasite stocks for provision of the in vitro test in an 80% when used to test traditional anticoccidial drugs. Regarding the universal solvents, no significant inhibitory effects were detected when incubating cell monolayers infected with *E. tenella* sporozoites for 2 and 44 hours with any of the solvents at different concentrations (10, 5, 1, and 0.5 µg/ml) compared with non-treated parasites. This optimised model is currently being transferred from the Royal Veterinary College (UK) to Saluvet-Innova (Spain) for its wider uptake by research groups and companies with an interest in developing novel anticoccidial compounds.

P-23

Detection of selected parasites in minced meat and meat products from retail using molecular methods

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The prevalence of food-borne parasitic diseases increases worldwide. This can occur due to the factors like variations in consumers' habits, increase in population hence predisposed individuals and also advance in detection methods. Parasites like Cryptosporidium parvum, Giardia intestinalis and Toxoplasma gondii do not belong amongst routinely inspected pathogens in meat intended for human consumption. Nevertheless, eating raw or undercooked meat with cyst stages of these parasites can cause their transmission to humans. The aim of this study was to investigate the possible presence of named parasites in 123 samples of minced meat and meat products from retail in the Czech Republic. Quantitative real time PCR method for the detection of specific genes was used. Only one (1,2 %) of all tested products was found to be positive. The results show that minced meat and meat products available in the Czech retail are safe. However, intermittent monitoring is necessary to assure the food safety for the consumers.

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P-24

DEVELOPMENT, INTER-LABORATORY SOP EVALUATION AND APPLICATION OF A MOLECULAR METHOD FOR DETECTION OF *TOXOPLASMA GONDII* OOCYSTS IN READY-TO-EAT LEAFY-GREEN SALADS IN A MULTICENTRE STUDY IN EUROPE

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In Europe, the majority of acquired *Toxoplasma gondii* infections are assumed to be foodborne. Yet the relative importance of different food matrices as a source of human infection, such as fresh produce, is largely unknown. Consumption of unwashed raw fruits or vegetables contaminated with T. gondii oocysts is one possible transmission route and has been identified as a risk factor in Europe and as a probable source of infection in South American toxoplasmosis outbreaks. Despite this, studies on the occurrence of *T. gondii* oocysts in vegetables and fruits are scarce and difficult to compare due to the high heterogeneity among them, mainly influenced by the lack of standardized procedures for the recovery and detection of *T. gondii* oocysts.

In this study, a standard operating procedure (SOP) was developed that allows molecular detection of *T. gondii* oocysts in ready-to-eat (RTE) leafy green salads with a detection limit of 10 oocysts per 30 g of salad. The key analytical steps include oocysts recovery by sample washing followed by pelleting of the eluate by centrifugation, DNA extraction and DNA detection by triplex qPCR, targeting two multicopy fragments (529 RE element, B1 gene) and an internal amplification control. After successful implementation of the SOP in consortium laboratories across Europe, the efficiency of individual steps of the SOP as well as laboratory performance were evaluated in a ring trial (RT). In the ring trial, three sample panels were analyzed by nine consortium laboratories to evalute the performance of the overall procedure (salads spiked with oocysts), DNA extraction (salad pellet spiked with oocysts) and qPCR (DNA extracted from oocysts spiked salad pellets). This allowed critical steps in the procedure to be identified and confirmed robustness and sensitivity of the method as well as the comparability of results among participants. Overall, 83% (15/18) of biological replicates with 10 oocysts/30 g salad analyzed during the ring trial, tested positive for T. gondii DNA.

The SOP is currently being applied in a pilot multicentre study to assess the prevalence of *T. gondii* oocysts in RTE leafy green salads in ten countries representative of the North, West, East and South of Europe (Czech Republic, Denmark, France, Germany, Italy, Norway, Poland, Portugal, Spain, United Kingdom). The sampling started in October 2021 and is being performed weekly over a period of one year to potentially detect seasonal variations. Two categories of RTE salad mixes (baby leaves and cut leaves mixes) are sampled to explore potential associations between oocyst contamination and cultivation and growth conditions. In the study period, a total sample size of about 3,000 RTE salads from across Europe is expected to be tested. Positive samples will be confirmed and characterized by ITS-1 single tube nested PCR followed by Sanger sequencing.

This is the first European-wide study to assess the occurrence of T. gondii oocysts in RTE leafy green salads using a validated and standardized procedure to assess the associated potential risk for human infection. The developed SOP could provide a basis for the development of an ISO standard to enable reliable and internationally comparable prevalence estimates.

P-25

Development of *in vitro Cryptosporidium parvum* models using bovine intestinal organoids

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Cryptosporidium parvum is a zoonotic apicomplexan parasite, considered a severe public health concern and causing significant losses to the livestock industry globally. *Cryptosporidium* oocysts are resistant to most disinfectants, vaccines are not available and treatment options are insufficient. In vitro systems are a tool to explore host-parasite relationships without using live animals, but the parasite is unable to complete the life cycle in conventional cell systems. Among in vitro models available, stem cell-derived organoids, which mimic the in vivo function of organs better than conventional cell lines, offer interesting possibilities. In fact, infection of human and murine intestinal organoids (enteroids) with *C. parvum* has demonstrated completion of the entire life cycle, with production of infectious oocysts. Considering that *C. parvum* predominantly parasitizes cattle, the use of bovine enteroids offers significant advantages for studying pathogenesis in the relevant host. However, to our knowledge, nothing has been published on this so far.
The aim of the present study is to infect bovine enteroids with *C. parvum* oocysts and determine whether the full life cycle of the parasite can be recapitulated in this system. A panel of enteroid isolates will be used to investigate possible differences between hosts.

Several enteroid lines have been successfully isolated from Norwegian calves. To find a consistent model for infection with C. parvum we have tried different approaches already established for human enteroids. We will report findings from infections of disrupted enteroids and organoid-derived epithelial monolayers including using enteroids obtained from two different breeds of calves. We have used qPCR to monitor replication of *C. parvum* in the enteroids and IF staining will be employed to visualize infected cells. For successful infections, host responses will be further characterized by qPCR.
Further studies will include reversing the polarity of the organoids, so they their apical side is presented outwards. Infection of apical-out enteroids is a promising model, as, theoretically, mature, differentiated enteroids can be infected without further manipulation.

Bovine enteroids represent a novel, relevant in vitro system to study the underlying molecular basis controlling the development of *C. parvum* as well as its pathogenesis. Preliminary results of infection of disrupted enteroids and organoid-derived monolayers, and development of bovine apical-out enteroids will be presented at ApicoWplexa in Bern 2022.

P-26

Characterization of the translationally controlled tumor protein gene (tctp) in *Babesia bovis* and evaluation of its participation in the establishment of infection

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Babesia bovis is a protozoan of the phylum Apicomplexa that causes bovine babesiosis. This species causes a disease characterized by high virulence and mortality. It has been proposed that parasites such as B. bovis produce proteins that suppress the immune response. Translationally controlled tumor protein (TCTP) is a multifunctional protein; among its extracellular functions is its involvement in the regulation of the immune response, stimulating B cell proliferation and activation. In P. falciparum it has been postulated that it interferes with the immune response. It has been pointed out that structural differences cause TCTP to block the interaction of host TCTP with the receptor, preventing the activation and proliferation of B cells and therefore, the activation of the immune response. This protein has not been characterized in B. bovis. The aim of this work was to characterize TCTP and to evaluate its participation in the establishment of infection. The complete tctp gene was amplified by PCR and cloned in different isolates of B. bovis and sequenced; 100% amino acid sequence identity was observed in the four sequences obtained. Bioinformatics tools were used to predict secondary structure, which showed the characteristic alpha helix. By bioinformatics, the peptides with most probability to be B-cell epitopes were identified and peptides between 18 and 24 amino acids were selected, synthesized, and used to immunize rabbits and cattle. Transcription was assessed using RNA from intraerythrocytic parasites by an RT-PCR protocol, and expression was confirmed by confocal microscopy using specific rabbit antiserum. Subsequently, four Bos taurus steers were immunized 3 times every three weeks with the mixture of peptides dissolved in PBS emulsified using Montanide ISA 71 VG adjuvant while four Bos taurus steers were immunized only with the adjuvant and PBS. Twenty-four days after the last immunization the animals were challenged with a virulent strain of B. bovis, and a daily physical examination was performed for 15 days. Less severe clinical signs were observed in animals immunized with the peptides. A lower amount of antibodies was observed in the serum of the animals of the control group after the challenge, compared to the total antibodies in the serum of the animals immunized with the peptides, which indicates a possible interference in the bovine immune response caused by the TCTP of B. bovis. It is concluded that B. bovis has a tctp gen that is transcribed and expressed in intraerythrocytic stages, and that the protein contains conserved peptides with B-cell epitopes which induce antibodies in immunized cattle, and finally, B. bovis TCTP might have a role in the establishment of infection. By better understanding the participation of TCTP in the host-parasite interaction, important information will be generated to understand the pathogenic mechanisms through which Babesia bovis generates damage.
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P-27

Selection of conserved surface antigens of *Eimeria sp.* through a bioinformatic approach

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Coccidiosis caused by *Eimeria* spp. has an important economic significance for the poultry industry. *E. tenella (Et)* and *E. acervulina (Ea)* are 2 of the 3 most frequent species, the former associated with a high rate of mortality. Although there are many effective live vaccines available in the market, they have some disadvantages that promote the study of novel antigens and the development of new vaccines based on alternative technologies. Given that glycosylphosphatidylinositol (GPI)-anchored proteins, displayed on the surface of the parasite, are good vaccine candidates, the objective of this work was their selection in *Et* and *Ea* through reverse vaccinology and the study of polymorphisms from new native isolates.
A sequential pipeline, previously applied to related Apicomplexa, was followed and 3 *Et* and 3 *Ea* genes for polymorphism analysis, based on i. Presence of GPI anchor and absence of transmembrane sites; ii. Surface localization; iii. Evidence of sporozoite transcription, and iii. No of strong and weak binders in MHC I predicted sites. Specific oligonucleotides were designed for PCRs from *Eimeria*-DNA of Argentinian isolates (n=4). Amplicons were subjected to NGT sequencing, and sequence multiple alignments were done upon identity confirmation.
Fifteen *Ea* and 89 *Et* GPI-proteins were predicted from the proteomes and secretomes (6,873 and 428 *Ea* proteins, and 8,627 and 624 *Et* proteins, respectively) and a short list of 8 *Ea* or 22 *Et* proteins was produced from which *etsag1, etsag, ethyp, easag4, easag2* and *eahyp* were selected for sequencing. *ethyp*, *easag2,* and *eahyp* have demonstrated 100% of conservation among isolates and the reference sequence, *etsag1* from 1 isolate showed 99.91 %, containing 2 mutations; *etsag* from 3 isolates showed 99.88 % with 2 mutations and *easag2* from 1 isolate showed 99.66 % with 4 mutations. In conclusion through a bioinformatic approach, conserved potential vaccine antigens could be selected.

P-28

Cultivation of *Theileria annulata* transformed cells in serum-free media

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Tropical theileriosis is a protozoan disease caused by *Theileria annulata* that affects cattle in Northern Africa, the Middle East and Asia where vector ticks of the genus *Hyalomma* occur. Various measures are applied to control the disease, including vaccination with attenuated *T. annulata* schizonts. Cultivation of *T. annulata* schizonts is mainly conducted in media containing Foetal Bovine Serum (FBS), which has some disadvantages such as costs, batch-to-batch variation and ethical concerns. In this study, we conducted three experiments to evaluate the ability of 1) *T. annulata* strains grown in RPMI with 10% FBS (RPMI-FBS) to adapt and grow in serum-free media (i.e., HL-1, RPMI without FBS supplementation, ISF-1, and M199), 2) a *T. annulata* strain grown in ISF-1 and subsequently frozen in this medium to grow in ISF-1 again after long-term storage in liquid nitrogen and 3) a *T. annulata* strain freshly isolated from infected bovine lymphocytes to grow in ISF-1, also after cryopreservation. Cell numbers, schizont index, the viability and generation doubling time were calculated in all experiments. In the first experiment, the Hessiene and Beja cell lines from Tunisia previously cultivated in RPMI-FBS and adapted to serum-free media continued to grow significantly better in RPMI-FBS compared to the serum-free media. In the second experiment, a Tunisian cell line (Hessiene) cryopreserved in ISF-1 with 5%[v/v] dimethylsulfoxide (DMSO) grew better after thawing in RPMI-FBS compared to ISF-1 with a highly significant difference in cell growth ($p<0.001$), whereas the third experiment showed that the Ankara cell line had similar growth characteristics in both RPMI-FBS and ISF-1 before and after thawing, with a shorter generation doubling time in ISF-1 than in RPMI-FBS ($p=0.23$).

Our findings suggest that freshly isolated cells can be propagated, frozen and thawed in serum-free media such as ISF-1, but once cells are adapted to cultivation in the presence of FBS or resuscitated from frozen storage, propagation in serum-free media may not perform as well as cultivation in RPMI-FBS.

P-29

Comparative view on the asexual cycle of *Besnoitia besnoiti* and *Toxoplasma gondii in vitro*

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Besnoitia besnoiti, a protozoan parasite from the phylum Apicomplexa, is the causative agent of bovine besnoitiosis, a disease that leads to considerable economic losses in cattle farms and severely affects animal welfare (1). Although experimental infection of rodents and rabbits is possible (2), the parasite seems to be very host specific. In 2010, the European Food Safety Authority (EFSA) classified bovine besnoitiosis as an "emerging disease" (3).
Nevertheless, the molecular mechanisms of cell invasion and replication of this parasite have been poorly understood. One reason for this is the lack of efficient protocols for stable transfection to allow targeted genetic manipulation.
In contrast, the closely related species *Toxoplasma gondii* can infect almost every warm-blooded animal. In addition, it is a well-studied model organism for which a variety of methods for in vitro cultivation and genetic manipulation are available (4).
By comparatively examining the lytic cycle of both organisms, we want to gain insights that will contribute to a better understanding of the biology of *B. besnoiti*. In addition, to understand better the molecular mechanisms underlying our observations, we are trying to establish methods for stable transfection and targeted genome editing in *B. besnoiti*. As a first step, we successfully generated a *Besnoitia besnoiti* strain, which is deficient for the gene for Hypoxanthine-xanthine-phosphoribosyl-transferase to enable easy drug selection.

References:

1) Alvarez-García, G., Frey, C. F., Mora, L. M., & Schares, G. (2013). A century of bovine besnoitiosis: an unknown disease re-emerging in Europe. Trends in parasitology, 29(8), 407–415. https://doi.org/10.1016/j.pt.2013.06.002

2) Basso, W., Schares, G., Gollnick, N. S., Rütten, M., & Deplazes, P. (2011). Exploring the life cycle of Besnoitia besnoiti - experimental infection of putative definitive and intermediate host species. Veterinary parasitology, 178(3-4), 223–234. https://doi.org/10.1016/j.vetpar.2011.01.027

3) EFSA European Food Safety Authority (2010). Bovine Besnoitiosis: an emerging disease in Europe. EFSA J. 2010; 8:1499. https://doi.org/10.2903/j.efsa.2010.1499

4) Winiger, R. R., & Hehl, A. B. (2020). A streamlined CRISPR/Cas9 approach for fast genome editing in Toxoplasma gondii and Besnoitia besnoiti. Journal of biological methods, 7(4), e140. https://doi.org/10.14440/jbm.2020.343

P-30

Identification of *Sarcocystis* species present in diaphragm muscles from wild boars (Sus scrofa) in Switzerland

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Sarcocystis spp. are heteroxenous protozoan parasites, which form tissue cysts in muscles of intermediate hosts (IH) and sporulated oocysts in the intestinal mucosa of definitive hosts (DH). Wild boars (*Sus scrofa*) are IH for *Sarcocystis miescheriana* (with canids and racoons as DH) and *S. suihominis* (with humans and other primates as DH) and get infected by ingestion of sporocysts contaminating feed or water. The aim of this study was to identify and characterize the *Sarcocystis* spp. occurring in muscles from wild boars hunted and/or consumed in Switzerland. DNA was extracted from diaphragm muscle of 86 wild boars and tested by a PCR targeting the cytochrome c oxidase (COI) gene of *Sarcocystis suihominis*. Besides, 61 of these samples were tested by a further PCR targeting the 18S rRNA gene of *Sarcocystis* sp. and the obtained amplification products were sequenced; 25 samples were processed by homogenization and direct microscopic examination and 42 samples also by histopathology. All 86 samples resulted negative for *S. suihominis* DNA. By the 18S rRNA PCR, 77% (47/61) of the samples resulted positive and all sequences were 99-100% identical with GenBank sequences reported as *S. miescheriana*. By homogenization and direct microscopy, sarcocysts were observed in all 25 samples. By histopathology, *Sarcocystis* sp. cysts could be found in 35.7% (15/42) of the samples. Homogenization/direct microscopy and 18S rRNA PCR amplification seemed to be the most sensitive detection methods. Our results suggest a high prevalence of *Sarcocystis* spp. in wild boars in Switzerland. *S. miescheriana* was the only detected species, suggesting a frequent predator-prey interaction between wild boars and canids/racoons. A previous study showed the presence of *S. suihominis* in 1/100 wild boars from Italy. However, this study suggests a potentially low prevalence of *S. suihominis* in wild boars from Switzerland. Further studies including a higher number of samples and different muscles will be conducted to identify the presence of *S. suihominis* in wild boars in Switzerland.

P-31

Coccidia infection in Italian intensive swine breeding: epidemiological update and analysis of risk factors

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Coccidiosis, particularly those caused by *Cystoisospora suis*, are among the most common causes of diarrhea in piglets worldwide. In Italy, despite the economic importance of the swine breeding, there is a scarcity of updated epidemiological data on coccidia. Therefore, a study on coccidia infection in swine farrow to feeder operations under intensive system in northern Italy was planned.
Between April and November 2021, 10 sows with their litters and 15 weaners, when available, from 10 herds were included in the study. Faeces from 89 sows, 89 litters (fecal pool for each litter), and 135 weaners were sampled and analyzed with FLOTAC double technique® with a sugar-salt solution (specific weight 1.290). Coccidia oocysts were counted (OPG) and identified as *C. suis* or *Eimeria* spp. after sporulation. Individual and farm data were used for the analysis of risk factors by generalized linear mixed models.
54 samples (prevalence, P=17.25%) from 8 farms (intra-herd P=2.9-41.7%) scored positive for coccidia oocysts. Concerning suckling piglets, a P=26.9% was recorded, with a mean OPG ±standard deviation (s.d.) of 650.2±4390.4; oocysts were found only in piglets older than 8 days. In weaners, lower P (15.5%) but higher oocysts counts (mean OPG±s.d.= 1073.16±7283.01) than suckling piglets were recorded; weaners aged 30-44 days scored more frequently infected and had higher oocyst shedding (P=25.6%, mean OPG±s.d.= 2189.5±10564.7) than older ones (45-60 days, P=6.9%, mean OPG±s.d.=96.3±754.6). The lowest P and OPG values were recorded in sows (P=10.1%; mean OPG±s.d.= 80.31±399.6). Oocysts were identified as *C. suis* in suckling piglets and weaners and as *Eimeria* spp. in sows. In the latter, helminth eggs were also found: Strongylida (P=23.6%), *Ascaris suum* (P=7.8%), *Trichuris suis* (P=2.2%), and *Strongyloides ransomi* (P=2.2%).
Risk factors analysis did not show any association between coccidia infections and variables related to farm management. The previous records of diarrhea in suckling piglets resulted a predictor of *C. suis* infection (p-value=0.013), with higher OPG values in litters from farms where diarrhea was previously recorded (mean OPG±s.d.=1010.9+5470.1) if compared to those without any previous report (7.3±26.2). Similarly, animal cleanliness score resulted a predictor of infection in weaners (p-value=0.009) with higher OPG values in individuals showing 20-50% or >50% dirty body surface (DBS) (mean OPG±s.d.=2410.6±10825.6 and 4±27.9, respectively) if compared to those having <20% DBS (0±0).
The widespread of *C. suis* infection was depicted in piglets and weaners intensively reared in Italy. In sows, only *Eimeria* spp. were identified, confirming the environmental contamination as the main source of infection for piglets. The association between higher oocyst counts and the anamnestic report of diarrhea cases (in suckling piglets) or a worse animal cleanliness score (in weaners) endorsed the role of *C. suis* as a causative agent of enteric disease in pigs.

P-32

"Shedding light" on *Eimeria spp*. Dense Granules: fluorescent reporters as reliable biomarkers for subcellular localization studies.

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Eimeria tenella is an intracellular parasite that invades chicken intestinal epithelial cells, commonly causing production losses and, in some situations, host mortality. Control strategies should aim to interfere with the parasite at early stages (i.e., host-cell invasion and/or early first-generation schizont development) before significant damage is caused by second-generation schizonts. Secretory organelles play an essential role in these early stages; however, knowledge of some of these organelles and their secreted proteins is limited in *Eimeria*. This is the case for dense granules (DG), which have not been observed in *Eimeria* sporozoites, and related *E. tenella* DG (EtGRA) proteins, whose orthologues in other coccidia are critical in parasitophorous vacuole (PV) formation and parasite survival.

The study of the subcellular localization of proteins can be key to understanding their function, identifying organelles that might be involved in storage, and understanding protein dynamics, expression pathways and timing. Fluorescent proteins have been used as powerful tools for protein co-visualization when fused to a protein of interest; however, limitations around molecular tools to target endogenous proteins in *Eimeria* parasites commonly require transfection using plasmids including a cassette encoding the gene fusion as well as the native protein. We evaluated potential mislocalisation due to transgene overexpression by tagging known proteins with a fluorescent reporter and evaluating their localisation. mCherry-tagged *E. tenella* microneme protein 2 (EtMIC2) and refractile body protein (EtSO7) were targeted to the correct subcellular localisation, supporting the use of these cassette constructs to evaluate protein localisation and dynamics.

Here, we aimed to employ the tagged transgene system to elucidate the storage location for EtGRAs. We selected two candidate EtGRAs with orthologues and known functions described in other coccidian parasites, and generated stable transgenic populations of *E. tenella* expressing mCherry-tagged EtGRA candidate proteins. Extracellular transgenic sporozoites showed a dotted pattern compatible with intracellular vesicles. Intracellular sporozoites secreted EtGRAs through one lateral at the anterior end, accumulating in the PV on the side closest to the host cell nucleus. In parallel, a polyclonal antibody was raised and used for indirect immunofluorescence, illustrating equivalent patterns of protein localisation and dynamics. Immune-electron microscopy showed labelling compatible with vesicles neighbouring the refractile bodies in extracellular sporozoites and labelled protein was localized in an amorphous material after secretion into the PV. Although the nature of EtGRAs storage in *E. tenella* has not been characterized, we can confirm that this is not equivalent to classical DG observed in other coccidia.

P-33

Response to *Toxoplasma gondii in vitro* infection of macrophages and neutrophils from vaccinated sheep

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Toxoplasma gondii infection causes reproductive failure in small ruminants leading to approximately one million sheep abortions per year within the European Union. Currently, there is one commercial live-attenuated vaccine for veterinary use that confers protection against abortion, although the precise machinery responsible for its protection is yet poorly understood. In this sense, macrophages along with polymorphonuclear neutrophils (PMNs) are two important cell populations in the immune response against almost all pathogens. Thus, the aim of this study was to investigate the effect of vaccination against *T. gondii* infection in the response of ovine monocyte-derived macrophages (OvMØs) and PMNs.
A total of 10 adult Assaf sheep that tested seronegative against a wide panel of abortifacient agents were selected for this study. Animals were vaccinated with ≥10^5 *T. gondii* tachyzoites following manufacturer's instructions (Toxovax®). Three months post vaccination peripheral blood monocytes and PMNs were obtained from both vaccinated (n=5) and non-vaccinated (n=5) sheep. Monocytes, purified from peripheral blood mononuclear cells (PBMCs), were cultured *in vitro* until differentiation to OvMØs. Later, both PMNs and OvMØs were infected (MOIs of 3:1 and 1:1, respectively) and incubated (3 and 8 hours, respectively) with three different *T. gondii* isolates: (i) TgShSp1 (type II PRU-variant; ToxoDB genotype #3), (ii) TgShSp24 (type III, ToxoDB genotype #2) and (iii) the S48 vaccine strain. Subsequently, the transcription levels of different cytokines, toll-like receptors and iNOS were analyzed by qPCR. In addition, quantification of neutrophil extracellular traps (NETs) was carried out by measuring extracellular DNA with Pico Green® reagents.
OvMØs from non-vaccinated sheep showed significant up-regulation of IL-12, IL-10, TNFα, IL-6 and TLR2 when compared to the vaccinated counterparts (p<0.05), while the transcription levels of iNOS was upregulated in OvMØs from vaccinated sheep (p<0.05). The *T. gondii* isolate used for infection did not influence significantly the response of the OvMØs (p>0.05). Similarly, transcription levels of IL-8, IL-1β, TNFα and TLR2 were upregulated in PMNs from non-vaccinated sheep (p<0.05), although no differences in NETs quantification were observed (p>0.05). These results suggest that vaccination predispose OvMØs and PMNs towards a contained immune response upon stimulation with the studied parasites whereas could lead to a most efficient elimination of the parasite through the production of nitric oxide. However, further *in vitro* studies based on parasite quantification in these cell populations as well as co-cultures might help to better understand its role in the immune response after vaccination.
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P-34

Comparison of paromomycin efficiency on the *Cryptosporidium parvum* development by standard methods and impedance spectroscopy

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Cryptosporidiosis is an important zoonosis worldwide, causing intestinal infection to humans and a wide range of animals. *Cryptosporidium parvum* is one of the major causes of neonatal calf diarrhea leading to economic losses and compromised animal welfare. Despite the high prevalence, no vaccine or drug therapy is available yet. Research for new drugs against *Cryptosporidium* met difficulties due to its obligate intracellular parasite status requiring the support of a host cell and the absence of standardized/automated tools enabling the study of the parasite and drug screening.
In this study *Human ileocecal colorectal adenocarcinoma cells* (HCT-8) were grown to confluency and infected by *C. parvum* during 48 h with a range of paromomycin concentrations, the gold standard for *in vitro* screening. Results were obtained by standard methods such as real-time qPCR and microscopy. The level of infection was assessed using fluorescent images combined with an automatic detection of parasites using a *Fiji* plugin and morphometric data for each parasite were recorded. The *half maximal inhibitory concentrations* (IC_{50}) of paromomycin were obtained by both methods and will be compared to a new approach using an electrical impedance-based device to quantify infectivity of HCT-8 infected by *C. parvum* and for drug screening assessment. First results with the impedance signal shown a reproductible peak at 12 h post-infection which could be used as an infectivity sensor, faster than current methods.

P-35

Characterization of Intestinal mononuclear phagocyte subsets of young lamb at homeostasis by single cell RNA-Seq and during *Cryptosporidium parvum* infection by flow

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Intestinal mononuclear phagocytes are key immune cells that maintain intestinal homeostasis and participate in the protective immune responses toward pathogens.

Cryptosporidiosis is a poorly controlled zoonosis caused by an intestinal parasite, *Cryptosporidium parvum* (*Cp*), with a high prevalence in ruminant farms. 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 in controlling the acute phase of *Cp* infection and deciphered the role of different subsets of intestinal mononuclear phagocytes in this protective immune response.

The aim of this project was to better characterize intestinal mononuclear phagocytes in lamb at homeostasis and during *Cp* infection. 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 (IPP) (lymphoid tissue) that extends all along the ileum. We first performed a characterization of mononuclear phagocytes present in the IPP of a non-infected 10-day-old lamb by a single cell transcriptomic approach on CD11c+ MHCII+ sorted cells. This global approach allowed us to identify in the IPP of young lamb two main populations of macrophages and at least three different population of dendritic cells. Then, we carried out phenotypic and functional analyses of these different cell subsets by flow cytometry and transcriptomic methods in various compartments of the small intestine at homeostasis and during infection.

P-36

Eimeria Vaccine Development Through Graph Based Machine Learning

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Eimeria tenella is an intracellular apicomplexan parasite which can infect the chicken with absolute host specificity, causing a haemorrhagic variant of enteric coccidiosis. This disease can cause diarrhoea, blood loss, malnutrition, and increased risk of secondary infection, with a deleterious effect on egg and meat production. The financial impact of *E. tenella* is estimated at ~£10.4 billion/year worldwide and is predicted to rise as poultry becomes the primary sustainable food source for a growing global human population. The established method of rotating anticoccidial drug regimens has led to widespread resistance to all existing chemoprophylactics, prompting consumer and legislative pressure in both the US and EU to move towards vaccine-based interventions. Identification of subunit vaccine candidates has focused on leveraging *Eimeria* proteins to generate effective immune responses, promising higher efficiency and lower cost-per-animal compared to current live-oocyst vaccines which require the full *in vivo* infective process to occur.

This project seeks to generate an *in silico*, graph-based network (GBN) representation of the host-pathogen protein interactome to streamline candidate prioritisation in vaccine development. Mapped with RNAseq gene expression profiles generated from an infection time course experiment, interactome GBNs have been curated that comprise 7,432 unique proteins and 547,284 connections for the host, and 1,675 proteins with 98,666 connections for the pathogen.

A mixture of binding prediction algorithms along with curated interaction data are currently being employed to produce a novel, joint host-pathogen interactome for *E. tenella*. The joint model will allow us to highlight interfacing hotspots between organisms with vaccine target potential, supplemented by the VACCEED machine learning framework for target prioritisation based on a reverse vaccinology approach. Longitudinal, multi-omics data will be integrated and overlayed onto the network to exploit the GBNs ability to serve as a foundation for graph-based deep learning and produce a dynamic model of gene expression profiles over time. This model can then be used for *in silico* simulation of potential interventions, to be validated using downstream wet-lab techniques. The application of this pipeline will be tested and fine-tuned with RNASeq datasets collected from in-house chicken vaccine trials.

P-37

Are *Toxoplasma gondii* infected water voles (*Arvicola amphibius s.l.*) easy prey for cats?

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The obligate intracellular protozoan parasite *Toxoplasma gondii* is the cause of one of the most common parasitic infections on earth. There is evidence suggesting that *T. gondii* may manipulate the behavior of its rodent host to enhance transmission to its definitive feline host, thereby ensuring completion of its life cycle. The aim of this study was to test this hypothesis in the natural environment, given that most studies have been performed with hosts maintained under laboratory conditions. For this purpose, we analysed cat-hunted (n=82) and trap-captured (n=49) rodents of the species Arvicola amphibius s.l. (Syn. A. terrestris, A. scherman) sampled throughout Switzerland. Brain and skeletal muscle samples from each rodent were tested for T. gondii DNA, and positive samples were further genotyped using a multilocus nested-PCR-RFLP approach including 11 T. gondii genetic markers. All amplified markers were sequenced and subjected to in silico RFLP- analysis to enhance the discriminatory power of the method. The prevalence of T. gondii DNA in cat-hunted rodents was 11.1 % (7/63) whereas no animal was infected among the 49 trap-captured individuals (0%; Fisher's exact test: p = 0.0176). Nevertheless, these results should be considered with caution given the limited number of samples available and the many factors at play in the natural environment, involving both predators and prey. All detected T. gondii parasites, which could be completely genotyped, exhibited the ToxoDB #3 genotype, a Type II variant. The present data reveal the forthcoming need to further test this hypothesis in the natural environment and on different host species.

P-38

Toxoplasma gondii and *Neospora caninum* seroprevalence among cattle in Ukraine

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Toxoplasma gondii is a unicellular parasite that can infect humans and numerous warm-blooded animals, including cattle. *Neospora caninum* can affect different animal species, including cattle. The main aim of our research was to estimate the seroprevalence of *T. gondii* and *N. caninum* among cattle from the territory of Ukraine. Blood samples were collected from 174 cattle, from farms with 1300 to 4500 animals, from Volyn, Khmelnytskyi, Cherkasy, Poltava and Ternopil regions of Ukraine, during time period from December, 2021 to February, 2022. Of the samples, 17 of 174 tested positive for antibodies against *T. gondii*, yielding an apparent seroprevalence of 10.4%. The seroprevalence was 10.3% among animals from Volyn region, 18.9% from Khmelnytskyi region, 2.6% from Cherkasy region, 10.0% from Poltava region and 10.0% from Ternopil region. Of the samples, 50 of 164 tested positive for antibodies against *N. caninum*, yielding an apparent seroprevalence of 28.7%. The seroprevalence was 33.3% among animals from Volyn region, 48.9% from Khmelnytskyi region, 34.2% from Cherkasy region, 0.0% from Poltava region and 10.0% from Ternopil region. Two samples (1.2%) were positive for antibodies against both *T. gondii* and *N. caninum*. Based on questionnaire data, cats and dogs had access to the territory of the farms. The results indicate exposure to both pathogens.

These results were also presented at ICOPA 2022.

Abstracts Poster Session 2 (39-77)

P-39

Tropism and persistence of *Toxoplasma gondii:* from pork carcass to dry sausage

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Toxoplasma gondii is an important zoonotic foodborne parasite. Amid the possible transmission routes, meat of infected animals appears to be a major source of infection in Europe. Pork is the most consumed meat in France, with dry sausages well represented. The risk of transmission via consumption of processed pork products is largely unknown, mainly since processing will affect viability but may not entirely inactivate all *T. gondii* parasites.
We investigated the presence of *T. gondii* DNA in the shoulder, breast, ham and heart of six pigs orally inoculated with 1000 oocysts or tissue cysts and two naturally infected pigs, by means of magnetic capture qPCR (MC-qPCR). Muscle tissues of the experimentally infected pigs were further used to evaluate the impact of manufacturing processes of dry sausages, including different concentrations of nitrites (0–120 ppm) and NaCl (0–26 g/kg), ripening (2 days at 16–24°C) and drying (up to 30 days at 13°C), by a combination of mouse bioassay, qPCR and MC-qPCR. Additionally, results of MC-qPCR were used to calculate estimates of *T. gondii* parasite burden.
DNA of *T. gondii* was detected in all pigs, including in 41.7 % (10/24) of muscle samples and in 87.5 % (7/8) of hearts. The number of parasites per gram of tissue was estimated to be the lowest in the hams (M = 1, SD = 2) and the highest in the hearts (M = 147, SD = 233). However, the *T. gondii* burden estimates varied on the individual animal level, by the tissue tested and the parasitic stage used (oocyst or tissue cyst). Of dry sausages, 94.4 % (51/54) were positive for *T. gondii* by MC-qPCR or qPCR, with the mean *T. gondii* burden estimate equivalent to 31 parasites per gram (SD = 93). Only the untreated sausage sample collected on the day of production was positive by bioassay.
The results suggest an uneven distribution of *T. gondii* in the tissues examined, and possibly absence or a concentration below the detection limit in some of them. Moreover, the results indicate an effect of additives used in dry sausages on the viability of *T. gondii* as soon as the first day of the production process. The results will be used in a quantitative microbiological risk assessment aiming to estimate the relative contribution of different sources to *T. gondii* human infections.

P-40

Meat processing as part of a quantitative microbial risk assessment for *Toxoplasma gondii* infections in Europe

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Toxoplasma gondii is an important zoonotic protozoan parasite in Europe, with a high disease burden largely owing to cases of congenital toxoplasmosis. People mainly become infected either via the ingestion of oocysts shed by felines or by tissue cysts in a wide range of meat products. To develop effective intervention strategies, it is important to know the relative importance of the different sources of infection. An aim of the European project TOXOSOURCES was to identify the most important sources of *T. gondii* infection in nine European countries by quantitative microbial risk assessment (QMRA). The model output will be compared to data on prevalence in humans and risk factor information from the literature.
The QMRA food chain model consists of several steps, for which data has been gathered and models established: 1) Data on the prevalence of *T. gondii* infection in livestock and wildlife were collected from the literature and analyzed to obtain prevalence estimates at slaughter or hunting age. 2) Data on food habits and other exposure behavior was collected using an online consumer survey in the Czech Republic, Denmark, France, Germany, the Netherlands, Norway, Poland, Portugal, and Spain. 3) Processing information for generic and country-specific meat products were collected from handbooks, recipes, and product label information. 4) Previously developed models on the effect of heating, freezing and salting on the viability of *T. gondii* were updated with new data from the literature. 5) In the final step, a dose-response model quantified the probability of infection.
Prior to establishment of the full QMRA, a simplified food-chain model consisting of steps 1, 3, 4, and 5 was developed based on example products. In that way, the effect of the processing methods on the risk per portion was evaluated. This information will help to identify potential high-risk products. On the population level, the importance of each product will be influenced by frequency of consumption (step 2). However, information on potential high-risk products is important for individual consumer decisions, and for public health decision making to recommend or avoid certain food products.

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P-41

Diagnosis of *Sarcocystis spp.* in pigs and wild boars from Argentina

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Sarcocystis spp. are protozoans with heteroxeneous cycle, forming muscle cysts in their intermediate hosts (IH) and oocysts with sporocysts in their definitive hosts (DH). *Sarcocystis miescheriana* and *S. suihominis* have swine as IH and canids and hominids as DH, respectively. The objectives of this work were to identify and differentiate *Sarcocystis* spp. in pigs and wild boars muscles by microscopic and molecular methods. Muscles samples from 561 pigs and 240 wild boars were processed by homogenization and direct microscopic examination. Pigs were classified according to the breeding system in: intensive farming ([IF, n=295], animals kept in confinement during most of their productive cycle), or semi-extensive farming ([SEF, n=266], animals bred outdoors, generally family or backyard production). The PBS homogenization was performed with a mixer using 5-10 g of muscles, centrifuged and further observed in an inverted microscope. Cysts from 10 pigs and 11 wild boars were collected for transmission electron microscopy (TEM). Aliquots from homogenates were stored in microtubes for DNA extraction and subsequent PCR for the *Sarcocystis* spp.18S rRNA gene fragment. All wild boars samples were analyzed by PCR and for pigs 202 samples (n=104-IF and n=98-SEF) were randomly selected. Samples resulting positive were further processed by a *S. suihominis* specific PCR, targeting the cytochrome c oxidase (coxI) gene. Regarding pigs samples, 24.8% (139/561) were positive by direct microscopy, with a significantly higher prevalence in the SEF (34.6%; 92/266) than the IF pigs (15.9%; 47/295) ($p < 0.05$). Of the 202 samples analyzed by PCR, 80 were positive (39.6%) for the 18S rRNA fragment, and none for cox I. The infection rate obtained by PCR was significantly higher for SEF pigs (49%; 48/98), compared to IF pigs (30.8%; 32/104) ($p<0.05$). In all the pig samples analyzed by TEM, cyst wall ultrastructure was compatible with *S. miescheriana*. Out all wild boar samples, 48.3% (116/240) were positive by direct microscopy and 45.8% (110/240) by PCR. Of the PCR positive samples, 3.6% (4/110) were positives for coxI PCR, however, insufficient DNA concentration for proper sequencing was obtained. The ultrastructure of the cysts wall by TEM were consistent with *S. miescheriana* in all 11 wild boar samples, and one sample had a cyst identified as *S. suihominis*. The *Sarcocystis* spp. prevalence was higher in wild boars than in pigs. In conclusion, the finding of the zoonotic parasite *S. suihominis* in one wild boar muscle sample is the first report in South America, which confirms a certain risk of infection for humans, especially through the consumption of raw or insufficiently cooked meat. In relation to pigs, the type of breeding could be a determining factor when considering infection by *Sarcocystis* spp. To our knowledge, this is the first study that analyzes the infection rate and differentiate *Sarcocystis* spp. in pigs and wild boars from Argentina.

P-42

Evaluation of risk of infection by *Toxoplasma gondii* from retail meat in São Paulo city, Brazil

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Toxoplasma gondii is an important zoonotic parasite with global impact capable of infecting all warm-blooded animals. Toxoplasmosis is recognized by the World Health Organization as one of the most important foodborne diseases worldwide. Infection occurs through ingestion of contaminated food or water or undercooked infected meat, or vertically from mother to foetus during a primary infection. There is geographical variation in the population structure of *T. gondii*, with an abundance of non-archetypal strains being reported in South America. In Brazil, the seroprevalence of *T. gondii* in humans can be as high as 97% and severe ocular toxoplasmosis is diagnosed more frequently than in Europe or the USA. In Brazil, exposure of livestock to *T. gondii* is very high (30-100%) yet there is a notable paucity of data on the relative risk of infection from retail meat. This is a significant knowledge gap as meat consumption in Brazil is amongst the highest in the world and there are non-archetypal and potentially virulent strains of *T. gondii* circulating in this region. This ongoing research aims to conduct a study of the incidence, viability, genetic diversity and virulence of *T. gondii* in retail meat to assess the risk of foodborne infection to consumers in the city of São Paulo, Brazil. For this study, a total of 304 samples (113 pork, 103 chicken and 88 beef) have been purchased from different retail outlets. Meat samples (50g/sample) were digested in acid pepsin, DNA extracted using the Wizard® Genomic DNA purification Kit (Promega Corp.), and the quantitative real time PCR (qPCR) performed using the 529 bp fragment as a target for *T. gondii* and myostatin gene as endogenous control. Positive meat samples were bioassayed in mice to determine *T. gondii* viability by parasite isolation. Ten meat samples (3.3%) were qPCR positive (4 chicken, 3 beef and 3 pork). Five bioassays were performed in mice, but with no parasite isolation. The risk of retail meat consumption determined by viability of the parasite has not been confirmed, although the parasite has been detected using molecular diagnostic techniques. This study is still ongoing with a sample size of about 1,000 retail meats to be analysed. The results from this study will provide information on the relative risk of infection with *T. gondii* from the consumption of raw (or undercooked) meat from different livestock species in the city of São Paulo.

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Detailed anatomical distribution of *Toxoplasma gondii* in tissues of infected pigs

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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 represents a potential risk for consumers as one of the most consumed meats in France. Therefore, we aimed to investigate and evaluate the anatomical distribution of *T. gondii* within tissues of experimentally and naturally infected pigs and to identify the predilection sites.

A set of muscles (n=36) and organs (n=14) was collected from naturally infected pigs (n=2) and pigs experimentally infected with 1000 oocysts (n=3) or 1000 tissue cysts (n=3). The anatomical distribution of the parasite was investigated in the three groups of pigs by means of qPCR.

Proportion of muscle tissues positive for *T. gondii* DNA was 21.5 % (23/107) in the pigs infected with oocysts, 60.2 % (65/108) in those infected by tissue cysts, and 43.6 % (27/62) in the naturally infected pigs. The most frequently infected muscle was *musculus quadriceps femoris* (75 %, 6/8), and the most frequently infected organ were the eyes (66.7 %, 4/6).

The results provide a base for an estimate of parasitic burden in tissues, and suggest an uneven concentration of tissue cysts within the tissues of the tested animals. Importantly, the results revealed interesting distribution pattern by the infective stage used, reflecting different sources of infection.

P-44

Comparison of *Toxoplasma gondii* distribution in tissues of experimentally infected pigs

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Toxoplasma gondii is a protozoan zoonotic parasite with a worldwide distribution. In Europe, the majority of human infections are estimated to be food-borne, with meat of the infected animals playing an important role in the transmission. Pork is one of the most frequently consumed meats in Europe, and therefore represents a potential risk if consumed raw or undercooked. We aimed to investigate and evaluate the anatomical distribution of *T. gondii* within the tissues of experimentally infected pigs with various *T. gondii* isolates and infectious stages.

A total of 22 pigs were divided in four groups based on the combination of different *T. gondii* isolates (type II or III) and infectious stages (400 oocysts or 10 tissue cysts) used during the experimental infection. A set of meat cuts (n=3) and organs (n=6), was collected from each pig, and investigated by means of qPCR.

Proportion of meat cuts positive for *T. gondii* DNA was 50.0 % (11/22) in the shoulders, 54.5 % (12/22) in the loins, and 54.5 % (12/22) in the hams. Proportion of the organs positive for *T. gondii* DNA was 0.0 % (0/22) in the livers, 4.5 % (1/22) in the spleens, 13.6 % (3/22) in the lungs, 13.6 % (3/22) in the kidneys, 27.3 % (6/22) in the hearts, and 63.6 % (14/22) in the brains. Overall, *T. gondii* DNA was confirmed in 16.7 % (15/90) of the pigs infected with oocysts, and in 42.6 % (46/108) of the pigs infected with tissue cysts. Infection with type II resulted in 14.8 % (16/108) of positive tissues, and in 47.8 % (43/90) of positive tissues in the pigs infected with type III.

The results suggest an uneven concentration of tissue cysts in the tested tissues. The 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.

P-45

Molecular detection of different *Sarcocystis* spp. in cattle carcasses affected by bovine eosinophilic myositis, including a putative new species

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Bovine eosinophilic myositis (BEM) is an inflammatory myopathy occasionally observed in striated muscle of affected cattle at slaughter, resulting in carcass condemnation. Due to the considerable economic losses associated to the myopathy, during the last years there has been an increase interest in its etiology, despite the low prevalence. There is evidence linking these lesions to the presence of protozoan parasites belonging to the genus *Sarcocystis*, which includes at least six species using cattle as intermediate hosts, that is *S. hominis*, *S. cruzi*, *S. hirsuta*, *S. bovini*, *S. bovifelis* and *S. heydorni*. Yet, the high prevalence of *Sarcocystis* spp. in cattle carcasses does not correlate with the low prevalence of BEM. Researchers have thus focused on the possible association between BEM and particular *Sarcocystis* species. The goal of the present study was to use molecular methods to identify *Sarcocystis* spp. inside and outside BEM lesions in condemned cattle carcasses, in order to evaluate the possible role of different *Sarcocystis* spp. in BEM etiology.
To reach this aim, from January 2019 to January 2020, heart and striated muscle samples were collected from 25 BEM condemned carcasses. Gross lesions were categorized into two groups: "green focal lesions" (GFL) and "green diffused patches" (GDP). One to five samples with lesions and two without lesions were collected from each carcass, for a total of 94 samples. Genomic DNA was extracted and analyzed by multiplex-PCR targeting 18S rDNA and *cox1* mtDNA genes. PCR products amplified using the genus specific primer set in absence of the specific fragment for *S. bovifelis*, *S. hominis*, *S. cruzi* or *S. hirsuta*, were sequenced to achieve species identification. Unidentified species were molecularly characterized through the amplification and sequencing of the complete 18S rDNA gene and the partial cox1 gene.
Out of 25 carcasses, 24 revealed the presence of at least one *Sarcocystis* spp. (96%; 95% C.I.: 78.86 - 99.99 %). The majority of intralesional *Sarcocystis* spp. were found to be *S. hominis*, followed by *S. bovifelis*, *S. cruzi* and *S. hirsuta*. The presence of *S. bovifelis* and *S. hominis* was significantly higher in intralesional samples (43.2% and 52.3%, respectively) than in samples without lesions (2% and 14%, respectively), while there was no significant difference between the presence of *S. cruzi* or *S. hirsuta* in intralesional (27.3% and 2.3%, respectively) and extralesional (30% and 2%, respectively) samples. Moreover, a putative *Sarcocystis* n. sp. was detected in one carcass and molecularly characterized.
The present study contributes to our understanding of the importance of different *Sarcocystis* spp. in the BEM pathogenesis. The results emphasize the association of *S. hominis* and *S. bovifelis* with bovine eosinophilic myositis and highlight the presence of a new *Sarcocystis* sp. using cattle as intermediate hosts.

P-46

Detection of viable *Toxoplasma gondii* in game meat

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Consumption of infected, undercooked meat is a well-known risk factor for transmission of *Toxoplasma gondii*, and when comparing foodborne pathogens, *Toxoplasma* has been ranked as one of the most significant causes of disease burden both in Europe and the USA. Despite this, there have been few studies assessing the risk of infection from retail meat samples. The aim of this study was to investigate the presence of *T. gondii* in commercially available meat cuts, and to assess parasite viability in higher risk meats. Initially, 300 meat samples (beef, chicken, lamb, pork and venison) were purchased from retail outlets, and screened for *T. gondii* using quantitative PCR. Results revealed *T. gondii* DNA was present in 35.4% venison samples, 6.9% lamb samples, 4.8% chicken samples, 4.2% pork samples and 0% beef samples. Partial PCR-RFLP genotyping revealed non-clonal genotypes. Given the high incidence of *T. gondii* in venison and the propensity to consume this meat undercooked, parasite viability was determined in this meat type. Twenty-three fresh venison products were purchased, and *T. gondii* DNA was detected in 5 samples (21.7%). For each of the positive samples, 6 outbred CD-1 mice were inoculated and monitored for 28 days. Viable *T. gondii* was detected in 2 venison products. Tachyzoites were isolated and cultured from the positive mice, and PCR-RFLP analysis revealed a Type II-variant (#3) in both samples. These results highlight the potentially important role of game meat, specifically venison, in foodborne transmission of *T. gondii*.

P-47

Efficacy of paromomycin against calves cryptosporidiosis

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The objective of this study was to test the efficacy of paromomycin on two large Slovenian dairy farms with a known problem with cryptosporidiosis. Thirty calves naturally infected with *Cryptosporidium* sp. from each farm were enrolled in the study (15 test calves – treated with paromomycin and 15 control calves – treated conventionally – fluid therapy and NSAID + antibiotic if indicated). Calves were separated from their mothers immediately after birth and raised individual boxes until they moved to group boxes at about 3 weeks of age. In farm A, paromomycin treatment was started on day 4 after birth (in clinically healthy calves with positive HuveCheck® Crypto test) for 7 consecutive days; in farm B, treatment with paromomycin was started after cryptosporidiosis was confirmed in calves with diarrhoea. Dosing was according to the manufacturer's instructions (Huvepharma, Belgium). All calves were weighed at birth and at one month of age. In addition, oocysts were counted (average in 8 high-powered fields) in faeces smears on days 11 and 16 using modified Ziehl Neelsen staining. In farm A, we found statistically significantly better weight gain and fewer cryptosporidia oocysts in faeces smears in the treated calves compared to the control calves (receiving conventional therapy). Although weight gain and oocyst count were on average better in the treated calves on farm B, the difference was not statistically significant. A better effect of paromomycin was observed when it was given before the onset of diarrhoea, but since paromomycin is an antibiotic, prophylactic treatment cannot be recommended. However, metaphylactic use is beneficial during the calving peaks given cryptosporidiosis has been detected.

P-48

In vitro anticoccidial effect determination of naringenin and dehydrated grapefruit peel (*Citrus x paradisi*)

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Anticoccidial drugs are among the preferred resources to control coccidiosis, but the need to have natural alternatives has prompted research into plant products that improve animal performance and reduce *Eimeria* oocyst shedding while avoiding problems of drug residues. Two studies were conducted to evaluate the effects of naringenin and dehydrated grapefruit peels (GDP) on *Eimeria* infection and intestinal health. In the first study, BUVEC cells were cultured and infected with *Eimeria ninakohlyakimovae* sporozoites and then exposed to different concentrations of a naringenin solution. Sporozoites mortality was observed as well as a diminished infection rate. In the second study, an in vitro culture system was used to evaluate the integrity of caprine epithelial cells infected with *Eimeria ninakohlyakimovae* and incubated with GDP, as well as the infection rate and schizont development of parasites exposed to GDP. Transepithelial electric resistance (TEER) values were increased in GDP and TTZ groups, as well as a decreased cell invasion rate of sporozoites of *E. ninakohlyakimovae* and a reduced number and size of schizonts/mm^2 of the intestinal cells culture. These findings demonstrate the beneficial activities of naringenin and GDP on caprine coccidiosis *in vitro*.

P-49

Identification of the phosphodiesterase inhibitor beminafil as a potential anticryptosporidial drug.

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Cryptosporidiosis, a disease caused by the intestinal apicomplexan parasite Cryptosporidium, is a major cause of life-threatening diarrhoea in young children globally, inflicts chronic diarrhoea in AIDS patients, and is the most common cause of waterborne diarrhoeal outbreaks in the United States. Nitazoxanide, the only FDA-approved anticryptosporidial in the United States, is only modestly effective in children and equivalent to a placebo in AIDS patients. Cryptosporidiosis also presents an economic concern for the livestock industry, predominantly affecting young ruminant animals and decreasing productivity. Similarly, the only approved treatment for prevention of bovine cryptosporidiosis, halofuginone lactate, does not eliminate the infection but decreases diarrhoeal symptoms in cattle. Notably, this treatment is currently not approved for therapeutic use in other food animals like pigs, sheep, or goats.
To identify new anticryptosporidial leads with novel targets and illustrated mode-of-action, we used a microscopy-based assay to screen two publicly available EMD-Serono target-based compound libraries for Cryptosporidium growth inhibitors. These screens identified a human phosphodiesterase (PDE)-5 inhibitor, beminafil, as a potent C. parvum growth inhibitor. Beminafil and chemical analogues sourced from EMD-Serono had comparable parasiticidal activity against C. parvum and C. hominis (i.e., the species that cause most human and livestock infections), significant in vivo efficacy in a cryptosporidiosis mouse model, and promising structure activity relationships for drug optimisation. Meanwhile, chemically unrelated PDE5 inhibitors, including the potent human PDE5-inhibitor sildenafil, lacked anticryptosporidial activity, suggesting that beminafil likely targets a parasite PDE. The function of PDEs in Cryptosporidium remains unknown, so, in addition to employing traditional medicinal chemistry for drug optimization, we are working to elucidate the molecular target and mode-of-action of beminafil using a combination of recombinant PDE expression for in vitro enzyme assays, life cycle-based phenotypic assays, and generation of transgenic C. parvum parasites using CRISPR. Our findings should illuminate a currently unexplored area of Cryptosporidium cyclic-nucleotide PDE signalling while facilitating target-based drug optimisation.

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Toxoplasma gondii MOB1 protein at the crossroad of cytokinesis and tissue homeostasis

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Monopolar spindle One Binder1 (MOB1) proteins are conserved kinase adaptors and key components of the yeast Mitotic Exit Network and the metazoan Hippo Pathway, signaling pathways that share a common core kinase module and regulate fundamental cellular processes as cytokinesis and ploidy maintenance. The Hippo Pathway regulates tissue homeostasis, organ growth and stem cell maintenance. The life cycles of Apicomplexa rely on the parasites' ability to differentiate between different life cycle stages and govern its proliferation, thus Hippo signaling could play an important role in regulating the apicomplexan life cycle. We identified one *mob1* gene in *Toxoplasma gondii* whose expression is drastically downregulated during tachyzoite replication in human fibroblasts. To characterize its function, we obtained gain-of-function transgenic lines with ligand-controlled destabilization domain and CRISPR/Cas9 generated loss-of-function clonal lines. The *mob1* overexpression and knockout induced a discreet, but significant, decrease and increase of tachyzoite replication *in vitro*. The effect of *mob1* knockout is surprising as most *mob1* genes studied are essential for cell survival by ensuring accurate cytokinesis. However, this result was confirmed using an alternative homologous recombination *T. gondii* knockout system and is consistent with the result of an *in vitro* genome wide CRISPR/Cas9 screen (DOI: 10.1016/j.cell.2016.08.019) for *mob1* that indicates this is not an essential gene in the tachyzoite stage during human fibroblast infection. Tachyzoites expressing endogenously C-terminal tagged *mob1* exhibit MOB1-HA expression with a dot-like pattern, localized between the newly individualized daughter nuclei at the end of mitosis, pointing to a multifaceted function of this gene in *T. gondii*. Indeed, using proximity-biotinylation, we identified a MOB1 interactome with both conserved and novel components. The analysis of MOB1 sequences shows a lack of conservation of some key amino acid residues in coccidians, indicating a possible explanation for the divergent function of the *T. gondii mob1*. Analyzing the effect of *mob1* knockout in strains that spontaneously form bradyzoite cysts *in vitro*, we identified a marked decrease of cyst formation. Additionally, public datasets show that *mob1* expression is highly regulated at the life cycle level presenting highest levels in feline enterocyte and oocyst stages (DOI:10.1038/s41598-018-37671-8, DOI:10.1371/journal.pone.0029998). In sum, our results regarding the *T. gondii* MOB1 protein indicate an intricate evolutionary history that may be conserved among coccidians.

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Quantification of integrated selection markers in *Toxoplasma gondii* knockouts

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Herein, we developed a single and a duplex TaqMan quantitative PCR (qPCR) for absolute quantification of copy numbers of integrated dihydrofolate reductase-thymidylate synthase (*mdhfr-ts*) drug selectable marker for pyrimethamine resistance in *Toxoplasma gondii* knockouts (KOs). The single TaqMan qPCR amplifies a 174 bp DNA fragment of the inserted *mdhfr-ts* and of the wild-type (WT) *dhfr-ts* (*wt-dhfr-ts*) which is present as single copy gene in *Toxoplasma* and encodes a sensitive enzyme to pyrimethamine. Thus, the copy number of the *dhfr-ts* fragment in a given DNA quantity from KO parasites with a single site-specific integration should be twice the number of *dhfr-ts* copies recorded in the same DNA quantity from WT parasites. The duplex TaqMan qPCR allows simultaneous amplification of the 174 bp *dhfr-ts* fragment and the *T. gondii 529-bp* repeat element. Accordingly, for a WT DNA sample, the determined number of tachyzoites given by *dhfr-ts* amplification is equal to the number of tachyzoites determined by amplification of the *Toxoplasma 529-bp*, resulting thus in a ratio of 1. However, for a KO clone having a single site-specific integration of *mdhfr-ts,* the calculated ratio is 2. We then applied both approaches to test *T. gondii* RH mutants in which the *major surface antigen* (SAG1) was disrupted through insertion of *mdhfr-ts* using CRISPR-Cas9. Results from both assays were in correlation showing a high accuracy in detecting KOs with multiple integrated *mdhfr-ts*. Southern blot analyses using BsaBI and DraII confirmed qPCRs results. Both TaqMan qPCRs are needed for reliable diagnostic of *T. gondii* KOs following CRISPR-Cas9-mediated mutagenesis, particularly with respect to off-target effects resulting from multiple insertions of *mdhfr-ts*. The principle of the duplex TaqMan qPCR is applicable for other selectable markers in *Toxoplasma*. TaqMan qPCR tools may contribute to more frequent use of WT *Toxoplasma* strains during functional genomics.

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Elucidating the cellular and molecular mechanisms of stage progression in cyst-forming Apicomplexa

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The Apicomplexa phylum is a controversial group of single-celled obligate intracellular protozoan parasites species. They are known to cause severe diseases in both humans and animals and are estimated to cause over 1.000.000 deaths and agricultural loss of over 1.000.000.000$ per year. This organisms' success is achieved thanks to their heteroxenous life cycles and multiple life stages, which allow them to persist in a variety of environments. Notably, this is evident in the cyst forming Apicomplexa which comprise, among the others, of *Toxoplasma gondii* and *Besnoitia besnoiti*.
*Toxoplasma gond*ii is the causative agent of toxoplasmosis, a disease of particular dangerous for immunocompromised and pregnant patients. Toxoplasma positive population has a high prevalence worldwide. It is characterized by a lifelong persistence in the form of tissue cysts which are refractory to any kind of treatments. The ability to form long-lived reservoirs in the form of tissue cysts is an evolutionary trade off that reduces virulence while increasing transmission and survival. The mechanisms of stage transition and tissue cyst formation are driven by host-specific signals, which can differ from species to species and depend on different host-pathogen interactions. Still little is known about the specific mechanisms of stage transition and to which extent they are conserved among different cyst-forming species.

Therefore, the aim of my project is to investigate the molecular triggers that induce stage transition and to elucidate the effect of the host environment on these processes. We will study *T. gondii*, for which there are robust *in vitro* culturing systems for tachyzoites and bradyzoites and extend these investigations to the closely related species *Besnoitia besnoiti* that causes bovine besnoitiosis. We are currently improving *in vitro* platforms for large-scale production of mature *T. gondii* tissue cysts. We will then exploit these innovations to characterize our *in vitro* cultures through molecular and transcriptomic analyses as well as to use an inoculum to infect 3D and 2D organoid intestinal culture systems mimicking the cat intestine to produce sexual stages *in vitro*. In parallel, we are developing an *in vitro* culture systems of the chronic stage in *Besnoitia besnoiti,* that have not been established to date. To this end, we use multiple strategies involving different host cells as well genetic manipulation of the parasite. Understanding the regulation of stage conversion in different cyst-forming Apicomplexa will represent a key milestone to address fundamental questions about the biology of these organisms and identify possible therapeutic targets.

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A novel preconoidal complex identified in a sCas9 phenotypic screen is essential for motility in *T. gondii*

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The presence of an apical complex has been the basis for the classification of the phylum Apicomplexa. Within this complex, the conoid is an important structure for parasite gliding motility, invasion, and egress. In a recent phenotypic screen in *Toxoplasma gondii*, we found two novel conoidal proteins, conoid gliding protein (CGP) and a putative lysine methyltransferase (PCKMT), which localized at the preconoidal rings and are important for motility, invasion, and egress of parasites but had no effect on microneme secretion. Here, we show that the presence of CGP and PCKMT determined the localization of the actin nucleator formin1 (FRM1) at the parasite conoid. To find new interactors of this complex, proximity-dependent biotin identification (BioID) was conducted in parasites where CGP, PCKMT and FRM1 were endogenously tagged with TurboID. According to mass spectrometry results, FRM1 was found in CGP and PCKMT BioID samples and vice versa. Additional apically localized proteins were also substantially enriched, including ICAP16. Confocal/STED images illustrate that CGP colocalised with FRM1, PCKMT and ICAP16 at the preconoidal rings. Interestingly, the conoidal localisation of these proteins depended on the presence of CGP in mature cells. In contrast, other components, important for gliding motility, such as MyoH or the gliding connector GAC were unaffected. Collectively, our results suggest that two independent complexes at the conoid are required for the initiation of gliding motility

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Global proteomic analysis of different in vitro stages of *Cystoisospora suis*

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Infections with the apicomplexan parasite, *Cystoisospora suis* are a frequent cause of diarrheal disease in neonatal piglets worldwide. As with all coccidian parasites, the lifecycle is characterized by asexual multiplication followed by sexual development with two morphologically distinct cell types that presumably fuse to form a zygote from which the oocyst arises. The aim of this project was to investigate protein expression in *Cystoisospora suis* stages cultivated *in vitro* and identify stage-specific proteins, especially of asexual stages (merozoites) and early and late sexual stages (microgametes, macrogametes and early oocysts). Epithelial cells were infected with *C. suis* sporozoites and supernatants were harvested at five time points (T) from 6 to 14 days of cultivation to obtain merozoites as well as gamonts and oocysts [1,2] . Extracted peptides were analyzed by mass spectrometry on a nano-HPLC Ultimate 3000 RSLC system (Dionex). Pig proteins were excluded by applying the Proteome Discoverer Software 2.4.0.305 (Thermo Fisher Scientific) and searching the UniProt *Sus scrofa* (pig) protein database. Parasite proteins were identified using ToxoDB *Cystoisospora suis* database. We analyzed total protein extracts from day 7, 8, 10, 12 and 14. We identified 1331 proteins, of which 234 uncharacterized proteins (17.5%). Overall, proteins with important roles in gametes biology, oocyst wall biosynthesis, DNA replication and axonema formation as well as proteins with important roles in merozoite invasion biology were identified, matching these results with our previous transcriptome analysis of these stages [2]. In addition to that, a sperm associate antigen was found in sexual stages. This protein is associated with microtubules being a constituent part of the cytoskeleton but also, it is important for structural integrity of the flagella, which is a morphological part of the microgametes. We evaluated inhibition of sexual development by using antiserum specific to this protein to evaluate whether it could be exploited as a candidate for control strategies against *C. suis.* Based on these results, targets can be defined using the transcriptome and proteome data for future strategies to interrupt parasite transmission during sexual development of *C. suis* and related parasite species.

References:

1. Feix AS, Cruz-Bustos T, Ruttkowski B, Joachim A. Characterization of Cystoisospora suis sexual stages in vitro. Parasit Vectors. 2020;13:143. doi:10.1186/s13071-020-04014-4.
2. Cruz-Bustos T, Feix AS, Lyrakis M, Dolezal M, Ruttkowski B, Joachim A. The transcriptome from asexual to sexual in vitro development of Cystoisospora suis (Apicomplexa: Coccidia). Sci Rep. 2022;12:5972. doi:10.1038/s41598-022-09714-8.

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Imaging the ultrastructures of *Toxoplasma gondii* by expansion microscopy

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In the 1950s, electron microscopy played a central role in describing the ultrastructural appearances of cells. The knowledge obtained from EM on the characteristic ultrastructural morphology of eukaryotic parasites led to the foundation of the phylum of Apicomplexa (Levine 1980).
Members of this phylum group intracellular parasites that share an apical complex composed of cytoskeletal elements and secretory organelles termed rhoptries and micronemes. In coccidian-subgroup of Apicomplexa, including *Toxoplasma gondii*, the conoid is made of a cone of spiraling tubulin fibers, two preconoidal rings (PCRs) and two intraconoidal microtubules (ICMTs). The conoid is attached to the apical polar ring (APRs) from which the subpellicular microtubules (SPMTs) emerge.
In the recent years, conventional fluorescence and the super-resolution microscopy have offered great insights at high spatial and temporal resolution of protein localization, typically at maximum resolution of 200 nm dependent on the diffraction limit. Expansion microscopy (ExM), which is based on the physical expansion of the cellular structure of interest, bypasses the diffraction limit and enable super-resolution imaging at a resolution level of ≃70 nm (Gambarotto et al. 2019).
When applied to the apical complex of *T. gondii*, ExM allowed the precise localization of proteins to the individual substructures including the cone, APR, ICMTs and PCRs. In combination with reverse genetics, we begin to delineate the role to be attributed to these substructures (Dos Santos Pacheco et al. 2022).

Dos Santos Pacheco, N., L. Brusini, R. Haase, N. Tosetti, B. Maco, M. Brochet, O. Vadas & D. Soldati-Favre (2022) Conoid extrusion serves as gatekeeper for entry of glideosome components into the pellicular space to control motility and invasion in Apicomplexa. *BioRxiv*.
Gambarotto, D., F. U. Zwettler, M. Le Guennec, M. Schmidt-Cernohorska, D. Fortun, S. Borgers, J. Heine, J. G. Schloetel, M. Reuss, M. Unser, E. S. Boyden, M. Sauer, V. Hamel & P. Guichard (2019) Imaging cellular ultrastructures using expansion microscopy (U-ExM). *Nat Methods,* 16**,** 71-74.
Levine, N. D. (1980) Some Corrections of Coccidian Nomenclature. *J Parasotol*.

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Structure-based functional investigation of the glideosome associated connector of *Toxoplasma gondii*

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The obligate apicomplexan parasite *Toxoplasma gondii* critically depends on gliding motility to invade and egress from host cells using a molecular machine called the glideosome. This machine conserved across the Apicomplexa consists of several membrane anchored proteins that localize to the pellicular space between the parasite inner membrane complex and plasma membrane. Actin filaments generated at the preconoidal rings are translocated to the apical polar ring and to the basal pole by the motors myosin H and myosin A, respectively. Motility is powered by the translocation of parasite adhesin/host receptor complexes that are linked to the actomyosin system by the glideosome associated connector (GAC). This large multi-task protein presumably binds F-actin, the cytoplasmic tails of adhesins and phosphatidic acid (PA). Combination of cellular and biochemical assays established that the N-terminal region of GAC mediates contact with F-actin while the C-terminal plextrin-homology (PH) domain interacts presumably with the inner leaflet of the plasma membrane. These molecular interactions are predictably essential to transmit the power generated by motors and propel the parasite forward.

We have solved the X-ray crystal structure of GAC. This 286 kDa protein presents mostly alpha-helical structures, forming a long helical arch that bridges an extended N-terminal actin-binding surface with the C-terminal PH domain facing away from the core protein. The interaction between the PH domain of GAC and PA-enriched membranes was scrutinized by NMR and biochemical investigations to identify the key residues critical for membrane binding. *In vitro* liposome binding assays with mutated PH domain or within the full-length GAC confirmed the importance of those residues for membrane affinity and for GAC activity *in vivo* by functional complementation. Based on the high-resolution structure, several mutants have been designed and are going to be tested *in vitro* and *in vivo* to shed light on the contribution of distinct protein domains on parasite fitness and specific GAC functions.

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A synergistic approach identifies host factors essential for the survival of intracellular Apicomplexa

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Intracellular apicomplexan parasites, including Plasmodium, Toxoplasma and Theileria are amongst the most morbidity-causing pathogens of humans and livestock worldwide, and the occurrence of resistance to established drugs is a major clinical hurdle. In this project we aim to identify host factors that are essential to apicomplexan parasites by combining genome wide genetic screens with chemoinformatic modelling. To achieve this, we designed a novel bovine CRISPR/Cas9 library targeting >22,000 protein-coding genes and performed drop out screens in Theileria-infected and non-infected bovine macrophages. After stringent validation, we identified 111 genes that are exclusively essential for Theileria-infected cells but not for uninfected macrophages, with one third of these involved in metabolic pathways. Next, we performed gene knock-out simulations of these candidate genes in a human hepatocyte-Plasmodium falciparum metabolic model. Among the genes predicted to be dispensable for hepatocytes but essential for Plasmodium-infected cells, we identified hydroxymethylbilane synthase (HMBS), an enzyme involved in host cell heme biosynthesis. To confirm this prediction, we generated HMBS knockout clones in the human haploid cell line HAP1 by CRISPR/Cas9 technique. Upon infection of these HMBS knock out clones with the widely used rodent model organism Plasmodium berghei, we observed a significant decrease in parasite numbers, accompanied by a striking reduction in parasite liberation from infected cells. This phenotype was completely reverted upon re-expression of the wild-type protein, excluding off-target effects of Cas9 expression. In conclusion, our results indicate that both apicomplexan parasite species tested depend on host HMBS for their survival. It will now be interesting to test whether host HMBS is also essential for other apicomplexan parasites like Toxoplasma, Neospora or Cryptosporidium.

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BLT-1 blocks apicomplexan parasite replication *in vitro*

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Coccidia are obligate intracellular apicomplexan parasites that affect humans and animals.
During replication, cholesterol is an essential molecule for membrane biosynthesis and offspring formation. Apicomplexans are considered defective in de novo synthesis of cholesterol and they must be scavenged it from host cells, mainly via LDL particles from extracellular sources. We studied the effect of the SR-BI-specific blocker BLT-1 on the in vitro replication of fast (Toxoplasma gondii, Neospora caninum, Besnoitia besnoiti) and slow (Eimeria bovis and Eimeria arloingi) replicating coccidian species.
BLT-1 treatments significantly inhibited in vitro replication of all five parasites indicating a common SR-BI-related key mechanism in the development process of these coccidian parasites. BLT1-mediated blockage of SR-BI induced enhancement of host cell lipid droplet abundance and neutral lipid content, thereby confirming the importance of this receptor in general lipid metabolism of the host cell. However, SR-BI gene transcription was not affected by T. gondii, N. caninum and B. besnoiti infections. Interestingly, BLT-1 treatment of infective stages reduced invasive capacities of all fast-replicating parasites paralleled by a sustained increase in cytoplasmic Ca++ levels. In conclusion, the data suggest a conserved role of SR-BI for successful coccidian infections.

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Deciphering the pathogen-host interaction at the placenta in the bovine neosporosis

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Our understanding on bovine neosporosis has achieved relevant milestones in the last decade, but mechanisms underlying the occurrence of abortions –or the protection against them– remain elusive. Previous studies have proposed that foetal and placental lesions resulting from parasite multiplication are key factors triggering abortion. However, the shift from the Th2-antiinflamatory response, that prevails during gestation, towards a Th1-proinflamatory response upon infection has also been postulated as a crucial phase for abortion. In a recent study, placentas from heifers challenged with the high virulence isolate Nc-Spain7 exhibited focal necrosis and inflammatory infiltrates as soon as 10 days post-infection (dpi), although parasite detection was minimal. These lesions were more frequent at 20 dpi, coinciding with higher rates of parasite detection and the occurrence of foetal death in some animals. By contrast, such lesions were not observed in placentas from animals infected with the low virulence isolate Nc-Spain1H, where the parasite was only detected in one animal at 20 dpi. This work aimed to study which mechanisms are involved during the changes that occurred in the placentas (caruncles and cotyledons) of pregnant heifers at early-stages of infection (10 and 20 dpi) through whole-transcriptome analysis (RNA-sequencing). In caruncles, infection with the high virulence isolate provoked a strong proinflammatory response at 10 dpi. This was not observed in heifers infected with the low virulence isolate, where the "IL-6/JAK/STAT3 signaling" and the "TNFα signaling via NF-κB" pathways were both down-regulated. At 20 dpi, more pronounced proinflammatory gene signatures were detectable in heifers infected with the high virulence isolate. In cotyledons, the response to the infection with the high virulence isolate was similar to that observed in caruncles, although the low virulence isolate also induced mild proinflammatory signals at 20 dpi. Interestingly, gene expression of E2F target genes, involved in the restraining of the inflammatory response, was higher in caruncles infected with the low virulence isolate at 10 dpi. In addition, a marked proinflammatory response was detected in heifers carrying dead foetuses compared to those carrying live ones. In line with this and based on a deconvolutional analysis of gene signatures from cotyledons and caruncles, tissues infected with the high virulence isolate displayed a markedly higher fraction of activated natural killers, M1 macrophages and CD8+ T cells. Therefore, our transcriptomic analysis supports the hypothesis that an intense immune response likely triggered by the parasite multiplication could be a key contributor to the abortion. However, more studies are required to determine the mechanisms that govern the distinct interactions between high and low virulence isolates and the host, which could contribute to decipher the molecular processes underlying the pathogenesis of neosporosis in cattle.

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Toxoplasma gondii-driven host cell DNA damage in primary human cells through the effector protein HCE1/TEEGR

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Toxoplasma gondii is an obligate intracellular coccidian parasite which is well-known for its extraordinary capacity to modulate its host cell. Recently, T. gondii infections were reported to affect host cell cycle progression, chromosome segregation and cytokinesis, thereby leading to polyploid host cells with two or more nuclei. So far, it remains unclear whether this kind of modulation is host cell type- or origin-dependent. Therefore, analyses on cell cycle progression and binucleated phenotype formation were performed in different primary human and bovine *T. gondii*-infected cell types (e. g. HUVEC, FHs74, HFF and BUVEC). In line with recent data in bovine endothelial cells, the host cell cycle arrest was in the S-phase and an increased proportion of binucleated cells was consistently observed in all cell types. To identify underlying mechanisms, we furthermore studied if *T. gondii* infection triggers DNA damage in host cells thereby potentially causing S-phase stasis. Using Comet assays, we showed that *T. gondii*-infected primary human endothelial cells experience more DNA strand breaks than non-infected cells. Furthermore, detection of histone H2A.X phosphorylation revealed an enhanced proportion of nuclear DNA damage foci in *T. gondii*-infected host cells. Given that host cells physiologically respond to DNA damage, we also studied the activation of DNA damage response-related repair pathways in *T. gondii*-infected HUVEC. Interestingly, parasite infections mainly activated the homologous recombination-related DNA repair pathway (at 12 hours p. i.) affecting only the first step proteins in the non-homologous end-joining pathway in host cells. To test whether the presented data may be modulated directly by the parasite, we studied two *T. gondii* mutants previously described to be related to the host cell cycle modulation (*T. gondii* ΔMYR1 and ΔHCE1). *T. gondii* ΔHCE1 is not able to induce the cyclin E1 in the host nuclei, a crucial S-phase entry regulator, in a myr1-dependent manner. Current data showed that *T. gondii* ΔMYR1 and ΔHCE1 mutants both failed to trigger the formation of DNA damage foci and activation of the DNA homologous recombination repair pathway in host cells.
In conclusion, our data suggest that *T. gondii* infection alters host cellular cell cycle progression and cytokinesis in a host cell type- and origin-independent manner. In addition, *T. gondii*-driven host cellular DNA strand breaks and activation of the homologous recombination pathway seem - at least partially - dependent on functional MYR1 and HCE1 expression in the parasite.

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The antigen recognition portion of African buffalo class I MHC is extremely polymorphic consistent with a complex pathogen challenge environment and the 3' region suggest distinct haplotype configurations

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African Cape buffalo (*Syncerus caffer*) have been distinct from the Auroch lineage leading to domestic cattle for five million years and are reservoirs of multiple pathogens that affect domestic cattle such as the apicomplexan parasite Theileria parva. To date there has been no analysis of the class I MHC locus in Cape buffalo. We present the first data on Cape buffalo class I MHC, which demonstrates that gene and predicted protein coding sequences are approximately 86-87% similar to that of African domestic cattle in the antigen recognition region. The study also shows concordance in the distribution of codons with elevated posterior probabilities of positive selection in the buffalo class I MHC and known antigen binding sites in cattle. Overall, the diversity in buffalo class I sequences appears greater than that in cattle, perhaps related to a more complex pathogen challenge environment in Africa over time. However, application of NetMHCpan suggested broad clustering of peptide binding supertypes between buffalo and cattle. Furthermore, in the case of at least 20 alleles, critical peptide-binding residues appear to be conserved with those of cattle, including secondary anchor residues. Alleles with six different length transmembrane regions were detected. This preliminary analysis suggests that like cattle, but unlike most other mammals, Cape buffalo appears to exhibit configuration (haplotype) variation in which the loci are expressed in distinct combinations.

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Effect of the deletion of NcBpk1 or NcRop2 on the virulence of *Neospora caninum*

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Bovine neosporosis is one of the main causes of transmissible abortion worldwide. However, the knowledge of the interaction between *Neospora caninum* and cattle is scarce, and the molecular bases that determine parasite virulence remain unknown. Recent transcriptomic and proteomic studies that compared high and low virulence isolates have evidenced a higher expression of NcBpk1 and NcRop2 in high virulence isolates, suggesting their implication in parasite virulence. Therefore, this study aimed to evaluate the role of these proteins in the virulence of *N. caninum* by knocking-out the NcBpk1 and NcRop2 genes in the highly virulent Nc-Spain7 isolate through CRISPR-Cas9. Mutant parasites were analysed in vitro, using a model of bovine monocyte-derived macrophages, and in vivo, using a well-established pregnant BALB/c mouse model. NcΔRop2 parasites displayed lower infection rates at 48 hours post-infection (hpi) and a decreased proliferation in bovine macrophages. In contrast, proliferation of NcΔBpk1 parasites in macrophages was higher at 72 hpi. In the murine model, deletion of NcRop2 was associated to a partial loss of virulence. Specifically, higher survival rates of the offspring and lower parasite burdens in dam's brains were recorded. Interestingly, infection with NcΔBpk1 parasites resulted in a slight exacerbation of the infection in the offspring. Overall, these studies confirm that NcROP2 is a virulence factor in *N. caninum.* Future studies will focus on unravelling the specific functions of NcROP2 and NcBPK1 to understand the molecular and cellular mechanisms triggered by these proteins during N. caninum infection.

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A new highly-performant competitive ELISA for the detection of *Besnoiti besnoiti* antibodies in cattle

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Besnoitia besnoiti (*Bb*), an apicomplexan parasite closely related to *Toxoplasma gondii* and *Neospora caninum*, is the cause of bovine besnoitiosis, mainly transmitted by blood sucking insects. It causes economic loss (reduced milk production, weight loss, infertility) and increased mortality. Besnoitiosis was recently classified as an emerging disease in Europe.
Identify seropositive animals is a key to control the disease. Serological tools such as ELISA, western blot (WB) or IFAT play an essential role for *Bb* diagnosis. Most of the commercially available ELISA kits are based on an indirect method using a *Bb* purified antigenic extract. To improve *Bb* serological diagnosis, Innovative Diagnostics (IDvet) has generated several monoclonal antibodies to Bb. The most promising one was used to develop a new blocking ELISA. The present results show the performances of this test.
Diagnostic specificity was evaluated on 500 cattle serum samples from bovine besnoitiosis free herds without history of besnoitiosis and/or with regular negative serological results. Measured specificity was 100.0 % (CI95%: 99.2 % - 100.0 %), n=500.
Diagnostic sensitivity was evaluated on 200 cattle serum samples from France. The positive status of the samples was determined by WB. Measured specificity was 100.0 % (CI95%: 98.1 % - 100.0 %), n=200. The percentage of correlation was 100% indicated very high agreement with WB.
In order to check the absence of cross-reactions with other apicomplexan protozoa, the following sera from *Toxoplasma gondii* and *Neospora caninum* seropositive animals were tested:

- 10 bovine sera from France, identified as positive for Neosporosis during a prophylaxis campaign and confirmed positive by the ID Screen® Neospora caninum Indirect ELISA;
- 5 bovine sera from France, identified as positive for Toxoplasmosis during a prophylaxis campaign and confirmed positive by the ID Screen® Toxoplasmosis Indirect Multi-species ELISA.

All the sera were found negative with the competitive ELISA. On the tested panel, no cross-reaction was observed. Repeatability, reproducibility and robustness gave good results.
The ID Screen® Besnoitia Competition ELISA efficiently detects positive animals, demonstrates very high specificity and excellent correlation with the Western blot, using as confirmation test for *Bb* diagnosis.
The new ID Screen® Bb cELISA is a reliable tool for the detection of cattle antibodies directed against *Bb*.

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First description of an immunogenic recombinant protein for *Besnoitia besnoiti* antibody detection in cattle

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Besnoitia besnoiti (*Bb*), an apicomplexan parasite closely related to *Toxoplasma gondii* and *Neospora caninum*, is the cause of bovine besnoitiosis, mainly transmitted by blood sucking insects. It causes economic loss (reduced milk production, weight loss, infertility) and increased mortality. Besnoitiosis was recently classified as an emerging disease in Europe.
For years, besnoitiosis has been underestimated and less studied than Toxoplasmosis or Neosporosis. Antigens or proteins with potential interest for diagnosis are still not identified or well documented. Most of the serological tools used for *Bb* diagnosis, such as ELISA, western blot or IFAT, are based on a purified, semi-purified or non-purified extract from *Bb*.
Indirect techniques, such as ELISAs, offer excellent performances and are able to deliver highly reliable results. Nevertheless, the use of semi-purified antigens of undefined composition could potentially lead either to specificity issues liked to indirect formats, cross-reactivities and sometimes antigen batch-to-batch reproducibility issues.
Therefore, the identification of a specific and highly immunogenic recombinant protein could improve *Bb* diagnosis in terms of specificity, exclusivity and sensitivity.
These results show the characterization of a new recombinant protein, evaluated for its diagnostic potential for bovine besnoitiosis.
Different Bb proteins (Bbrec17, Bbrec30, Bbrec47) were over-expressed using baculovirus/insect cell expression system. The yield of production was verified on crude extracts by Western blot with anti-His antibodies. Preliminary results indicated that BBrec30 was a target of potential interest.
Therefore, Bbrec30 protein was then produced in large batch, and purified by His-tag affinity chromatography. Purity and size were checked by gel electrophoresis (SDS-PAGE).
Bbrec30 protein immune reactivity was then evaluated by indirect ELISA on:

- 4 cattle sera from France, previously qualified as positive by Western blot and using another highly performant indirect ELISA based on a purified antigen from *Bb* (ID Screen® Besnoitia indirect 2.0).
- 30 cattle sera from bovine besnoitiosis free areas and confirmed negative with the ID Screen® Besnoitia indirect 2.0.

All negative sera gave low OD values, whereas all positive sera were strongly reactive on the Bbrec*30* protein. For each of the positive samples tested, a high and significant positive to negative ratio was obtained, indicating a high discrimination between positive and negative samples.
As a conclusion, Bbrec30 protein is an immunogenic protein with high potential for serological diagnosis. Panels are being extended to better assess its sensitivity and specificity. This is the first description of the potential use of a recombinant protein for Bb serological diagnosis ; further work will allow to better understand how Bbrec30-based serological results could be interpreted.

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Seroprevalence of *Neospora caninum* in cattle in the Madeira Island (Portugal)

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Neospora caninum is an apicomplexan protozoan responsible for important reproductive losses in cattle. Although this parasite has a worldwide distribution, the prevalence of bovine neosporosis in the Autonomous Region of Madeira is poorly known. The aim of the present study was to provide an estimate of the prevalence and distribution of *N. caninum* in the Madeira Island.
Sample size was determined considering published test sensitivity (100%) and specificity (93%) data, an estimated prevalence of 20%, a precision in the estimate of 5% and a confidence level of 95%, for an unknown population size. A total of 348 sera from cattle in 18 civil parishes belonging to 8 municipalities were randomly selected and tested by a commercial ELISA test (IDEXX) following the manufacturer's instructions.
Antibodies to *N. caninum* were detected in 72 samples, resulting in an apparent prevalence of 20.7% (95% CI: 16.8 – 25.3) and a true prevalence of 15% (95% CI: 10.8 - 19.9) when considering test performance. Positive animals came from 12 of the civil parishes (66.7%) and 7 of the municipalities (87.5%). Seropositivity was significantly associated to sex and municipality ($p<0.05$), but not to age or animal origin. Kuldorff's spatial scan statistics revealed no significant clustering of infection.
The seroprevalence determined in the present study is in the same range as that previously observed in continental Portugal. Results point to the need for including bovine neosporosis in the differential diagnosis of cattle abortive diseases in the Madeira Island.

P-66

Exploring serological and hematological markers for diagnosis and monitoring of *Besnoitia besnoiti* in a naturally infected herd in the early phases of infection

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Despite its widespread in Europe, bovine besnoitiosis is considered a neglected and misdiagnosed disease, particularly in the acute phase of the infection when the clinical signs are nonspecific and when commercially available serological tests targeting IgG may fail in the detection of recently infected animals. Since few data are available on the dynamics of *Besnoitia besnoiti* infection in its early phases, a longitudinal study was conducted in a naturally infected dairy cattle herd (northern Italy) with the aim to explore the serological and hematological changes from the first appearance of the infection.
Sera of four cows showing clinical signs (fever, respiratory distress) resembling those of *B. besnoiti* acute infection (May 2021, T0) and of all cows sampled in July (T1, n=117) and November (T2, n=125) were analyzed for IgM (MegaScreen® FLUO *Besnoitia besnoiti*, Megacor) and IgG (ID Screen® *Besnoitia* Indirect 2.0, IDVet). Animals were classified as acutely (IgM+, IgG-), recently (IgM+, IgG+) or chronically (IgM-, IgG+) infected. On blood samples, molecular (Cortes et al., Vet. Parasitol. 2007; 146:352–356) and hematological analyses were performed. Values of hematological parameters were compared among groups (acutely, recently and chronically infected) using the non-parametric Mann-Whitney U-test.

All suspicious cases at T0 were confirmed as acutely (n=1) or chronically (n=3) infected by *B. besnoiti*. At T1, 21 infected cows out of 117 (prevalence, P=17.9%) were registered: 9 in the recent and 12 in the chronic phase of infection. At T2, 24 animals out of 125 scored positive (P=19.2%), with six new cases of infection (incidence=4.8%), three in the recent phase of infection. By PCR, circulating DNA of the pathogen was found in two animals, in the acute and in the recent phase of infection, respectively.
Considering the hematological parameters, at T1 mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) resulted lower in seropositive (MCV=48.2 fL, MCH=16.3 pg) than seronegative cows (MCV=49.6 fL, MCH=16.8 pg) (p-value: 0.018 and 0.03, respectively), while at T2 lower values of hematocrit (Ht) and hemoglobin (Hb) were recorded in recent infected (Ht=27.2%, Hb=9.1 g/dL) than chronic positive or negative cows (Ht=31.3%, Hb=10.5 g/dL) (p-value: 0.021 and 0.026, respectively).

The combination of IgM and IgG, distinguishing acute or recent vs. chronic phase of infection, allowed to estimate the rate of the infection spread among the herd during the study period. The hematological changes found were similar to those previously described in cows in the early stages of infection (Langenmayer et al., BMC Vet. Res. 2015; 11:32), while the alterations described in the chronic phase were not still detected.
The combination of serological and hematological analyses should be better evaluated for the determination of the epidemiological situation of infected herds and thus for the set up of personalized control programs.

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Toxoplasma gondii genetic diversity in the South Region of Brazil and study of its population structure in the country

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Brazil has one of the highest seroprevalence rates of *Toxoplasma gondii* in humans in the world, and one of the highest genetic diversities of the agent. Free-range chickens have been used in studies of genotypic characterization of *T. gondii* isolates to understand the population structure of the parasite and its biological characteristics in different regions of the world. The present study aimed to determine the frequency of occurrence of anti-*T. gondii* antibodies from free-range chickens in the South Region of Brazil using the Modified Agglutination Test (MAT, titer ≥ 10), to isolate the parasite by mouse bioassay, and genotypically characterize the *T. gondii* isolates by PCR-Restriction Fragment Length Polymorphism (PCR-RFLP, 11 markers) and Microsatellite analysis (MS, 15 markers). Serum samples from 607 free-range chickens from 61 municipalities were evaluated. Antibodies anti-*T. gondii* were found in 54.9% (333/607) of the chickens, with a frequency of occurrence of 51.4% (110/214), 52.1% (100/192), and 61.2% (123/ 201) in the states of Paraná, Santa Catarina and Rio Grande do Sul, respectively. The bioassay in mice was performed with the tissues of chickens with titers ≥ 20 in the MAT (304 birds) and 160 isolates were obtained (69 from Paraná state, 39 from Santa Catarina state and 52 from Rio Grande do Sul state). Complete PCR-RFLP genotyping was obtained from 158 *T. gondii* isolates revealing 48 genotypes (20 from Paraná, 17 from Santa Catarina and 22 from Rio Grande do Sul states); the classic clonal Types I, II and III (from Santa Catarina and Rio Grande do Sul states), and the lineages widely distributed in Brazil (Types BrI, BrII and BrIII) were observed; of the other genotypes found, 19 were previously described in different regions of Brazil and 17 were new genotypes. Genotyping by MS was possible in 158 *T. gondii* isolates; examining the eight typing markers, 60 genetic types were identified, however, searching the set of 15 markers, a total of 118 genotypes were revealed and 40 represented clones; in Paraná state, 24 types and 53 genotypes (16 clones) were identified, in Santa Catarina, 18 types were observed, corresponding to 26 genotypes (13 clones), and, in Rio Grande do Sul, 30 types and 39 genotypes (11 clones). MS typing corresponding to clonal types I variant, II and III was identified, in addition to non-archetypal types: Africa 1, Caribbean1, and South America1,2,3,4, and the other isolates were also identified as non-archetypes, considering the eight typing markers. The study corroborates the high diversity of *T. gondii* in Brazil. A phylogenetic network constructed with 200 PCR-RFLP genotypes described in Brazil, including those from the present study, has showed *T. gondii* Brazilian population is structured in three major groups and corroborates the high divergence of Type II and Type II variant classical strains.

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Genotyping *Toxoplasma gondii*: whole genome sequences of isolates across Europe reveal diversity within European type II strains

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Toxoplasma gondii is a zoonotic protozoan parasite with an apparently largely clonal population structure in Europe. Few fine-scale typing methods exist for *T. gondii*. Whole genome sequence analysis of *T. gondii* offers new opportunities for the development of new discriminatory genotyping methods possibly with higher resolution and throughput for this parasite.
We assessed the genomes of more than 70 *T. gondii* Type II isolates, including sequences of more than 60 European field isolates, by whole genome sequencing (WGS) and compared to the Type II reference strain Me49.
Comparative analyses revealed up to four separate clusters of European type II *T. gondii*. The spatial distance between sites of isolation partially explained the detected genetic distance between isolates.
We conclude that European *T. gondii* type II isolates are indeed genetically diverse. Based on our experiences, WGS may be useful to better understand the molecular epidemiology of the parasite in Europe, to trace infection sources in outbreaks and to detect the introduction of exotic or the emergence of recombinant strains.

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Detection of anti-*Toxoplasma gondii* antibodies in meat juice from retail meat cuts

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Toxoplasma gondii is one of the most studied parasites due to its medical and veterinary importance. It is estimated that 30% of the human population has had contact with this zoonotic pathogen. The dissemination of *T. gondii* is caused by transplacental transmission through tachyzoites, through the ingestion of food and water contaminated with sporulated oocysts and by consumption of animal tissues containing infective cysts, an important route of transmission to humans involving the ingestion of raw or undercooked contaminated meat. The detection of antibodies against this parasite has been previously achieved by the analysis of meat juice, an exudate from musculature, in epidemiological monitoring studies in Europe, but the effectiveness of this matrix considering the serological methods available, the type of muscle and the prevalence situation in Brazil also needs to be adequately validated. The aim of this report is to evaluate the occurrence of anti-*T. gondii* antibodies in meat juice samples from beef chuck and pork loin collected from markets in São Paulo city. Analysis of samples were performed using the indirect fluorescent antibody test (IFAT) against meat juice obtained from experimentally infected animals (bovine and swine) as positive controls. Samples of this matrix were considered positive using the cutoff titers of ≥16 for pork and ≥8 for beef. Partial results from 66 pork and 30 beef samples showed three (4.5%) and eight (26.7%) positive samples for IgG anti-*T. gondii* antibodies, respectively. It seems that the use of IFAT is suitable for the analysis of meat juice, however the overall specificity of the test for surveillance purposes is yet to be determined after the analysis of a larger number of samples. The present study is going to contribute to new information about the risk of toxoplasmosis infection and food safety.
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First identification of *Sarcocystis spp*. in synanthropic and wild rodents from Argentina

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Sarcocystis spp. are cyst-forming intracellular protozoan parasites with an obligate two-host prey-predator type life cycle. *Sarcocystis* spp. in muscles of different rodent species have been described in several countries. The objective of this study was to determine the presence of *Sarcocystis* spp. in synanthropic and wild rodents from rural areas in the Humid Pampa Region, Argentina. A total of 158 rodents were captured, euthanized and sampled from dairy cattle farms between 2021 and 2022. The animals were 107 synanthropic (103: *Mus musculus*, 3: *Rattus rattus*, and 1: *R. norvegicus)* and 51 wild rodents (28: *Oxymycterus rufus*, 12: *Necromys lasiurus* and 11: *Akodon azarae*). Samples of different skeletal muscles (tongue, masseter, heart, and semitendinosus) were collected and assessed by histopathological analysis and homogenization followed by direct microscopic examination. Also, PCR targeting a 18S rRNA gene fragment and the internal transcribed spacer 1 (ITS1) were performed from muscles homogenates from *Sarcocystis* spp. microscopically positive samples. The positive amplicons were further sequenced and analysed. Histopathology and direct microscopy revealed the presence of thin-walled cysts consistent with *Sarcocystis* spp. in 12.6% (20/158) of rodents. Sarcocysts were more frequently observed in semitendinosus muscle (8 *M. musculus*, 6 *O. rufus,* 2 *A. azarae,* 2 *N. lasiurus),* followed by the masseter (5 *O. rufus,* 3 *M. musculus,* 1 *N. lasiurus),* heart (1 *M. musculus*, 1 *N. lasiurus)* and tongue (1 *M. musculus).* By 18S rRNA fragment PCR 16/20 samples were positive, and seven were selected for further ITS1 PCR and sequencing. Six of the 18S rRNA sequences showed a 99.48-99.64% identity with *S. dispersa* (3 *M. musculus*, 1 *A. azarae*, 1 *N. lasiurus* and 1. *O. rufus*) a species with a rodent-owl cycle, and one (*O. rufus*) was only 95.5-96.6% similar to other *Sarcocystis* spp. The last sample showed a 100% identity with *S. attenuati* at ITS1 sequence fragment (954bp), a species with snakes as putative definitive hosts. The remaining ITS1 sequences (around 1450 bp) showed a high similarity among them and a 100% identity with several *Sarcocystis* spp. but with a low coverage (10-13%), possible due to the lack of ITS1 sequences in the database. Our results indicate a relatively high proportion of *S. dispersa*-like in different rodents from Argentina, potentially related with an owl predation of rodents. In addition, a *S. attenuati*-like species was detected in a *O. rufus*, suggesting a potential rodent-snake cycle. This is the first study to identify *Sarcocystis* spp. by molecular methods in rodents from Argentina and probably the first report of ITS1 sequences for *S. dispersa*-like. Further studies are needed to unravel the role of synanthropic and wild rodents in the epidemiology of cyst forming coccidia parasites.

P-71

Exposure of Galapagos birds to *Toxoplasma gondii* suggests different transmission pathways

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Felids serve as the definitive hosts of *T. gondii*, shedding millions of environmentally resistant oocysts that retain viability in soil and water for months. In the Galapagos archipelago (Ecuador, Pacific Ocean), domestic cats were introduced in 17th century and nowadays can be found in the four populated islands: Isabela, Floreana, Santa Cruz and San Cristobal. Previous surveys found that cats from Isabela and San Cristobal are seropositive for *T. gondii*, which rises the risk of exposure to *T. gondii* of naïve endemic species. Antibodies against *T. gondii* have been detected in three endemic bird species: Galapagos penguins and flightless cormorants (both seabirds), and Galapagos hawks (raptors). These findings suggest that other bird species could be at risk of exposure to *T. gondii* in the archipelago. The magnitude of this risk is assumed to be a function of the birds' diet (carnivore > granivore > piscivore > insectivore) and the extent of their contact with oocyst-contaminated soil. The aim of this study was to compare the prevalence of *T. gondii* in Galapagos bird species that differ in diet and contact with oocyst-contaminated soil to infer the exposure factors involved. In Santa Cruz, where cats are present, 92 sera samples (66 pooled and 26 non-pooled) were collected from four ground bird species (tree finches, ground finches, Galapagos mockingbirds and yellow warblers). In Daphne, Seymour North and South Plaza islands, where cats are absent, 124 non-pooled plasma samples were collected from six seabird species (swallow-tailed gulls, magnificent frigatebirds, great frigatebirds, red-billed tropicbirds, Nazca boobies and blue-footed boobies). All samples were tested using the modified agglutination test (MAT) for the detection of antibodies against *T. gondii*, with a titer ≥1:20 considered as positive. At least one individual of each species of ground birds was seropositive for *T. gondii*. By contrast, swallow-tailed gulls (0/23), great frigatebirds (0/25), and blue-footed boobies (0/15) were negative for MAT while antibodies were found in magnificent frigatebirds (4/10), red-billed tropicbirds (1/26) and Nazca boobies (2/25). The species of seabirds found to be seropositive in this study are also consumers of fish known to transport oocysts in their digestive tracts. These results confirm that foraging birds in contact with oocysts-contaminated soil are at a higher risk of exposure to *T. gondii* than piscivorous seabirds, but also that the consumption of paratenic host fish is a valid transmission route. A diet that relies mostly on these fish could be a crucial risk factor for the exposure to *T. gondii*, especially in pelagic seabirds that have little to no contact with contaminated soil. A total of 63 recently obtained seabirds' plasma samples will be included in the study to complete the statistical analyses relating prevalence and precise the exposure risk factors.

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Neospora caninum, Sarcocystis spp. and *Toxoplasma gondii* infections and their relationship with milk production in goats from Argentina.

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Goats are intermediate hosts of *Neospora caninum*, *Sarcocystis* spp. and *Toxoplasma gondii*. Neosporosis and toxoplasmosis in goats have been extensively studied in different regions of the world related to reproductive losses. *Sarcocystis* spp. infection is generally asymptomatic and rarely cause clinical signs and abortions. In general, goat seroprevalence of these infections is high, although the impact on milk production is unknown. The aim of this study was to evaluate and relate the antibodies titers to these protozoa with the goat milk production in the province of Buenos Aires, Argentina. Blood samples were obtained from 336 Saanen breed goats from 2 dairy farms: E1 (N=146) and E2 (N=190). Sera were tested for *N. caninum*, *Sarcocystis* spp. and *T. gondii* antibodies by indirect immunofluorescence antibody test (IFAT) and processed to end dilution. In both dairy farms, milk production volume was measured once a month from August to December. The total production per lactation was calculated and adjusted to 240 lactation days. Differences in antibody titers were explored considering age, body condition and FAMACHA© grade as covariates using a multifactorial ANOVA test. The average total milk production of goats by lactation was 429.3 l (IC±17.8). The seroprevalence of *N. caninum*, *Sarcocystis* spp. and *T. gondii* was 81.8% (275/336), 96.4% (324/336) and 66.7% (224/336), respectively. Fifty-four percent (181/336) of goats were seropositive to three protozoa, 37.5% (126/336) to two and 8.3% (28/336) to only one. No significant differences in milk production were found between seropositive goats to three and two protozoa and positives to one or negatives to all three. *Neospora caninum* seropositive goats at cutoff titers ≥200 and ≥400 showed significantly higher milk production than the rest. Similarly, *Sarcocystis* spp. seropositive goats with cutoff titers ≥200 and ≥400 evidenced higher milk production than seronegative and lower titer goats. On the other hand, seropositivity to *T. gondii* at different titer cutoff levels showed no association with differences in milk production, except for 16 goats with titers ≧1600 which produced significantly less milk than the rest. Results confirm that *N. caninum*, *Sarcocystis* spp., and *T. gondii* infections and coinfection are common in dairy goats from the province of Buenos Aires, Argentina. High seropositivity at the three protozoa may determine a significant economic impact because of the reproductive failures. However, and contrary to our hypothesis, seropositivity was not related with lower milk production and even the seropositive animals with higher titers produced more than seronegative and low titers goats. Possibly, the high seropositivity observed influences the statistics, since seronegative goats are underrepresented. The animal's age, body condition and anemia degree (FAMACHA©) are important factors determining milk production in goats, and therefore, should be considered as in our study to avoid misinterpretations.

P-73

Toxoplasma gondii-abortion outbreak in dairy sheep from Argentina

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Toxoplasmosis is a parasitic disease caused by the Apicomplexan parasite *Toxoplasma gondii.* This intracellular protozoan is worldwide distributed, infecting a broad range of birds and mammals, including humans. *Toxoplasma gondii* is considered one of the most common causes of reproductive failure in sheep. In Argentina, a case of abortion due to *T. gondii* has been reported in a sheep flock raised for meat production. Furthermore, *Toxoplasma* genotypes present in Argentinean sheep flocks have not been published yet. The objectives of the present study are to describe an outbreak of *T. gondii*-abortions in a dairy sheep flock from Argentina and to do the molecular characterization of the genotype of *T. gondii* involved. Between May and June 2020, one hundred and seventeen sheep aborted in a dairy flock of 633 sheep located in Pehuajó, Buenos Aires province, Argentina. Thirty-three foetuses and placentas were recovered and submitted to the Specialized Veterinary Diagnostic Service (SDVE) of INTA Balcarce, in order to establish an etiological diagnosis. These tissues were examined by standard gross pathology procedures and were tested using serology, histopathology and immunohistochemistry. Moreover, DNA was obtained from foetal tissues and a PCR assay on the B1 gene was used to detect *T. gondii*. *Toxoplasma* genotyping by nested PCR-RFLP was performed over the positive PCR samples. In addition, milk (n=63) and blood samples (n=207) were obtained from sheep (aborted and non-aborted) for serological and PCR analysis. Compatible histological lesions with toxoplasmosis including encephalitis, pneumonia, and severe multifocal necrotizing placentitis were observed. *Toxoplasma gondii* was immunolabeled in placenta, brain tongue, intestine, lung, and liver. Antibodies against *T. gondii* and DNA were detected in 33/33 foetal fluids and in 30/33 brain samples, respectively. Preliminary genotyping analysis using the molecular markers SAG1 and 5'-3´SAG2 identified the Type II allele. *Toxoplasma gondii*-seroprevalence was 93.47% (193/207) with end-point titres higher than 1/12.800 in some sheep. Although 92.06% (58/63) of the analysed milk samples from aborted sheep evidenced antibodies against *T. gondii*, *Toxoplasma*-DNA was not found in any of these samples. No evidence of other abortifacient infectious agents were detected in the aborted foetuses. Scarce information of *T. gondii*-abortion outbreaks has been published in Argentina, being this study the first *Toxoplasma*-genotyping of a natural case. Further investigations at a larger scale are required to continue providing detailed information about the *T. gondii* genotypes involved the reproductive failure that will allow us to better understand the epidemiology of *T. gondii* in Argentina.

P-74

Detection of *Toxoplasma gondii* specific antibodies in pigs using an oral fluid-based commercial ELISA: advantages and limitations

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Toxoplasma gondii is a major food-borne parasite and undercooked meat of infected pigs represents an important source of infection for humans. Since infections in pigs are mostly subclinical, adequate diagnostic tests for use at the farm level are pursued. Oral fluid (OF) was shown to be a promising matrix for direct and indirect detection of infections with various pathogens in pigs. The objective of this study was to assess, whether *T. gondii* infections in pigs could be diagnosed using an indirect ELISA kit adapted for OF samples (OF-ELISA). Routine serology and OF-immunoblot (IB) were used as standards for the comparison. For this, serial OF samples from sows (n = 8) and fatteners (n = 3) experimentally inoculated with *T. gondii* oocysts, individual field samples from potentially exposed sows (n = 9) and pooled OF samples from potentially exposed group-housed fatteners (n = 195 pig groups, including 2,248 animals) were analysed for antibodies against *T. gondii* by ELISA. For individual animals, OF-ELISA exhibited a relative diagnostic specificity of 97.3% and a relative diagnostic sensitivity of 78.8%. In experimentally infected animals, positive OF-ELISA results were observed from 1.5 weeks post inoculation (wpi) until the end of the experimental setup (8 to 30 wpi); however, values below the estimated cut-off were occasionally observed in some animals despite constant seropositivity. In potentially exposed individual animals, OF- and serum-ELISA results showed 100% agreement. In group-housed fatteners, antibodies against *T. gondii* could be reliably detected by OF-ELISA in groups in which at least 25% of the animals were seropositive. This OF-ELISA, based on a commercially available serum ELISA, may represent an interesting non-invasive screening tool for detecting pig groups with a high exposure to *T. gondii* at the farm level. The OF-ELISA may need further adjustments to consistently detect individual infected pigs, probably due to variations in OF antibody concentration over time.

P-75

Difficulties in the control of bovine neosporosis in Terceira Island, Azores, Portugal

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In Terceira, one of the 9 islands belonging to the archipelago of Azores, milk production represents 25% the total income, therefore, any aspect affecting reproduction efficacy impacts dramatically on health and the economy. With the present – and first - serological survey (ELISA) for *Neospora caninum,* an overall prevalence of 14.83% was identified in dairy cattle, with animals in 62,43% of farms testing positive. IFAT showed that the prevalence of infected dogs living in farms was 64.52%, while only 8.33% of dogs from urban areas were infected. Thus, there is a higher risk of infection in farm dogs compared to urban dogs ($p=1,419 \times 10^{-9}$). The type of feeding place (p=0,01139) and the type of associated infrastructure (p=0,006303), also had a positive association with the presence of dogs infected with this agent. In the present work, more than proposing solutions, we focus on the most important difficulties in controlling the spread of *N. caninum* in dairy cattle and dogs in Terceira Island. Almost all dairy production is based on free ranging cattle on green fields. The delivery of a newborn calf mostly is happening in the grass pens, thus, fetal membranes are not removed, both because they are not readily found and, because farmers do not recognize it as an important health management measure. Also, an obstacle to control the spread *N. caninum* is the fact that, there are no physical barriers capable of preventing dogs from free-roaming between farms and terrains, and they can access water sources and food storage places, Stray dogs are a problem. There is, as well, a great need for professional qualification and raising awareness among producers on the impact that neosporosis exerts on the productivity of a farm, both dairy and beef cattle, and the risks involved in dogs having unrestricted access to any area. Concerning the dogs from urban areas, feeding of cattle raw meat is widely accepted, and increases the prevalence of infected and diseased dogs. The implementation of control measures for neosporosis in Terceira is necessary and is dependent on a higher level of changes in the management system, with promoting the land consolidation as one of the factors, for the improvement of dairy health management.

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Typing of European *Toxoplasma gondii* type II strains by a novel Ion AmpliSeq-based technique

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Introduction

Toxoplasma gondii is a protozoan parasite with a largely clonal population structure in the northern hemisphere. The clonal type II lineage prevails in Europe. A genotyping method, based on 15 microsatellite (MS) regions, represents the referencing standard, being able to distinguish between clonal lineages of *T. gondii* as well as to differentiate strains within these lineages using MS fingerprinting markers. We aimed to establish a highly discriminatory next-generation sequencing (NGS)-based typing method for *T. gondii* able to differentiate closely-related type II strains, to trace back infection sources and to monitor for exotic (non-canonical) or recombinant *T. gondii* strains.

Methods

Toxoplasma gondii strains (n=110) were isolated and collected from different parts of Europe. Most of the strains (n=93) belonged to type II, two were type I, ten type III and five type II x III recombinants, based on MS typing. For many isolates whole genome sequences were obtained and their alignment to ME49 reference strain allowed the identification of highly polymorphic regions (HPRs). These regions showed a considerable number of single nucleotide polymorphisms (SNPs). In a second step, the HPRs identified by whole genome sequencing (WGS) analyses were confirmed by Sanger sequencing. Finally, 18 HPRs were used to design a multiplex primer panel suitable to establish an Ion AmpliSeq-based typing tool. For the implementation of the novel typing technique, the isolates analysed by WGS were used. In addition, the sensitivity of the method was tested with serially diluted reference DNA, using three different cycling conditions in the multiplex PCR.

Results

The 18 HPRs are located on 11 chromosomes. Clonal lineages type I, II and III were clearly separated by the 18 HPR Ion AmpliSeq technique and two of the recombinant strains were identified as type II x III strains. Among the type II strains, the 18 HPR Ion AmpliSeq technique was able to differentiate a larger number of haplotypes compared to MS fingerprinting as a reference. The numbers and identities of SNPs from isolates originating from the same outbreak were identical, suggesting a common source. Furthermore, almost all SNPs identified by the 18 HPR Ion AmpliSeq technique corresponded to those

that were expected based on WGS. By testing serially diluted DNA samples, the 18 HPR Ion AmpliSeq technique exhibited a similar analytical sensitivity compared to MS typing.

Conclusion

The results of our study suggest that reliable typing of *T. gondii* strains using an NGS-based technique is feasible. The developed 18 HPR-based Ion AmpliSeq method has a high typing resolution, which appears promising for tracing infection sources in outbreaks and detecting recombinant strains. Moreover, the developed method provides the option to further improve typing resolution by increasing the number of included HPRs.

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Evaluation of *Eimeria spp.* parasites as surrogates for the study of *Toxoplasma gondii* oocyst inactivation

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Foodborne diseases are a global public health concern. *Toxoplasma gondii* is considered as one of the most of concern parasites in the world due to its multiple routes of transmission, high prevalence, and global distribution. The *T. gondii* oocyst the environmental form of the parasite, is known to remain infective for very long periods in soils and waters. These reservoirs can be sources of contamination during the primary production of vegetables, fruits, and seafood.
In order to reduce human exposure, food industries implement control measures whose efficiency to inactivate the pathogens in the food matrix requires evaluation.
However, for *T. gondii*, such studies face methodological and financial constraints. Indeed, sourcing infective oocysts in large quantities and measuring their infectivity are costly, require specific expertise and authorizations, and raise ethical and biohazard issues. Therefore, identifying and characterizing organisms that could be used as surrogates for *T. gondii* oocysts is a critical issue for the agri-food industry.
The ideal surrogate should be non-pathogenic, easy to produce in large quantities and its viability/infectivity easily measured in the laboratory to assess control measures inactivation efficiency. The surrogate should display comparable or less inactivation to *T. gondii* oocysts, as well as similar inactivation kinetics. Whenever possible, the model should be equivalent in structure and size to the target pathogen and have a similar mode of transmission in the matrices to be studied.
In this study, oocysts of the genus *Eimeria* were evaluated as surrogates of *T. gondii*. Physical and chemical treatments were applied to *Eimeria acervulina* and *Toxoplasma* oocysts and their impact on their structure was evaluated by flow cytometry and microscopy. The following parameters were studied: integrity (size/granulometry) and permeability, measured by the incorporation of different markers such as lectins.
The first data obtained suggest that *Eimeria acervulina* would be a good model to study *T. gondii* for certain treatments.

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In vitro model to screen plant extracts and evaluate anticoccidial activities of innovative compounds

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Coccidiosis is the most prevalent parasite infection in poultry industry and its control in broilers production relies mainly on coccidiostat usage, as feed additives. However, coccidiosis control efficacy is threatened by resistance development in parasite field populations. Todays, consumers are looking for food production with low input and respectful of animal welfare. To reach this goal, a research program was developed to evaluate plant extracts efficacy to control coccidiosis. A meta-analysis was conducted with Pubmed publications (with keywords: coccidiosis, *Eimeria*, broiler, chicken, plant extract). Data from 250 publications were analysed and generated a library of 296 compounds or plant extracts. Each publication was scored according to relevance of the test (*in vitro* vs *in vivo*), of the experimental design, of the statistical analysis, etc. The consortium selected 45 plants, based on this evaluation. Dry plant extracts were extracted from water subcritical process or using ethanol as solvent. Toxicity of plant extracts was determined according to ISO 10993-5 standard (no toxicity was assumed for at least 80% of cell viability). Plant extracts were solubilised in DMSO 10%, and efficacy to inhibit cell invasion by *Eimeria tenella* was evaluated at the maximal non-cytotoxic dose. CLEC213 cells, avian lung epithelial cell line, were used for the *in vitro* screening. Engineered *E. tenella* strain that expresses Nano-luciferase reporter gene under actin promoter control, was used to quantify cell invasion.
Thirteen plant extracts out of 45, reduced significantly cell invasion (between 5% to 50 % of invasion, after treatment). From the seven most efficient extracts, a half-maximal inhibitory concentration, IC50 was calculated (from 0.064 to 2.084 mg dry mater / mL), which supports a dose-dependent effect, the high efficacy of the plant extracts and the reliability of the *in vitro* screening. Some possible synergy between plant extracts remains to be tested *in vitro*. Those very promising results have to be evaluated *in vivo*: galenic preparations and concentration ranges will be tested to cope with possible *in vivo* degradation of bioactive compounds.

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