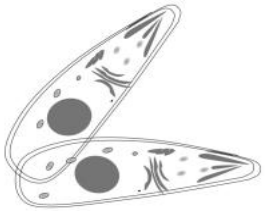
2024



ApiCOWplexa

LA PLATA, ARGENTINA

VII INTERNATIONAL MEETING OF

APICOMPLEXAN PARASITES

IN FARM ANIMALS

October 23-25, 2024

LA PLATA

ARGENTINA

APICOWPLEXA

APICOWPLEXA

Institutional support

Laboratorio de
INMUNOPARASITOLOGÍA
(LAINPA)

Facultad de Ciencias
VETERINARIAS

UNIVERSIDAD
NACIONAL
DE LA PLATA

CONICET

INTA

I P A D S

Sponsors

CABAÑA LOS PINOS

BIO-OPTIC
Excelencia tecnológica y calidad de servicios

INBIO HIGHWAY
-BIOLOGIA MOLECULAR-

SERIVET
DIVISIÓN ANIMAL

ID Innovative Diagnostics

Apicomplexan parasites are an important economic constraint to the food and agricultural industries. Apicomplexan parasites are a diverse group of protozoan organisms, which have acquired a parasitic lifestyle and infect vertebrates and non-vertebrates. Among apicomplexans are numerous species that are responsible for a variety of serious diseases in humans, companion animals and/or farm animals, and they are thus of outstanding medical and socio-economic importance.

ApiCOWplexa 2024 will be an in-person meeting and will provide a forum for researchers, scientists, students, industrial and governmental partners with an inherent interest in apicomplexan parasites in livestock. The meeting will provide an excellent opportunity for networking and scientific exchange. Topics of this meeting will cover the different areas of research on apicomplexan parasites, from One-Health to molecular and cell biology, host-parasite interactions, immunology, diagnosis, epidemiology, drug development, and vaccines and control of infections with these parasites.

Organizing Committee

- María Cecilia Venturini (National University of La Plata, Argentina)
- Magdalena Rambeaud (National University of La Plata, Argentina)
- Lais Pardini (National University of La Plata, Argentina)
- Andrea Dellarupe (National University of La Plata, Argentina)
- María Laura Gos (National University of La Plata, Argentina)

Scientific Committee

- Andrew Hemphill (University of Bern, Switzerland)
- Alexandre Leitão (University of Lisbon, Portugal)
- Luis Ortega Mora (Complutense University of Madrid, Spain)
- Juan Manuel Unzaga (National University of La Plata, Argentina)
- Dadín Prando Moore (Innovation Institute for Agricultural Production and Sustainable Development, Argentina)
- Mariana Bernstein (National University of La Plata, Argentina)
- Gastón Moré (University of Bern, Switzerland)
- Lucía María Campero (Innovation Institute for Agricultural Production and Sustainable Development, Argentina)
- Walter Basso (University of Bern, Switzerland)
- Luis Fernando Pita Gondim (Federal University of Bahia, Brazil)
- María Eugenia Francia (Institut Pasteur de Montevideo, Uruguay)
- Chunlei Su (University of Tennessee, USA)
- Gema Álvarez García (Complutense University of Madrid, Spain)

Welcome to the VII International Meeting of ApicoWplexa in farm animals

Today, we have the opportunity to welcome you in La Plata, since two years ago, at the Bern meeting, Argentina was proposed as the venue for the VII International Meeting of ApicoWplexa.

It was a challenge, considering the political and economic changes that have occurred during this time in Argentina.

We would like to thank the Immunoparasitology Laboratory of the Faculty of Veterinary Sciences, National University of La Plata, and the IPADS of Balcarce, for their Institutional Support. Both groups began research on toxoplasmosis and neosporosis in farm animals in Argentina back in the 90s, when our first local scientific reports on these topics were published. Since then, both laboratories have had a fruitful and interesting exchange

We would like to thank the Organizing and Scientific Committees for their commitment during this time, as well as the sponsors of this event, for their support. We would especially like to thank our international colleagues, many of whom are present today. Our joint projects have greatly enhanced our understanding of Apicomplexan parasites. And to you, the participants of this Meeting, from Argentina and other countries, for your interest in sharing this event. To the foreign participants, some of whom traveled so many hours to reach the southern tip of America, I hope you also get the chance to see some of Argentina's beautiful landscapes.

We invite you all to actively participate in the SESSIONs, exchange ideas, and contribute to the advancement of knowledge in Apicomplexa research.

Let's make the most of these next few days!

María Cecilia Venturini

Dadin Prando Moore

PROGRAM

WEDNESDAY, OCTOBER 23

-14:00-16:00: Registration

-16:00: Opening ceremony. Speakers: Dr. Cecilia Venturini (Argentina) and Dr. Dadin Prando Moore (Argentina).

-16:15-17:00: Symposium: *"ApicoWplexa meeting: History, achievements and consequences ..."* Speaker: Dr. Andrew Hemphill (Switzerland).

SESSION 1: ONE HEALTH

Chairs: Dr. María Cecilia Venturini (Argentina) and Dr. Dadín Prando Moore (Argentina).

17:00-17:30: Keynote*: "A One Health Perspective of Apicomplexan Parasites in Europe."* Speaker: Dr. Frank Katzer.

-17:30-19:00: Oral Presentations and electronic posters.

Oral Presentations:

17:30-17:45: OH1. Cryptosporidiosis of newborn calves in Argentina: overview and surprising findings. Presenting author: Schnittger, L.

17:45-18:00: OH2. Diagnostic biomarkers of *Toxoplasma gondii* early infection in experimentally infected sheep. Presenting author: Huertas-López, A.

E-posters:

OH3. Investigating transmission of *Cryptosporidium parvum* between Greylag geese and livestock. Presenting author: Hamilton, C.

OH4. Identification of *Sarcocystis* infecting the musculature of the invasive African green monkey in St Kitts. Presenting author: Freeman, M.

OH5. Detection and molecular characterization of *Toxoplasma gondii* in American minks (*Neogale vison*) introduced in Patagonia, Argentina. Presenting author: Runco, M. (*)

OH6. Survival of *Toxoplasma gondii* oocysts in a semi-arid climate in northeastern Brazil. Presenting author: Ferreira Feitosa, T. (*)

OH7. Serological insights *Toxoplasma gondii* and *Neospora caninum* in free-ranging wild boar and *Axis deer* populations in Uruguay. Presenting author: Cabrera, A.

OH8. Molecular characterisation of *Cryptosporidium* spp. from the intestine of synanthropic rodents from urban and peri-urban areas of La Plata, Argentina. Presenting author: Fitte, B.

OH9. Elucidating immune responses to *Toxoplasma gondii* in porcine skeletal muscle and neurons. Presenting author: Chandrasegaran, P. R. G. (*)

OH10. Prevalence of anti-*Toxoplasma gondii* antibodies in slaughterhouse workers in the state of Paraíba, Brazil: epidemiological assessment and occupational risks. Presenting author: Ribeiro Vilela, V. L. (*)

OH11. Analysis of free-living seabirds from Brazil as potential hosts of *Toxoplasma gondii*. Presenting author: Chiebao, D.

-19:15: Welcome reception. Amau Hotel.

THURSDAY, OCTOBER 24

SESSION 2: EPIDEMIOLOGY AND ECONOMIC IMPACT

Chairs: Dr. Gastón Moré (Switzerland) and Dr. Alexandre Leitão (Portugal).

- 09:00-9:30: Keynote: *"Apicomplexan parasites associated with reproductive losses in South America"*. Speaker: Dr. Germán Cantón (Argentina).

- 9:30-11:00: Oral Presentations and electronic posters.

Oral Presentations:

-9:30-9:45: EEI1. Confirmation of the dog (*Canis lupus familiaris*) as definitive host of *Sarcocystis aucheniae* under natural conditions by microscopy and molecular studies. Presenting author: Florin-Christensen M.

-9:45-10:00: EEI2. *Sarcocystis* spp. and *Toxoplasma gondii* in muscles from wild boars (*Sus scrofa*) in Switzerland. Presenting author: Moré G.

E-posters (1st part):

EEI3. Estimation of direct economic and productive losses due to abortions caused by *Neospora caninum* in the primary dairy sector of Uruguay. Presenting author: Carrillo Parraguez, M.

EEI4. Natural infection with *Besnoitia akodoni* (protozoa: Sarcocystidae) in synanthropic (*Muridae*) and wild (*Cricetidae*) rodents of Argentina. Presenting author: Bentancourt Rossoli, J. V. (*)

EEI5. *Neospora caninum* and *Toxoplasma gondii* in naturally infected synanthropic (*Muridae*) and wild (*Cricetidae*) rodents from argentina. Presenting author: Bentancourt Rossoli, J. V. (*)

EEI6. Detection of *Toxoplasma gondii* in retail meat from São Paulo megacity, Brazil. Presenting author: Chiebao, D.

EEI7. Molecular detection of agents transmitted by vector arthropods in buffalos (*Bubalus bubalis*) and associated ectoparasites in southeastern Brazil. Presenting author: Zacarias Machado, R.

EEI8. Molecular occurrence of *Theileria parva* in cattle and wild buffalos in Mozambique. Presenting author: Zacarias Machado, R.

EEI9. Bovine coccidiosis: retrospective study of 63 outbreaks at INTA Balcarce (1998-2024). Presenting author: Vilatuña, E. (*)

EEI10. *Cryptosporidium* spp. in outbreaks of bovine neonatal diarrhea registered at INTA Balcarce (2001-2023). Presenting author: Vilatuña, E. (*)

E- poster SESSION continues after SESSION 5 (18:20-19:15 hs)

-11:00-11:30: *Coffee break.*

SESSION 3: DIAGNOSIS

Chairs: Dr. Lucía María Campero (Argentina) and Dr. Magdalena Rambeaud (Argentina).

-11:30-12:00: Keynote: *"Serodiagnosis of Toxoplasma gondii infection: recent advances and a One Health approach"*. Speaker: Dr. Gema Álvarez García (Spain).

-12:00-13:30: Oral Presentations and electronic posters.

Oral Presentations:

-12:00-12:15: D1. Development of a real-time LAMP for the specific and sensitive detection of *Cryptosporidium parvum* in water samples. Presenting author: de Alba, P. (*)

-12:15-12:30: D2. Modernizing diagnosis of coccidiosis: challenges faced in adding molecular species differentiation and enumeration to OPG counts from litter or fecal samples. Presenting author: Lindo, D. P. (*)

E-posters:

D3. Cryptosporidiosis in dairy cattle from Buenos Aires, Argentina. Presenting author: Basset, C. M. (*)

D5. Development and validation of a new IgM-ELISA to detect anti-*Toxoplasma gondii* antibodies in sheep sera. Presenting author: Huertas-López, A.

D6. Exploring NcSAG1 and NcSRS2: Recombinant Proteins of *Neospora caninum* and their role in pathogenesis and diagnostics. Presenting author: Echeverría, S.

D7. First report of congenital toxoplasmosis in guanacos (*Lama guanicoe*) from Los Glaciares National Park, Argentina. Presenting author: Campero, L. M.

D8. Assessment of diagnostic techniques of bovine abortion by *Neospora caninum* at INTA Balcarce. Presenting author: Sosa, E. (*)

D9. A putative new *Besnoitia* species in the southern black-eared opossum *Didelphis aurita*. Presenting author: Moré, G.A.

D10. Detection of *Sarcocystis capracanis* and *Sarcocystis hircicanis* in goats from Argentina. Presenting author: Steffen, K. D.

-13:30-14:45: *Lunch*.

SESSION 4: TREATMENT AND CONTROL

Chairs: Dr. Pita Gondim (Brazil) and Dr. Dadin Prando Moore (Argentina)

-14:45-15:15: Keynote: *"Current and potentially novel options for control of diseases caused by apicomplexans affecting farm animals."* Speaker: Dr. Andrew Hemphil (Switzerland).

-15:15-16:45 Oral Presentations and electronic posters.

Oral Presentations:

-15:15-15:30: TC1. Effects of the novel bumped kinase inhibitor BKI-1708 against *Toxoplasma gondii*, *Neospora caninum* and *Besnoitia besnoiti*: differences and similarities. Presenting author: Ferreira de Sousa, M.C. (*)

-15:30-15:45: TC2. Tartrolon E: a new drug candidate for *Cryptosporidium parvum* infections. Preliminary results. Presenting author: Fumuso, F.G. (*)

E-posters:

TC3. *In vitro* and *in vivo* activities of a trithiolato-diruthenium complex conjugated with sulfadoxine against the apicomplexan parasite *Toxoplasma gondii*. Presenting author: Hänggeli, K.P.A. (*)

TC4. Marine compound tartrolon E rapidly blocks *Toxoplasma gondii* host cells invasion capacity without affecting viability, morphology, nor attachment capabilities. Presenting author: Fumuso, F.G. (*)

TC5. Repurposing of covid box and kinetic box to discover potential drugs against *Besnoitia besnoiti* infection. Presenting author: Álvarez, G.

TC6. Drug discovery against *Sarcocystis neurona* infection. Presenting author: Kubota, R.

TC7. *Cryptosporidium* specific IgY antibodies reduce severe diarrhea in an experimental calf model of infection and disease. Presenting author: Mira, A.

TC8. *In vitro* assessment of antimicrobial peptides as potential agents against *Toxoplasma gondii*. Presenting author: Amdouni, Y.

TC9. *In vitro* anticoccidial effect of naringenin and dehydrated grapefruit peel (*Citrus x paradisi*). Presenting author: Pérez-Fonseca, A.

TC10. Reduction of abortion rates in an Argentinean beef herd by culling *Neospora caninum* seropositive heifers. Presenting author: Moore, D.P.

TC12. Efficacy of a calcium-dependent protein kinase 1 inhibitor, BKI-1708 in animal infection models of *Cryptosporidium*. Presenting author: Ojo, K.K.

-16:45-17:15: *Coffee break.*

SESSION 5: *IN VITRO* AND *IN VIVO* MODELS

Chairs: Dr. Gema Alvarez García (Spain) and Dr. Andrea Dellarupe (Argentina).

-17:15-17:45: Keynote: *"Ruminant models for tissue cysts-forming apicomplexan parasites: Reflections on the parasite and the host… and a plea for consistency."* Speaker: Dr. Luis Ortega Mora (Spain).

-17:45-18:30: Oral Presentations and electronic posters.

Oral Presentations:

-17:45-18:00: IVIVM1. Contrary to the accepted paradigm that type III *Toxoplasma gondii* strains show remarkable diversity of virulence degrees evaluated in a harmonized mouse model. Presenting author: Salas-Fajardo, M.Y. (*)

-18:00-18:15: IVIVM2. Differences in virulence and oocyst shedding profiles in lambs experimentally infected with different isolates of *Cryptosporidium parvum*. Presenting author: Bartley, P.

E-posters:

IVIVM 3. Experimental infection model in calves with *C. parvum:* infectious dose, diarrhea and oocyst excretion. Presenting author: Garro, C. (*)

-18:20-19:00: Epidemiology and economic impact (2nd part)

EEI11. Sheep, Toxoplasma, and genetic diversity: insights from Uruguay. Presenting author: Tana-Hernández, L.

EEI12. *Toxoplasma gondii* and *Neospora caninum* in ruminants from Somalia. Presenting author: Barros, L.D.

EEI13. *Neospora caninum* congenital transmission and abortion rates in dairy and beef cattle of central Argentina (1998–2024). Presenting author: Cantón, G.

EEI14. Equine piroplasmisis: molecular detection of *Theileria equi* and *Babesia caballi* in Corrientes, Argentina. Presenting author: Ganzinelli, S. (*)

EEI15. PCR detection of cattle cyst-forming coccidian in fecal material of canids from South-eastern Buenos Aires, Argentina. Presenting author: Soto Cabrera, A. (*)

FRIDAY, OCTOBER 25

SESSION 6: PARASITE-HOST INTERACTIONS AND PATHOGENESIS

Chairs: Dr. Juan M. Unzaga (Argentina) and Dr. Luis M. Ortega Mora (Spain).

-09:00-9:30: Keynote: *"The role of dogs as definitive hosts of Neospora caninum and several other cyst-forming coccidian parasites."* Speaker: Dr. Pita Gondim (Brazil).

-9:30-11:00: Oral Presentations and electronic posters.

Oral Presentations:

-9:30-9:45: PHIP1. Identifying functional genomic variants and genes associated with *Theileria* infection in cattle. Presenting author: Powell, J.

-9:45-10:00: PHIP2. Early dynamics of *Toxoplasma gondii* infection in pregnant sheep. Presenting author: Sánchez-Sánchez, R.

E-posters:

PHIP3. Cytokine expression in bovine fetuses spontaneously aborted by *Neospora caninum*. Presenting author: Sosa, E. (*)

PHIP4. Immune response differences in cattle breeds revealed by differential gene expression analysis following experimental *Neospora caninum* infection. Presenting author: Fiorani, F. (*)

PHIP5. An overexpression screen identifies interferon stimulated genes that control *Toxoplasma gondii* in porcine cells. Presenting author: Ungogo, M.A.

PHIP6. Characterization of *Toxoplasma gondii* non-archetypal isolates provide new insights into their phenotypic traits and confirm the

laboratory adaptation phenomena. Presenting author: Ortega-Mora, L.M.

PHIP7. Exploring NCROP2 role in *Neospora caninum* virulence through transcriptomic analysis of bovine macrophages. Presenting author: Hassan M. A

PHIP8. Modulation of host cell membrane fluidity by *Neospora caninum*: a study using laurdan fluorescence and hyperspectral imaging. Presenting author: Cabrera, A.

-11:00-11:30: *Coffee break.*

SESSION 7: CELLULAR AND MOLECULAR BIOLOGY

Chairs: Dr. María Eugenia Francia (Uruguay) and Dr. Lais Pardini (Argentina).

-11:30-12:00: Keynote: *"Advances in population genetic studies of Toxoplasma gondii and their applications in the molecular epidemiology of toxoplasmosis"*. Speaker: Dr. Chunlei Su (USA).

-12:00-13:00: Oral Presentations and electronic posters.

Oral Presentations:

-12:00-12:15: CMB1. An iswi-related chromatin remodeler orchestrates parasite life cycle progression by insulating gene expression in a densely packed genome. Presenting author: Pachano, B. (*)

-12:15-12:30: CMB2. *Neospora caninum* invasion and replication are modulated by cathelicidins in mouse bone marrow-derived macrophages. Presenting author: Fiorani, F. (*)

E-posters:

CMB3. Mitochondrial damage and il-1 production of monocyte by *Neospora caninum* infection is mediated by dense granule protein 7 and prohibitin. Presenting author: Nishikawa, Y.

CMB4. Innate immune response in *Neospora caninum* and BoAHV-1 co-infection: the interaction of TLRs and cathelicidins. Presenting author: Plá, N. (*)

CMB5. Virulence studies of *Neospora caninum* in goat trophoblast primary culture. Presenting author: Alvarez, B.E. (*)

-13:00-14:15: *Lunch*

SESSION 8: IMMUNOLOGY AND VACCINES

Chairs: Dr. M.C. Venturini (Argentina) and Dr. Andrew Hemphill (Switzerland).

-14:15-14:45: Keynote: *"Modulating type and localization of adaptive immunity to improve protection against Neospora caninum and Toxoplasma gondii."* Speaker: Dr. Alexandre Leitão (Portugal).

-14:45-16:15: Oral Presentations and electronic posters.

Oral Presentations:

-14:45-15:00: IV1. Evaluation of the humoral immune response of pigs immunized by intradermal and intramuscular routes with a multigene DNA vaccine (ROP18 + SAG1) against *Toxoplasma gondii*. Presenting author: García J.L.

-15:00-15:15: IV2. Development of next generation vaccine against *Toxoplasma gondii*. Presenting author: Nishikawa, Y.

E-posters:

IV3. Identification of conserved putative surface antigens expressed in infective stages of *Eimeria tenella* and *E. acervulina* through reverse vaccinology. Presenting author: Tomazic, M.

IV4. Immunization with plant-based vaccine expressing *Toxoplasma gondii* SAG1 fused to plant hsp90 elicits protective immune response in lambs. Presenting author: Campero, L.M.

E- posters: Epidemiology and economic impact (3rd part)

OH12. Enhancing One Health outcomes: the importance of data structure and distribution. Presenting author: Helman, E. (*)

EEI16. Everything about neosporosis in Colombia. Presenting author: Rivas López, C. P. (*)

EEI17. Seroepidemiology of bovine neosporosis in the dairy herd at INTA Balcarce. Presenting author: García, C. E. (*)

EEI18. High prevalence cluster of *Neospora caninum* identified in the Mar y Sierras dairy basin, Argentina. Presenting author: Soto Cabrera, A. (*)

EEI20. *Sarcocystis* cysts burden distribution in striated muscles of lambs. Presenting author: Moore, D. P.

-16:15-16:45: *Coffee break.*

SESSION 9: BIOLOGICAL SYSTEMS (GENOMICS, PROTEOMICS) AND EVOLUTION

Chairs: Dr. Chunlei Su (USA) and Dr. Frank Katzer (Scotland).

-16:45-17:15: Keynote: *"From the Bench to the Field: Translating Functional Genomics Discoveries into Practical Solutions for the Productive Sectors."* Speaker: Dr. María Eugenia Francia (Uruguay).

-17:15-17:45: Oral Presentations.

Oral Presentations:

-17:15-17:30: BSE1. The tiny, but diverse, mitochondrial genomes of eimeriid and adeleorinid coccidia. Presenting author: Barta, J.R.

-17:30-17:45: BSE2. Genomic analyses of *Goussia degiustii* and *Goussia leucisci,* coccidian parasites infecting fish. Presenting author: Cohen, B. (*)

-18:30: Awards. Closing comments.

-20:30: Congress dinner. Land Plaza Hotel.

Oral Presentations and E-posters marked with (*) participate for the Junior Scientist Awards.

SYMPOSIUM

"ApiCOWplexa meeting:

History, achievements and consequences....."

Andrew Hemphill

Institute of Parasitology, Vetsuisse Faculty, University of Bern, Switzerland

Apicomplexan parasites cause a variety of serious diseases in humans, pets and/or farm animals, and they are thus of outstanding medical and socio-economic importance. The most prominent representative of the phylum Apicomplexa is *Plasmodium* spp., with an estimated 249 million human cases and around 600'000 deaths in a single year. Thus, during the last 10-20 years, major research funding and finances for networking activities have gone into malaria research. However, ApicoWplexa pays tribute to the outstanding importance of another group of apicomplexan parasites, namely those causing diseases in farm animals. These include *Toxoplasma, Cryptosporidium, Eimeria, Neospora, Sarcocystis, Besnoitia, Babesia and Theileria*, which are responsible for important diseases that affect the health and productivity of cattle, pork, poultry, sheep, goats, as well as of pets, game and wild animals, with some of them exhibiting a considerable zoonotic potential. For many years, research and networking activities on apicomplexan parasites in farm animals have received only a fraction of the financial support compared with similar activities in the malaria field, reflecting the fact that parasitic food-borne diseases in general are under-recognized. Nevertheless, globalization of the food supply has rendered food-borne disease more common, and increased international travel, higher numbers of persons at risk, and changes in culinary habits, coupled with improvements in diagnostic tools, have contributed to the augmented awareness of food-borne diseases. Apicomplexans play a major role in food security and

food safety, both of which represent pressing issues as the human population on a global scale is steadily increasing, with an increasing demand in sufficient, safe and nutritious food. It is evident that apicomplexan parasites are an important economic constraint to the food and agricultural industries and represent a threat for the continuous sustainability of food supplies worldwide.

ApicoWplexa was founded in 2012 (basically as an "idea" that started with a few beers in a park in Lisbon), with the goal to create an informal network dedicated to researchers, students and many others specifically working on these topics. Since the first meeting in Lisbon in 2012, organized by Alexandre Leitao and his colleagues, 5 additional in-person meetings have been held in Kusadasi, Edinburgh, Madrid, Berlin and in Bern. Researchers, students and representatives of pharmaceutical companies and government agencies have readily taken the opportunity to meet their peers, especially since the research on apicomplexan parasites in farm animals is often "diluted" in larger scientific conferences. During 2020 and 2021, Covid has taken its toll, but during this period, online meetings were held every 2 to 3 months, which were attended by many researchers worldwide, underlining the continuous interest in the topic. This presentation will provide information on activities originating from this network.

ApicoWplexa is a purely voluntary and informal nertwork, open to those who are interested in the topic, with no strings (and unfortunately also no funding) attached. However, this is probably the reason this network stayed alive during the last 12 years, driven mainly by the scientific interest of participants, and dependent on the initiative of those who accepted the monumental task to organize the bi-annual meetings. With this in mind, we thank our hosts in La Plata, which have made it possible that for the first time this conference can take place on South American soil.

SESSION 1

One Health: a view from Parasitology in Europe

Keynote. A One Health Perspective of Apicomplexan Parasites in Europe

Katzer F.*, Bartley P.

Moredun Research Institute, Edinburgh, Scotland

*frank.katzer@moredun.ac.uk

The main apicomplexan parasites that are of One-Health importance in Europe will be discussed. *Toxoplasma gondii* is recognised by the European Centre for Disease Prevention and Control for its importance due to foodborne outbreaks but waterborne transmission should not be forgotten. *Cryptosporidium parvum* is another important zoonotic species due to outbreaks associated with contaminated drinking water or direct contact with infected lambs or calves, while the foodborne transmission of *Cryptosporidium* has also been highlighted in recent years. There are several other zoonotic *Cryptosporidium* species including *C. cuniculus*, which was linked to a large drinking water outbreak in England in 2008, and a recently described *Cryptosporidium* species called *C. mortiferum*, previously known as chipmunk genotype I, which is considered an emerging zoonoses in Sweden. Zoonotic apicomplexan parasites in Europe are also transmitted by ticks, these include *Babesia* spp. in particular *Babesia* divergens, which is well known for infections in cattle, where it cases red-water, but in immunosuppressed people it can be fatal if not diagnosed and treated early enough. Other *Babesia* spp. that are sporadically found in humans in Europe are *B. mictoti*, *B. venatorum*, *B. odocoilei* and *Babesia* spp. of

the MO1 clade. The genus *Sarcocystis* also contains zoonotic parasites, for two of these species, humans are the definitive hosts namely: *S. hominis* and *S. suihominis*. Finally, *Giardia duodenalis* commonly causes disease in humans in Europe, and human infectious assemblages/sub-assemblages can be found in many livestock, companion animal and wildlife species. However, the role of many of these host species in the transmission of *Giardia* to humans still requires further investigations/confirmations.

This presentation will give an overview of the main zoonotic apicomplexan parasites that are present in Europe, highlighting their importance, transmission routes and possible intervention/control strategies.

ORAL PRESENTATIONS

OH1- Cryptosporidiosis of newborn calves in Argentina: overview and surprising findings

de Alba P.[1,2], Garro C.[1], Florin-Christensen M.[1,2], Schnittger L.[1,2]*

[1] *Instituto de Patobiología Veterinaria, INTA-CONICET, Los Reseros y Nicolas Repetto s/n, Hurlingham 1686, Argentina*
[2] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ciudad Autónoma de Buenos Aires C1033AAJ, Argentina*

*Presenting author: leoschnittger@googlemail.com

Cryptosporidium spp. are enteroparasitic protozoans that cause cryptosporidiosis in newborn calves. Clinical signs of the disease are diarrhoea and dehydration leading to decreased productivity and economic losses in dairy farms around the world. In addition, cryptosporidiosis by *C. parvum* is an important zoonotic disease of public health concern. This review aims to integrate existing knowledge on prevalence and risk factors of calf cryptosporidiosis in Argentina, as well as *C. parvum* GP60 subtype diversity. Depending on the study

region and applied diagnostics, prevalence among calves up to 20 days of age varied in Argentina between 25.2% and 42.5%, while prevalence rates of 16.3 to 25.5% were observed at the age period of 1–90 days. These values are above those observed in most countries. Furthermore, exclusively *C. parvum* was identified in pre-weaned calves. In Argentina, *C. parvum* infection has been shown to be the major cause of calf diarrhoea, followed by rotavirus A (RVA), while the remaining enteropathogens played a marginal role. Calf age of 20 days or less, incidence of diarrhoea, poorly drained soils, and large farm size were identified as risk factors for *C. parvum*-infection. Altogether nine GP60 subtypes (IIaAxxG1R1, where xx corresponds to the trinucleotide TCA repeated 16 to 24 times) were identified. Since identified GP60 alleles have a similar genetic background, we hypothesize that the continuous trinucleotide repeat array has been generated by stepwise repeat expansion of A16. As GP60 subtypes with fewer TCA repeats show a wider global distribution, the IIaA16G1R1 subtype appears as the primordial allelic variant. Finally, 45.8% (77 of 168) of GP60 subtypes identified in Argentina are known to be zoonotic (IIaA16G1R1, IIaA20G1R1, and IIaA22G1R1) suggesting a potentially high risk for public health. Our findings contribute to an improved understanding of bovine cryptosporidiosis in and beyond Argentina.

OH2- Diagnostic biomarkers of *Toxoplasma gondii* early infection in experimentally infected sheep

Velasco-Jiménez N.[1], Huertas-López A.[1,2]*, Sánchez-Sánchez R.[1], Horcajo P.[1], Peres Rubio C.[3], Cerón J. J.[3], Álvarez-García G.[1], Calero-Bernal R.[1], Ortega-Mora L. M.[1]

[1] SALUVET, Animal Health Department, Complutense University of Madrid, Madrid, España
[2] Animal Health Department, University of Murcia, Murcia, España
[3] Interlab-UMU, Animal Medicine and Surgery Department, University of Murcia, Murcia, España

*Presenting author: ana.huertas@um.es

Pregnant sheep experimentally challenged with *Toxoplasma gondii* oocysts may suffer from early abortions, which occur during the second week post-infection, prior maternal IgG seroconversion and the presence of parasite DNA in placental and fetal tissues. This panorama justifies the search for new biomarkers of recent infection and abortion. In this study, the utility of acute phase proteins (APPs) (haptoglobin and serum amyloid A), oxidative stress biomarkers (OSBs), thrombosis-related cytokines (IFN-γ, CXCL9 and CXCL10) and specific IgM during early *T. gondii* infection in sheep were investigated. Two groups of sheep were orally dosed with 10 and 1000 *T. gondii* oocysts, respectively, while a control group remained uninfected. Several infected sheep suffered from early abortion on 8-12 days post-infection (dpi). Sera were collected every two days until 14 dpi and then weekly until 28 dpi. APPs and OSBs were determined using an Olympus 400 analyzer, while cytokines were measured with commercial ELISA kits. IgM and IgG levels were determined by in-house ELISAs based on lyophilized *T. gondii* tachyzoites. In all infected sheep, APPs significantly raised on 7-8 dpi, while the increase of cytokines was observed on 5-10 dpi. Specific IgM significantly increased earlier than IgG (12 vs. 21 dpi with 10 oocysts, and 12 vs. 14 dpi with 1000 oocysts). Only in sheep infected with 1000 oocysts, most of antioxidants decreased while oxidants increased on 14 dpi, which could be due to the depletion of antioxidants caused by the exacerbated oxidant response. Combination of unspecific biomarkers, such as APPs, OSBs and cytokines, with the specific IgM- ELISA could provide an indicator of early abortion caused by *T. gondii* infection. In the future, these promising biomarkers should be tested in sheep with natural infection by *T. gondii*.

E-POSTERS

OH3- Investigating transmission of *Cryptosporidium parvum* between Greylag geese and livestock

Thomson J.[1], Wells B.[1], Innes E.[1], Hamilton S.[1], Keegan K.[1]; McBean D.[1], Silva N.[1], Watson E.[1], Hamilton C.[1]*

[1]*Moredun Research Institute, Penicuik, Scotland, UK*

*Presenting author: clare.hamilton@moredun.ac.uk

Cryptosporidium parvum is an important zoonotic parasite causing diarrhoeal disease in neonatal animals, and humans. Migratory birds, such as geese, can carry pathogens that are transmissible to other wildlife species, farm animals, and humans. The aim of this research was to investigate transmission dynamics of *Cryptosporidium parvum* between livestock and a significantly expanded Greylag goose population in Orkney (Scotland, UK). Faecal samples were collected over three sampling periods: the goose migratory season (8 farms); the calving period when cattle were housed indoors (pre-turnout; 2 farms); and after cattle had been turned out to pasture following housing (post-turnout; 2 farms). DNA was extracted from all samples and screened for *Cryptosporidium* using the *18S* PCR, and speciated using sequencing. *Cryptosporidium* was detected in 25/200 geese during the migratory season. Thus far, *Cryptosporidium parvum* has been identified in 6/25 positive samples but speciation analysis is ongoing. During pre-turnout, *Cryptosporidium* was detected in 12/17 calves and 24/25 dams on Farm 1, and 3/20 calves and 10/25 dams on Farm 2. On Farm 1, 92% of positive calves and 67% of positive dams were shedding *C. parvum*. On Farm 2, only one calf was shedding *C. parvum*. Of 100 geese tested during pre-turnout, one was positive for *Cryptosporidium* (goose genotype I).
During post-turnout, *Cryptosporidium* was detected in 7/25 calves and 3/25 dams on Farm 1, and 3/25 calves and 0/25 dams on Farm 2. No *C.*

parvum was detected on either farm. Of 54 geese tested during post-turnout, two were positive for *Cryptosporidium* (species to be determined). Results indicate a low burden of *Cryptosporidium* spp. in Greylag geese, and limited evidence of transmission of zoonotic species to livestock on these study farms. Sampling different flocks of geese may be important to better assess the risk of transmission across the whole island.

OH4- Identification of *Sarcocystis* infecting the musculature of the invasive African green monkey in St Kitts

Freeman M.[1]*, Herbert N.[1], Bolfa P.[1]

[1]*Ross University School of Veterinary Medicine, Center for Conservation Medicine and Ecosystem Health, Basseterre, Saint Kitts*

*Presenting author: mafreeman@rossvet.edu.kn

The African green monkey (*Chlorocebus aethiops sabaeus*) is considered an invasive species in the federation of St. Kitts and Nevis, and shares the same habitat with people, domestic and wild animals. Muscle samples (tongue, heart, intercostal, diaphragm and biceps femoris) from 17 trapped monkeys were donated to RUSVM after being part of a terminal surgical study at the Behavioral Science foundation.
Histologically, intact intrasarcoplasmic protozoal cysts with crescent-shaped bradyzoites were seen in the sarcoplasm of myocytes. Some cysts were ruptured resulting in a focal myositis with muscle degeneration and necrosis.
DNA extractions were made from either frozen/fresh tissues or taken from wax-embedded tissues. PCR reactions were performed using apicomplexan primers targeting the small subunit ribosomal DNA (*SSU rDNA*) and the cytochrome c oxidase subunit 1 mitochondrial gene (*COI*). Amplicons of the expected sizes were sent for bidirectional sequencing and contiguous sequences assembled. BLAST searches in

the NCBI databases confirmed an apicomplexan origin for the PCR products and provided the highest identity to *Sarcocystis* spp. known in the databases.
DNA sequences from multiple tissues were 100% identical to each other. The *SSU rDNA* sequence had a 98.5% identity to *Sarcocystis bovifelis*, whereas the *COI* sequence had an 84.4% identity to *Sarcocystis bovini*.
Future work needs to identify other/definitive hosts that are infected with this species of *Sarcocystis* on St Kitts, potentially bovids and other livestock. In addition, we must consider the zoonotic potential of this species as it has been repeatedly recovered from primates.

OH5- Detection and molecular characterization of *Toxoplasma gondii* in American minks (*Neogale vison*) introduced in Patagonia, Argentina

Runco M.[1,5]*, Gos M. L.[1], Pardini L.[1,5], Bernstein M.[1,5], Rago M. V.[2], Monteverde M.[3], Guichón M. L.[2], Piudo L.[3], González A.[3], Campero L. M.[4], Venturini M. C.[1]

[1] *Cátedra Inmunología Veterinaria Básica, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Argentina, Laboratorio de Inmunoparasitología (LAINPA), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Argentina,*
[2] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina, Grupo de Ecología Terrestre de Neuquén, Instituto de Investigaciones en Biodiversidad y Medioambiente (INIBIOMA, UNCo–CONICET), Subsede Junín de los Andes Neuquén, Junín de los Andes, Argentina*
[3] *Grupo de Ecología Terrestre de Neuquén, Dirección de Ecosistemas Terrestres, Centro de Ecología Aplicada del Neuquén (CEAN), Junín de los Andes, Argentina.*
[4] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina, Instituto de Innovación para la Producción Agropecuaria y el Desarrollo Sostenible (IPADS Balcarce, INTA- CONICET), Balcarce, Argentina.*

[5] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), La Plata, Argentina*

*Presenting author: mrunco@fcv.unlp.edu.ar

The American mink (*Neogale vison*) is an invasive, non-native mammal first introduced in Argentina in the 30's. Its semi-aquatic behavior and broad carnivorous diet makes it a sentinel species for *Toxoplasma gondii*, as it is exposed to sporulated oocysts present in the environment and tissue cysts from prey animals. The knowledge of the genotypes present in the environment is essential for the application of preventive health measures. The aim of the study was to detect *T. gondii* and characterize the genotypes of *T. gondii* found in American minks captured in Neuquén province, Argentina. A commercial kit (Inbio Highway) was used for DNA extraction from brain samples, following manufacturer's recommendations. For the detection of the specific sequence of 529bp of *T. gondii* DNA, the quantitative PCR (qPCR) was carried out. DNA positive samples were further genotyped by multilocus nested PCR-RFLP analysis for SAG1, SAG2 (5´3´SAG2, altSAG2), SAG3, BTUB, GRA6, C22-8, C29-2, L358, PK1 and Apico markers. A total of 15% (6/40) of the analyzed brains were positive to *T. gondii*. Nonetheless, complete genotypification was possible in one sample, and the resulting genotype was characterized as new non-clonal genotype (ToxoDB #347), which has not been reported previously. Phylogenetic network was inferred using the software SplitsTree CE 6.0.0_alpha. The genotype ToxoDB #347 clustered with non-clonal genotypes from Misiones province, Argentina. The presence of the American mink represents a health risk, as it may act as a source of infection for scavenger animals, hence perpetuating the life cycle of this apicomplexan parasite. This is the first molecular detection of *T. gondii* in an American mink population in Argentina and the first genotypic characterization of *T. gondii* in American mink in South America.

OH6- Survival of *Toxoplasma gondii* oocysts in a semi-arid climate in northeastern Brazil

Ferreira T.F.[1]*, Silva A. L.P.[1], Silva Filho G.M,[1], Lima B.A.[1], Vilela V. L. R.[1]

[1] *Instituto Federal da Paraíba, Sousa, Brazil*

*Presenting author: feitosa.tf@gmail.com

Toxoplasma gondii is a protozoan that affects almost all warm-blooded animals, and susceptible hosts can become infected primarily through transplacental transmission, ingestion of animal tissues containing infective cysts, and ingestion of water and food contaminated with cat feces containing sporulated oocysts. Therefore, this study aimed to analyze the sporulation rate of oocysts subjected to natural environmental conditions in the semi-arid region of Northeast Brazil. For this purpose, four cats were infected with tissues containing *T. gondii* cysts, and fecal analysis was performed using Centrifugal Flotation in Sucrose Solution (CFSS) through optical microscopy at 400x magnification to search for oocysts. When the feces tested positive, they were transferred to containers with environmental sand and divided into two groups: Group 1 – positive fecal samples on the ground, and Group 2 – control with fecal samples stored in Biochemical Oxygen Demand (BOD) at a temperature of 28°C and 80% humidity. The following time intervals were used to evaluate sporulation: t = 0, 12, 24, 36, 48, 60, and 72 hours after fecal excretion. CFSS was used to observe oocysts. In the first 12 hours, it was observed that 91% of the oocysts had already sporulated in Group II, but no oocysts were seen in Group I. The sporulation rate remained stable in Group II during the analyzed times, and no oocysts were observed in Group I at any of the experimental moments. In Group I, soil temperatures ranged from 27.2 to 55.4°C, and humidity from 22 to 38%. It is concluded, therefore, that high temperature and low humidity are decisive in destroying *T. gondii* oocysts and that this protozoan is rapidly inactivated when exposed to

sunlight and the temperatures of the semi-arid region of Northeast Brazil.

OH7- Serological insights *Toxoplasma gondii* and *Neospora caninum* in free- ranging wild boar and *Axis deer* populations in Uruguay

Cabrera A.[1]*, Echeverría S.[2], Ruppel F.[2], González F.[2], Tomasina R.[1,2], Castro G.[3], DeBrun L.[4], Cravino A.[5], Cancela F.[6], Puentes R.[4], Basmadjián Y.[1], Robello C.[2,7], Mirazo S.[6]

[1] *Unidad Académica de Parasitología y Micología, Instituto de Higiene, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay.*
[2] *Laboratorio Interacciones Hospedero-Patógeno Institut Pasteur de Montevideo, Montevideo, Uruguay.*
[3] *proJAb Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay.*
[4] *Microbiología, Departamento de Patobiología, Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay.*
[5] *Instituto de Ecología y Ciencias Ambientales. Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.*
[6] *Unidad Académica de Bacteriología y Virología, Instituto de Higiene, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay.*
[7] *Unidad Académica de Bioquímica, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay*

*Presenting author: cabrera@pasteur.edu.uy

This study focuses on the seroprevalence of *Toxoplasma gondii* and *Neospora caninum*, two significant apicomplexan parasites, in free-ranging wild boar and *Axis deer* in Uruguay. *Toxoplasma gondii*, the causative agent of toxoplasmosis, can lead to serious health issues in

humans and animals, including reproductive failures and neurological disorders. *Neospora caninum*, responsible for neosporosis, primarily affects cattle, causing economic losses due to reproductive issues. Both parasites have complex life cycles involving multiple hosts, and wildlife species play a crucial role in maintaining these parasites in the environment. This study aims to provide insights into the prevalence of these pathogens in two invasive species in Uruguay. Samples were collected from 254 wild boars and 90 *Axis deer* across 10 departments in Uruguay between 2020 and 2022. Blood samples were analyzed for antibodies against *T. gondii* and *N. caninum* using commercial and "in house" ELISA kits and confirmed with Western blotting. Statistical analysis was performed to compare seroprevalence rates using Chi-square and Kruskal-Wallis tests, considering p-values < 0.05 as significant.

The study found a seroprevalence of 48.4% for *T. gondii* in wild boars and 7.8% in *Axis deer*. Conversely, *N. caninum* was detected in 2.4% of wild boars and 53.3% of *Axis deer*. Coinfection was observed in a small number of cases in both species. The results indicate a significant presence of both pathogens in the studied wildlife populations, with notable differences in seroprevalence between species and regions. The high seroprevalence of *T. gondii* in wild boars suggests active circulation of the parasite in the natural environment of Uruguay. The presence of *N. caninum*, although lower in wild boars, was significantly higher in *Axis deer*, indicating a potential role of these species in the epidemiology of these pathogens. The study highlights the importance of monitoring wildlife diseases to understand their impact on both animal and human health, as well as their economic implications. The findings provide valuable data for developing strategies to control and mitigate the spread of these diseases in wildlife and livestock populations.

OH8- Molecular characterisation of *Cryptosporidium* spp. from the intestine of synanthropic rodents from urban and peri-urban areas of La Plata, Argentina

Dellarupe A. [1,4], Fitte B.[1,4]*, De Felice L.[1,2], Sella M. E.[1], Steffen K. D.[1,2,4], Robles M. del R.[3,4], Venturini M. C.[1], Unzaga J. M.[1,2]

[1] *Laboratorio de Inmunoparasitología (LAINPA), Facultad de Ciencias Veterinarias-Universidad Nacional de La Plata, La Plata, Argentina*
[2] *Cátedra de Parasitología y Enfermedades Parasitarias, Facultad de Ciencias Veterinarias- Universidad Nacional de La Plata, La Plata, Argentina*
[3] *Centro de Estudios Parasitológicos y Vectores – CONICET, Universidad Nacional de La Plata, La Plata, Argentina*
[4] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ciudad Autónoma de Buenos Aires, Argentina*

*Presenting author: fittebruno@gmail.com

Cryptosporidiosis is a zoonotic disease with a global distribution. Transmission is via the fecal-oral route through contaminated water, food, or direct contact between hosts. Synanthropic rodents serve as hosts for this parasitic disease, acting as a source of infection for other hosts, including humans. The objective of this study was to detect the presence of *Cryptosporidium* spp. DNA in 85 intestinal samples from synanthropic rodents (16 *Rattus rattus*, 25 *Rattus norvegicus*, and 44 *Mus musculus*). The samples were collected in urban and peri-urban areas of the city of La Plata, Province of Buenos Aires, Argentina, during the spring-summer and autumn-winter periods of 2014-2015, following the occurrence of flooding. The intestinal samples were collected during the necropsy at CEPAVE and subsequently processed at LAINPA. The DNA was extracted using a commercially available kit (AND Puriprep- suelo kit-INBIO HIGHWAY). Subsequently, the nested-PCR technique was employed for the amplification of the *18S rRNA* subunit gene, which is genus-specific. The samples that tested positive by PCR were purified

and sequenced. Subsequently, the sequences were aligned and analysed, and the consensus sequences were compared with those previously reported in GenBank using the BLAST tool. Of the 85 samples subjected to PCR analysis, 14.11% (12/85) yielded positive results. Of these, 9.1% (4/44) derived from the intestines of *M. musculus*, 16% (4/25) from *R. norvegicus*, and 11.8% (4/34) from *R. rattus*. Of the 12 positive samples, 11 underwent sequencing. The comparative analysis using BLAST revealed that two samples were identity with *Cryptosporidium* sp., three with *C. muris*, one with *C. occultus*, one with *C. tyzzeri*, and four with *C. parvum*. These findings indicate the presence of cryptosporidiosis in synanthropic rodents in urban and peri-urban areas of La Plata, as well as the presence of some zoonotic species that represent a significant risk to the population.

OH9- Elucidating immune responses to *Toxoplasma gondii* in porcine skeletal muscle and neurons

Chandrasegaran P. R. G.[1]*, Hassan M. A.[1]

[1] *The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, UK*

*Presenting author: praveena.chandrasegaran@ed.ac.uk

Toxoplasma gondii infection poses a significant risk to food safety and animal health. Over 40% of human toxoplasmosis cases are linked to the consumption of contaminated pork. Acute infection can cause up to 57% mortality in 3-week-old piglets, while chronic infection can compromise the immunity of pigs to other devastating intracellular pathogens, such as classical swine fever virus (CSFV). There are no treatments or approved vaccines to control *Toxoplasma* in pigs, making the development of new *Toxoplasma* control strategies an important research priority.

Chronic infection, characterised by semi-dormant *Toxoplasma* cysts in muscle tissues and central nervous system, is critical to both parasite transmission and clinical disease in humans. The cytokine interferon gamma (IFN**γ**) is indispensable to the effective control of both acute and chronic *Toxoplasma* infection in virtually all vertebrates. Thus, understanding the IFN**γ**-induced immune factors that control chronic infection in pigs is essential for developing One Health strategies to reduce the prevalence of *Toxoplasma* in pigs, in turn to reduce the disease burden in humans.
Here, we aim to identify the IFN**γ**-induced immune responses to *Toxoplasma* in porcine skeletal muscle cells (SkMCs) and neurons. To do this, SkMCs or neurons will be stimulated with IFN**γ** and infected with *Toxoplasma*. We will then perform RNA sequencing to compare the differential expression of interferon-stimulated genes (ISGs) during different infection stages, acute and chronic. ISGs that show significant changes will be confirmed by targeted knockout and/or overexpression in SkMCs and neurons followed by detailed functional characterization. We anticipate that this study will provide novel insights into host-parasite interactions that will be relevant to both pigs and humans.

OH10- Prevalence of anti-*Toxoplasma gondii* antibodies in slaughterhouse workers in the state of Paraíba, Brazil: epidemiological assessment and occupational risks

Vilela V. L. R.[1]*, Silva J. O.[1], Batista S. P.[2], Matos T. S.[3], Bison I.[3], Parentoni R. N.[3], Santos J.
R. S.[3], Brasil A. W. L.[1], Feitosa T. F.[1]

[1] *Federal Institute of Paraíba – IFPB, Sousa, Brazil.*
[2] *Federal University of Campina Grande – UFCG, Patos, Brazil.*
[3] *Federal University of Paraíba – UFPB, João Pessoa, Brazil.*

*Presenting author: vilelavlr@gmail.com

Toxoplasmosis, a zoonosis widely distributed around the world, is caused by the protozoan *Toxoplasma gondii* and can affect humans through various transmission routes. Slaughterhouse workers, due to the nature of their activities, are considered a high-risk group because of their constant exposure to contaminated biological materials. Therefore, understanding the factors associated with *T. gondii* infection in vulnerable populations, such as these professionals, is essential for the implementation of preventive measures and health education. This study aimed to assess the prevalence of anti-*T. gondii* antibodies in slaughterhouse workers in the state of Paraíba, Brazil, and the possible factors associated with infection by this protozoan. Blood samples were collected from 170 slaughterhouse workers from six municipalities in the state, and during the collections, an epidemiological questionnaire was applied to the study participants. The samples were identified, refrigerated, and sent for serological analysis through the Immunofluorescent Antibody Test (IFAT). The prevalence of anti-*T. gondii* IgG among the workers was 77.6% (132/170; 95% CI: ±0.752), with titers ranging from 1:16 to 1:8192. The factors associated with infection included low education levels (illiterate: odds ratio (OR) 1.51; 95% CI 1.08–2.11; elementary education: OR 1.46; 95% CI 1.09–1.95) and difficulties in conceiving children (OR 1.23; 95% CI: 1.06–1.42). We concluded that there is a high seropositivity for anti-*T. gondii* antibodies among slaughterhouse workers in the state of Paraíba and that their workplace may pose a greater risk as they are directly exposed to contaminated tissues. Factors such as low education levels and difficulties in conceiving children underscore the need for preventive measures and workplace health education.

OH11- Analysis of free-living seabirds from Brazil as potential hosts of *Toxoplasma gondii*

Acosta I.[1], Chiebao D.[2]*, Serafini P.[3], Canani G.[4], Pena H.[5], Campolina C.[1], Marques C.[6], Neves T.[6], Efe M.[7], Nunes G.[8], Labruna M.[5], Bugoni L.[1]

[1] *Universidade Federal do Rio Grande, Rio Grande, Brazil*
[2] *Instituto Biológico, Sao Paulo, Brazil*
[3] *Universidade Federal de Santa Catarina, Florianópolis, Brazil*
[4] *Instituto Albatroz, Santos, Brazil*
[5] *Universidade de Sao Paulo, Sao Paulo,Brazil*
[6] *Instituto Albatroz, Santos, Brazil*
[7] *Universidade Federal de Alagoas, Maceio, Brazil*
[8] *Universidade Federal do Rio Grande do Sul, Imbe, Brazil*

*Presenting author: daniela.chiebao@sp.gov.br

Dispersal patterns of zoonotic pathogens can be strongly influenced by mobility and contact among hosts. *Toxoplasma gondii* infection has been documented in many avian species, however, there is little information regarding free-living seabird populations. The continental shelf of the southwestern Atlantic Ocean is a foraging area for seabirds that breed locally, as well as migratory seabirds wintering in the area, which may come into contact with each other in prey aggregation areas. Therefore, this study aimed to investigate the prevalence of this important zoonotic pathogen in free-living seabirds, including species that nest within sheltered or protected populations of domestic cats and houses introduced exotic rodents, as well as migratory visitors sampled at sea. Blood samples were collected from 322 birds of three local breeders (*Phaethon aethereus, Sula leucogaster* and *S. dactylatra*) in the eastern coast of Brazil *(Abrolhos archipelago*), and two migratory species using the area during the pre-laying (*Pterodroma arminjoniana*) and the non-breeding periods (*Thalassarche chlororhynchos*). Serological agglutination test for detection of anti-*Toxoplasma gondii* antibodies was performed. From the seabirds in this study, 34.5% (n = 111)

presented antibodies anti-*T. gondii*. Antibody titers in seropositive birds ranged from 10 to 640. There were seropositive birds in all sampled localities. This study provides the first records for *P. arminjoniana* and *T. chlororhynchos* as seropositive to *T. gondii*, suggesting their potential role as sentinels for the environmental contamination by *T. gondii* and also *T. gondii* infection. These findings indicate the circulation of the parasite in the Brazilian coastal and oceanic regions, probably due to the ingestion of *T. gondii* oocysts by the birds. The epidemiological involvement of migratory birds as hosts of pathogens, as well as the role of the historical introduction of human occupation on Brazilian islands could be related.

OH12- Enhancing One Health outcomes: the importance of data structure and distribution

Helman E.[1]*, Bernstein M.[1], Rozenblit V.[2], Flaibani N.[2], González Garello T.[2], Runco M.[1]; Fernández M. S.[2], Pardini L.[1], Unzaga J. M.[1]

[1] *Laboratorio de Inmunoparasitología (LAINPA), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata (FCV-UNLP), La Plata, Argentina*
[2] *Grupo de Bioestadística Aplicada, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina*

*Presenting authors: elisahelman@gmail.com

Effective implementation of the One Health concept requires rigorous analysis of epidemiological data. However, veterinary epidemiology often overlooks the nature and distribution of the data collected. This study compares three analytical approaches for evaluating the replication capacity of five *Toxoplasma gondii* strains (RH, ME49, VEG, TgMr, TgSb). The response variable was the number of replicated parasites, with three assays and three subsamples per assay conducted for strain. The three models evaluated were: A) a linear model assuming complete independence between subsamples, B) a linear model

averaging the number of parasites per assay, and C) a generalized linear mixed model with a Poisson distribution for count data. To address the clustering of data, we employed two strategies: for model B we averaged the subsamples for assay; meanwhile for model C, the subsamples were included as random effects. Results showed that model A detected significant differences in parasite replication across all strains (p-value<0.05), whereas models B and C identified significant differences in all strains except TgSb (p-value>0.05). The omission of clustering in Model A increased the probability of committing a type I error due to pseudoreplication. Furthermore, both models A and B demonstrated heteroscedasticity -variability in error variance across levels of explanatory variables (strain types)– violating a core assumption of general linear models and leading to inaccurate variability estimates and heightened risks of type I and type II errors. In contrast, model C managed data structure and distribution effectively, reducing the risks of pseudoreplication and providing more accurate variance estimates. This study highlights the importance of reflecting experimental design in data modeling to minimize type I and II errors, thereby strengthening the validity of epidemiological conclusions. Adequate modeling is crucial for sound outcomes in One Health.

SESSION 2

Epidemiology and economic impact

Keynote. Apicomplexan parasites associated with reproductive losses in South America

Cantón, G. J.

Instituto de Innovación para la Producción Agropecuaria y el Desarrollo Sostenible (IPADS, CONICET-INTA Balcarce), Buenos Aires, Argentina.

canton.german@inta.gob.ar

Ruminant production systems are key for the development of South American countries. Cattle stock in South America represent ~25% of the world population. Furthermore, small ruminant (sheep and goats) production systems is carried out in varying farming systems, including transhumant conditions. Wild and domestic South American camelids also represent an important income in some regions. Water buffaloes (*Bubalus bubalis*) also represent an important ruminant specie in some producing countries of South America. Farmed and wild deer species are also relevant in the region. Nevertheless, there is scarce information about the reproductive efficiency of these production systems. The causes of reproductive losses usually remained underdiagnosed under extensive conditions systems. *Toxoplasma gondii* and *Neospora caninum* have been diagnosed as causes of abortion/perinatal death in small ruminants (sheep and goats) in Argentina, Brazil and Uruguay, and remain as possible causes of abortions in Chile, Colombia, Peru and Venezuela. Contrastingly, some studies showed that *T. gondii* was the main cause of sheep abortion in Uruguay, and *N. caninum* the main cause of abortion in sheep of Argentina. *Sarcocystis* spp. was confirmed as a cause of abortion in sheep only in Brazil, and could be consider as a

possible cause in Argentina, Peru and Uruguay. There are no reports of *T. gondii* and *Sarcocystis*-abortion in cattle from South America. On the contrary, *N. caninum* was one of the main causes of abortion/perinatal death in cattle from Argentina, Brazil, Chile, Uruguay and Venezuela. *N. caninum* was confirmed as the cause of abortion in different camelids of Peru and there is a suspicion of abortion related with *T. gondii* in guanacos from Argentina. No reports of spontaneous water buffaloes abortion associated with apicomplexan parasites were described in the region. There is only reports of *N. caninum* abortion in farmed and zoo deer in Argentina. Although toxoplasmosis, neosporosis and sarcocystosis abortions have been confirmed in different ruminant species in the region, their impact on animal and public health, and the economy of the livestock sector are largely underestimated. Major limitations for identifying causes of abortion are associated with difficulty of acquiring quality samples for laboratory investigation under extensive field conditions; lack of access to reliable veterinary laboratories in remote or marginal areas; financial and strategic limitations for disease monitoring and surveillance programs.

ORAL PRESENTATIONS

EEI1- Confirmation of the dog (*Canis lupus familiaris*) as definitive host of *Sarcocystis aucheniae* under natural conditions by microscopy and molecular studies

Vargas Tacuri C.[1], Giuliano S.[2], Malaga J. I.[1], Chavez-Fumagalli M.[1], Florin-Christensen M.[3]*, Reategui Ordoñez J.[1]

[1]*Universidad Católica Santa María, Arequipa, Perú*
[2]*Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Argentina*

[3]*Instituto de Patobiologia Veterinaria, INTA-CONICET, Hurlingham, Argentina*

*Presenting author: jacobsen.monica@inta.gob.ar

Sarcocystis aucheniae is a coccidian parasite that uses South American camelids as intermediate hosts, yielding macroscopic sarcocysts in their skeletal muscles. The commercialization of llama, alpaca, and guanaco meat constitutes an important regional resource in several South American countries. Yet, it is hampered by the disagreeable aspect conferred to meat by *S. aucheniae* sarcocysts, which leads to the depreciation or confiscation of carcasses. Dogs have been described as definitive hosts of this parasite, based on observing oocysts in their intestinal mucosa or sporocysts in feces after feeding them with sarcocysts or sarcocyst-infected meat. However, no species molecular identification was carried out in these studies. Moreover, naturally infected dogs that could act as disseminators of the parasite in the field have not been reported. In this study, fecal samples of dogs from alpaca-breeding regions of Arequipa and Puno, Peru (n=46), were collected and subjected to sucrose flotation. Samples from the top of the tube were recovered on cover slides and microscopically observed (100X and 400X). Putative *Sarcocystis* sp. sporocysts were observed in one fecal sample from a female, adult dog from Puno. The whole sample was processed and the resultant sporocyst suspension was counted in a Neubauer chamber, yielding ~50 sporocysts per gram of feces. Genomic DNA was extracted using a commercial kit and used as template to amplify an S. *aucheniae*- specific fragment of the 18S rRNA gene by seminested PCR. An amplification band of the expected size was obtained, verifying the identity of the parasite. Notably, the excretion of sporocysts in the feces of the same dog was still detected two weeks later, suggesting that sporocyst dissemination in the environment by an infected dog is a long-lasting phenomenon. These results constitute the first confirmation of dogs as *S. aucheniae* definitive hosts under natural conditions.

EEI2- *Sarcocystis* spp. and *Toxoplasma gondii* in muscles from wild boars (*Sus scrofa*) in Switzerland

Moré G.[1]*, Filippini C. [1], Oehm A. [2], Ruetten M. [3], Frey C. F. [1], Basso W. [1]

[1] *Institute of Parasitology, University of Bern, Bern, Switzerland*
[2] *Institute of Parasitology, University of Zurich, Zurich, Switzerland*
[3] *PathoVet AG, Tagelswangen, Switzerland*

*Presenting author: gastonmore@gmail.com

Sarcocystis spp. and *Toxoplasma gondii* are heteroxenous protozoan parasites, which form tissue cysts in intermediate hosts (IH) and oocysts in the intestinal mucosa of definitive hosts (DH). Wild boars (*Sus scrofa*) are IH for *Sarcocystis miescheriana* (with canids and racoons as DH), *Sarcocystis suihominis* (with humans and other primates as DH) and for *T. gondii* (with felids as DH). The aim of this study was to identify and characterize *Sarcocystis* spp. and *T. gondii* in muscles from wild boars hunted for consumption in Switzerland. DNA was extracted from muscles of 286 wild boars and tested by a PCR targeting the 18S rRNA gene of *Sarcocystis* spp., the mitochondrial cytochrome c oxidase (coxI) gene of *S. suihominis* and by qPCR for *T. gondii*. Besides, 225 samples were processed by homogenization and direct microscopic examination, and 42 samples by histopathology. *Sarcocysts* were observed in 89.3% (201/225) and 35.7% (15/42) of the samples, respectively. By the 18S rRNA PCR, 91.25% (261/286) samples resulted positive, and all 62 sequences obtained were 100% identical among them and with GenBank sequences reported as *S. miescheriana*. The *S. suihominis*-coxI PCR was positive in 1.75% of samples (5/286), all five from animals hunted in Switzerland. A total of six coxI sequences (772bp primers trimmed) were obtained, being 97.3-99.6% similar among them and with a 97.5-99.8% identity with a sequence reported as *S.suihominis*. Despite the identity differences, all these sequences positioned together

in a phylogenetic tree, in a sister clade of *S. miescheriana* sequences. All samples resulted negative by *T. gondii* qPCR. A high prevalence of *Sarcocystis* spp. infection was observed and *S. miescheriana* was the most frequently detected species, suggesting a frequent predator-prey interaction between wild boars and canids/racoons. This study is the first to show the presence of *S. suihominis* in wild boars hunted in Switzerland.

E-POSTERS

EEI3- Estimation of direct economic and productive losses due to abortions caused by *Neospora caninum* in the primary dairy sector of Uruguay

Carrillo Parraguez M.[1,2,†*], Ponssa E.[3,†], Caffarena D.[1,4], Artagaveytia J.[5], Sotelo F.[6], Fariña S.[7], Mendoza A.[7,††], Giannitti F.[1,††]

[1]*Plataforma de Investigación en Salud Animal, Instituto Nacional de Investigación Agropecuaria (INIA), Estación Experimental La Estanzuela, Ruta 50 km 11, Colonia, 70006, Uruguay.*
[2]*Escuela de Medicina Veterinaria, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Concepción, Talcahuano, 4260000, Chile.*
[3]*Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Tandil, Buenos Aires, 7000, Argentina.*
[4]*Unidad Académica Salud de los Rumiantes, Departamento de Producción Animal, Facultad de Veterinaria, Universidad de la República, Montevideo, 13000, Uruguay.*
[5]*Instituto Nacional de la Leche (INALE), Montevideo, 11700, Uruguay.*
[6]*Instituto Nacional para el Mejoramiento Lechero (MU), Montevideo, 11800, Uruguay.*

[7]*Sistema Lechero, Instituto Nacional de Investigación Agropecuaria (INIA), Estación Experimental La Estanzuela, Ruta 50 km 11, Colonia, 70006, Uruguay.*
[†]*Equal contribution and share first authorship.* [††]*Equal contribution and share last authorship.*

*Presenting author: miguelfelipe.carrillo@gmail.com

Neospora caninum is one of the main abortive pathogens in dairy cattle in Uruguay; however, the economic losses caused by neosporosis in this country are unknown. The aim of this work was to estimate the direct annual economic losses for the primary dairy sector due to abortions caused by neosporosis in the Uruguayan herd. A bioeconomic model was used to estimate economic losses from second and third gestational trimester abortions, considering the present value of the future production that is lost, after deducting the costs of production (loss of profits). The average economic loss from an abortion due to neosporosis was US$ 868 if the aborting cow was retained in the herd, and US$ 1,866 if it was culled. Individual losses varied depending on the stage of gestation and the age of the aborting cow, the worst scenario being third semester abortion occurring in first-pregnancy heifers. The annual number of abortions was estimated based on the stock of cows and heifers, national seroprevalence of *N. caninum* in dairy herds, rates of vertical and horizontal transmission and different abortion risks. The losses at national level were calculated by multiplying the individual losses by the number of abortions distributed across different simulated scenarios and weighted according to a proportion of subscenarios (with and without culling of an aborting cow). The estimated economic losses reached nearly US$ 12 million. In physical terms, abortions resulted in a total loss of 62 million liters of milk, 3.3% of the volume industrialized annually in the country. The characteristics of the grazing dairy production system of Uruguay requires a specific approach in estimating the economic impact of bovine diseases. This information can be used

to evaluate the cost-benefit of the implementation of control and/or prevention strategies for bovine neosporosis on a farm or national scale.

EEI4- Natural infection with *besnoitia akodoni* (protozoa: Sarcocystidae) in synanthropic (Muridae) and wild (Cricetidae) rodents of Argentina

Bentancourt Rossoli J. V.[1,2,*], Moré G.[2,3], Soto-Cabrera A.[2,4], Moore D. P.[2,4], Morrell E. L.[4], Campero L. m.[2,4], Basso W.[3], Pedrana J.[2], Hecker Y. P.[2,5], Scioscia N. P.[1,2]

[1]*Instituto de Investigaciones en Producción, Sanidad y Ambiente (IIPROSAM), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata (FCEyN-UNMdP), Mar del Plata, Buenos Aires, Argentina*
[2]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina*
[3]*Institute of Parasitology, Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland*
[4]*Instituto de Innovación para la Producción Agropecuaria y el Desarrollo Sostenible (IPADS Balcarce), Balcarce, Argentina*
[5]*Grupo SALUVET, Facultad de Veterinaria, Universidad Complutense de Madrid, Madrid, España*

*Presenting author: judithbentancourt@mdp.edu.ar

Species of *Besnoitia* are cyst-forming parasites within the Sarcocystidae family (Apicomplexa), affecting mammals, birds, and reptiles globally. The objective of this study was to identify *Besnoitia spp*. in rodents captured on dairy cattle farms in the Humid Pampa of Argentina. A total of 356 rodents were captured, 212 belonging to the family Muridae and 144 to the family Cricetidae. Samples from the brain, masseter muscle, tongue, heart, semitendinosus muscle, and liver, were collected and analyzed by histopathology and molecular assessment. Additional

samples from other tissues were collected when macroscopic cysts compatible with *Besnoitia spp*. were observed. Histopathology results revealed cysts in 2% (7/356) of the rodents, with their presence detected mostly in the semitendinosus muscle, followed by the masseter, tongue, heart, liver, pleural cavity, testes, and brain. These samples were further analyzed by PCR targeting the ITS-1 marker and flanking regions, and the 18S rRNA and CoxI gene fragments. ITS-1 PCR products were obtained in all seven samples with varying sizes of 448-500 bp with a 100% identity with *B. akodoni*, previously identified in Akodon montensis from Brazil. The 18S rRNA sequences from five samples matched 100% with *Besnoitia* sp. from Chile and *B. jellisoni* sequences, and 99.27% with *B. darlingi* and *B. oryctofelisi* sequences. The CoxI sequences (n = 4), showed only 92-93% similarity to an uncharacterized *B. besnoiti* protein. This is the first study to identify *B. akodoni* in naturally infected *Mus musculus*, *Oxymycterus rufus*, *Necromys spp*., and *Akodon azarae*. Previous phylogenetic analyses suggested that felines could be definitive host. However, coprological and molecular studies on potential definitive hosts present in the region would be necessary to confirm this hypothesis and clarify the transmission cycle.

EEI5- *Neospora caninum* and *Toxoplasma gondii* in naturally infected synanthropic (Muridae) and wild (Cricetidae) rodents from Argentina

Bentancourt Rossoli J. V. [1,2]*, Campero L. M.[2,4], Moré G.[2,3], Soto-Cabrera A. [2,4], Moore D. P. [2,4], Basso W.[3], Morrell E. L.[4], Pedrana J.[2], Hecker Y. P.[2,5], Scioscia N. P.[1,2]

[1]*Instituto de Investigaciones en Producción, Sanidad y Ambiente (IIPROSAM), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata (FCEyN-UNMdP), Argentina*
[2]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina*
[3]*Institute of Parasitology, Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Switzerland*

[4]*Instituto de Innovación para la Producción Agropecuaria y el Desarrollo Sostenible (IPADS Balcarce), Ruta 226 Km 73,5, Balcarce, Buenos Aires, Argentina*
[5]*Grupo SALUVET, Facultad de Veterinaria, Avenida, Universidad Complutense de Madrid, Madrid, España.*

*Presenting author: judithbentancourt@mdp.edu.ar

Neospora caninum and *Toxoplasma gondii* are coccidian parasites (Phylum Apicomplexa) with canids and felids serving as their definitive hosts, respectively. Numerous studies worldwide have reported natural infections by these parasites in rodents. However, there is limited information available regarding the presence of these protozoans in rodents in our country. The objective of the present study was to detect *T. gondii* and *N. caninum* infections in rodents captured on dairy cattle farms in the Humid Pampa of Argentina. A total of 356 rodents were captured, including 212 from the family Muridae and 144 from the family Cricetidae, on dairy farms in three localities in the southeast of Buenos Aires province. Histopathological, serological and molecular analysis were performed. No cysts of either parasite was found in histological sections of organs and muscles. However, using IFAT, 1.4% (5/356) of the animals were seropositive for *N. caninum* (3/193 *Mus musculus*; 1/58 *Oxymycterus rufus*; 1/41 *Necromys* spp.) and 9.6% (34/356) were seropositive for *T. gondii* (19/193 *M. musculus*; 9/58 *O. rufus*; 3/41 *Necromys* spp.; 2/29 *Akodon azarae*; 1/11 *Oligoryzomys flavescens*). Only one individual (*M. musculus*) was positive for antibodies against both parasites. DNA was extracted from the brains of these 41 rodents. Using conventional PCR, *T. gondii* was detected in 9 out of 41 (22%) samples (5 *O. rufus*, 2 *Necromys* spp., 1 *A. azarae*, and 1 *M. musculus*), while *N. caninum* was negative in all samples. These results suggest that rodents may not play a role in the life cycle of *N. caninum* in dairy farms, whereas they could potentially act as reservoirs for *T. gondii*. This study represents the first investigation of *N. caninum* and *T. gondii* in cricetid

rodents from Argentina and adds to the existing knowledge on these parasites in murine rodents.

EEI6- Detection of *Toxoplasma gondii* in retail meat from São Paulo megacity, Brazil

Pontes Chiebao D.[1]*, Amaku M.[2], Miyagi S. A.[2], Wu X.[3], Catalano S.[3], Stewart C.[4], Smith D.[3], Burgess S.[3], Ewing D.[5], Innes E.[3], Hamilton C.[3], Pena H.[2]

[1]*Instituto Biologico, Sao Paulo, Brazil;*
[2]*University of Sao Paulo, Sao Paulo, Brazil;*
[3]*Moredun Research Institute, Midlothian, UK;*
[4]*Newcastle University, Newcastle, UK;*
[5]*Biomathematics and Statistics Scotland (BioSS), Edinburgh, UK*

*Presenting author: daniela.chiebao@sp.gov.br

Toxoplasmosis is recognized as a major foodborne disease transmitted by different routes, including the ingestion of *Toxoplasma gondii* cysts within raw or undercooked infected meat. Detection of *T. gondii* specific antibodies among humans shows a high prevalence in Brazil and can be associated with severe clinical symptoms. Brazil is one of the top 10 countries for meat consumption per capita. To address the gap in knowledge about the risk of human infection from retail meat, this study aimed to verify the prevalence of parasite DNA in three popular meat cuts consumed in a large urban location, Sao Paulo megacity, comparing the occurrence over five different macro regions and three types of commercial retailers. Fresh cuts of chuck, loin and thighs, each from cattle, pig and chicken, respectively, were analyzed using qPCR targetting the 529bp repeat element of *T. gondii* and myostatin was used for the internal control. The Exact Fisher´s test was used to compare proportions of positive results with 95% confidence level. After sampling over 3 year periods, 958 samples were obtained from five regions, 257,

322 and 379 from cattle, pig and chicken, respectively, purchased at 461 establishments (butcher shops, street markets and grocery stores). The quantitative analysis demonstrated 18 (1.9%) positive samples, being three, seven and eight from cattle, pig and chicken, respectively, with significant result regarding the city region (p=0.019), associated with the West Zone of Sao Paulo, which showed a higher proportion of positive samples (50% of all positives). These results suggest low probability of infection by *T. gondii* for consumers preferring to use these meat cuts and the prevalence of detection was comparable to previous surveys from other regions. Understanding the key transmission routes and risks associated with food-borne *T. gondii* infection will help in the design and implementation of prevention and control programs.

EEI7- Molecular detection of agents transmitted by vector arthropods in buffalos (*Bubalus bubalis*) and associated ectoparasites in southeastern Brazil

Tostes Secato C.[1], Gonçalves L. R.[1], Calchi A. C.[1], de Souza Ramos I. A.[1], da Silva T. M.[1]; Lemos W.[1], Zacarias Machado R.[1]*, André M. R.[1]

[1]*Vector-borne Bioagents Laboratory (VBBL), Department of Pathology, Reproduction and One Health (DPRSU), School of Agrarian and Veterinary Sciences (FCAV), São Paulo State University (UNESP), Jaboticabal, SP, Brazil*

*Presenting author: rzacariasmachado@gmail.com

The high occurrence of tick-borne diseases in ruminants has impaired the development of livestock-rearing in Brazil, which has one of the largest buffalo herds in Latin America. The aim of the present study was to investigate, though molecular methods, the molecular occurrence of piroplasmids (Anaplasmataceae) in buffalos in southeastern Brazil. DNA was extracted from blood samples from 81 buffalos and from 127 specimens of the tick *Rhipicephalus microplus* (113 adults, 14 nymphs

and one larva) and 92 specimens of the louse *Haematopinus tuberculatus*, which were collected from buffalos in the municipality of Passos, state of Minas Gerais. These samples were subjected to conventional PCR assaying (cPCR) based on the endogenous gene gapdh of mammals, 16S rRNA of ticks and cox-1 of invertebrates (for lice). The positive samples were subjected to PCR assays to *Ehrlichia* spp. (gene dsb) and nested PCR assaying directed towards the genes 18S rRNA of Piroplasmida, sbp-2 of *Babesia bovis* and rap-1α of *Babesia bigemina*. These were followed by sequencing of the amplicons and analysis using BLASTn. All the blood samples and ectoparasites of the buffalos were positive for the endogenous genes in the PCR assays. All the samples were found to be negative for *B. bigemina* (rap-1α) in nPCR. Regarding piroplasmids, 8/81 (9.9%) of the buffalo blood samples were positive, and the BLASTn analysis showed that these had > 99.9% similarity to B. bovis in one sample and to *B. bigemina* in seven samples; and one female adult tick (0.7%) was positive in the referred PCR, with 99.70% similarity to *B. bigemina*. In nPCR for *B. bovis* (spb-2), 1/81 (1.2%) of the buffalo samples was found to be positive. The present study demonstrated, for the first time, the occurrence of a diversity of blood parasites in buffalos and associated ectoparasites, in southeastern Brazil.

EEI8- Molecular occurrence of *Theileria parva* in cattle and wild buffalos in Mozambique

Tostes Secato C.[1], Matos C.A.[1], Geraldes Teixeira M. M.[2], Pereira C. L.[3], Gonçalves L.R.[4], Zacarias Machado R.[5]*, André M. R.[5]

[1]*Vector-borne Bioagents Laboratory (VBBL), Department of Pathology, Reproduction and One Health (DPRSU), School of Agrarian and Veterinary Sciences (FCAV), São Paulo State University (UNESP), Jaboticabal, SP, Brazil.*
[2]*Institute of Biomedical Sciences (ICB), University of São Paulo (USP), São Paulo, SP, Brazil.*
[3]*National Administration of Conservation Areas, Maputo, Mozambique.*

[4]*Imunodot Diagnósticos Veterinários, Rua Dr. Mario de Campos 1150, Jardim São Marcos I, CEP 14887-200, Jaboticabal, SP, Brazil.*
[5]*Vector-borne Bioagents Laboratory (VBBL), Department of Pathology, Reproduction and One Health (DPRSU), School of Agrarian and Veterinary Sciences (FCAV), São Paulo State University (UNESP), Jaboticabal, SP, Brazil.*

*Presenting author: rzacariasmachado@gmail.com

Mozambique is prominent among the countries that suffer most from economic losses due to pathogens transmitted by ticks, particularly the causative agents of theileriosis, ehrlichiosis, anaplasmosis and babesiosis. Theilerioses are diseases that cause great losses within livestock-rearing in tropical regions of the world and are associated with high mortality, diminished meat and milk production and expenditure on treatment. *Theileria parva*, *T. annulata*, *T. taurotragi*, *T. mutans*, *T. velifera* and *T. orientalis/T. buffeli* are species known to infect cattle, but with different levels of pathogenicity. The present study had the aim of detecting, through molecular methods, the occurrence of Theileria parva in cattle and wild buffalos (*Syncerus caffer*) sampled in Mozambique. DNA samples were extracted from blood from 89 wild buffalos (*Syncerus caffer*) (Marromeu Reserve, Sofala Province) and 219 cattle (districts of Boane, Magude, Matutuíne, Moamba and Namaacha, in Maputo). These were subjected to conventional PCR assaying (cPCR) with the aim of amplifying the endogenous gene gapdh of mammals. Samples that were positive through this cPCR were then subjected to real-time PCR assaying (qPCR) aimed at the 18S rRNA gene of *T. parva*. All the samples were found to be positive for the endogenous gene through cPCR. All the blood samples from cattle were negative for detection of *T. parva* through qPCR, while 25/89 (28.1%) of the samples from buffalos were positive. The number of copies of a fragment of the 18S rRNA gene of *T. parva* per microliter ranged from 2.41 x 100 to 1.08 x 102. The efficiency, R2, slope and Y-intercept of the reactions ranged from 91.6% to 94.3% (mean of 92.6%), -3.541 to -3.467 (mean of -3.513),

0.992 to 0.999 (mean of 0.997) and 36.401 to 37.919 (mean of 37.092), respectively, in accordance with the recommendations of the MIQE guidelines. These results from the present study demonstrate the importance that wild buffalos have as reservoirs of *T. parva*.

EEI9- Bovine coccidiosis: retrospective study of 63 outbreaks at INTA Balcarce (1998-2024)

Vilatuña E.[1]*, Ovelar M. F.[1], Azaldegui I.[1], Lloberas M. M.[1], García J. A.[1], Cantón G.[1]

Institute of Innovation for Agricultural Production and Sustainable Development (IPADS) (INTA Balcarce - CONICET), Argentina.

*Presenting author: nutricionvet1@gmail.com

Coccidiosis is caused by protozoa of the genus *Eimeria* spp. causing gastrointestinal disease in young cattle. This study evaluates the epidemiology, clinical and pathological findings of 63 outbreaks of bovine coccidiosis in Central Argentina, recorded at INTA Balcarce between 1998 and 2024. Anamnesis, coproparasitological and pathological information was assessed. Thirty-five autopsies were performed, and tissue samples were collected in 10% formalin solution for histopathological studies. Most of the outbreaks occurred in calves in cow-calf systems (72.1%), followed by feedlot (16.4%), pastoral wintering (9.8%) and dairy farms (1.6%). The affected calves were 1 to 4 (16.1%), 5 to 8 (55.4%) and 9 to 12 (28,6%) months-old. The average morbidity was 5%, with a mortality rate of 2.0% and a fatality rate of 60%. Diarrhea was observed in 81.6% of outbreaks, of which 60.0% were bloody. Associated neurological signs were also recorded in 53.3% of outbreaks. Other frequent clinical findings were weakness (15.0%) and sudden death (13.3%). In 10.0% of cases only neurological signs were recorded, without previously observing gastrointestinal signs. In 69.2% of the fecal samples, OPG was higher than 10,000; in 21.1% the count was lower

than 10,000 OPG and in 9.6% it was negative (later confirmed by pathological studies). In all autopsies, compatible lesions were registered: intestinal congestion (77.1%), thickening of the intestinal mucosa (22.9%), catarrhal (40.0%) or fibrinous enteritis (11.4%). Severe diffuse interstitial eosinophilic and histiocytic enterocolitis with necrotizing cryptitis, presence of intracellular and extracellular parasites morphologically compatible with *Eimeria* spp. were observed in all cases. This study reinforced the importance of an integrated diagnostic approach since OPG is not sensitive enough to confirm the initial presumptive diagnosis. Furthermore, the importance of including coccidiosis as a presumptive clinical diagnosis in young cattle showing compatible neurological signs.

EEI10- Cryptosporidium spp. in outbreaks of bovine neonatal diarrhea registered at INTA Balcarce (2001-2023)

Vilatuña E.[1], Louge Uriarte E. L.[1], Recalt A. V.[1], Fiorentino M. A.[1], Verna A.[1], Cantón G.[1]*

[1]*Institute of Innovation for Agricultural Production and Sustainable Development (IPADS). (INTA Balcarce - CONICET), Argentina.*

*Presenting author: canton.german@inta.gob.ar

Cryptosporidium spp. is a common zoonotic parasitosis. *Cryptosporidium parvum* is associated with clinical disease in neonatal calves being a common agent detected in cases of neonatal diarrhea. The objective of this work is to retrospectively evaluate the presence of *Cryptosporidium* spp. in outbreaks of neonatal diarrhea (0 to 20 days-old) in calves registered at INTA Balcarce between 2001 and 2023. Anamnesis information was revised, including geographical location, production system (dairy or beef), among others. During this period, 121 outbreaks of neonatal diarrhea were registered, and 291 samples were assessed for the presence of microorganisms associated with this

syndrome, including *Cryptosporidium* spp., by modified Ziehl- Neelsen staining or commercial lateral flow immunochromatographic assay (BoviD-5, Bionote Inc.). The most frequent pathogens detected in these 291 fecal samples were *Escherichia coli* (61.9%), *Cryptosporidium* (43.6%), rotavirus (30.6%), *Salmonella* (14.4%), coronavirus (2.1%) and *Eimeria* spp. (1.4%). In 49/121 outbreaks (40.5%), *Cryptosporidium* spp. was detected in fecal samples and associated with clinical outcome. Outbreaks where *Cryptosporidium* spp. was detected occurred in Buenos Aires (93.9%), Córdoba (4.1%) and Santa Fe (2.0%) provinces, in dairy (69.4%) or beef farms (26.5%) (no data available in 4.1% outbreaks). Outbreaks where *Cryptosporidium* spp. was confirmed affected calves of 1-5 (22.4%), 6-10 (55.1%), 11-15 (12.2%) and 16-20 (10.2%) days old. These results showed that *Crypstosporidium* spp. is the second more frequent pathogen associated with neonatal diarrhea syndrome in the first two weeks of age of calves in dairy and beef farms included in this retrospective study in Central Argentina.

EEI11- Sheep, *Toxoplasma*, and genetic diversity: insights from Uruguay

Tana-Hernandez L.[1], Valentin A.[1], Cabrera A.[2], Dorsch M.[3], Fierro S.[4], Giannitti F.[3], Arranz-Solis D.[5], Alvarez-García G.[5], Francia M.E.[1,2]

[1]*Laboratory of Apicomplexan Biology, Institut Pasteur de Montevideo, Uruguay.*
[2]*Department of Parasitology and Mycology, Faculty of Medicine, Institute of Hygiene, University of the Republic, Uruguay*
[3]*Animal Health Research Platform, National Institute of Agricultural Research (INIA), La Estanzuela Experimental Station, Colonia, Uruguay.*
[4]*Uruguayan Wool Secretariat (SUL), Uruguay.*
[5]*SALUVET, Animal Health Department, Complutense University of Madrid, Spain*

*Presenting author: ltana@pasteur.edu.uy

Toxoplasmosis is a globally prevalent foodborne zoonotic disease that significantly impacts public health and causes economic losses, especially in countries reliant on livestock industries. Sheep and goats are notably affected, often experiencing reproductive failures. Uruguay, a major exporter of sheep meat and combed wool, grapples with suboptimal reproductive performance in its sheep flocks. Previous studies identified that 27% of sheep abortions were attributable to *Toxoplasma gondii* among analyzed aborted fetuses and placentas. Despite this, comprehensive data on the parasite's prevalence and genotypes in Uruguayan sheep were lacking. This study aimed to: assess *T. gondii* seroprevalence using an in-house ELISA assay; elucidate prevailing serotypes and genotypes through molecular and serological typing; and characterize the impact of genetic diversity on parasite phenotype. Results revealed that 17% (636/3744) of sheep were seropositive for *T. gondii*, with significant genetic variability observed in strains from aborted fetuses. Predominantly, type III strains were detected, followed by types II and I, alongside atypical and previously unreported genotypes. Two strains, TgUru1 and TgUru2, isolated from aborted sheep, exhibited distinct characteristics. TgUru1 demonstrated high virulence and rapid growth, whereas TgUru2 showed slow growth with high rates of spontaneous cyst formation. These phenotypic differences were consistent in both in vivo and in vitro analyses and correlated with gene-specific expression patterns during developmental stages. Ongoing research focuses on the genetic and transcriptomic foundations of these variations to understand their implications on disease outcomes. Furthermore, the study extends to examining the correlation between these animal findings and the human population in Uruguay concerning the prevalent strain's genetic background. Adopting a One-Health approach, this research seeks to comprehensively understand the dynamics between *T. gondii*, livestock, and potential human health implications in the region.

EEI12- *Toxoplasma gondii* and *Neospora caninum* in ruminants from Somalia

Kakimori M. T. A.[1], Osman A. M.[2], Santos Silva A. C.[1], Ibrahim A. M.[3], Shair M. A.[3], Cavallieri A. C.[1], Barros L. D.[4]*, Garcia J. L.[1], Jayme Vieira T. S. W.[5], Hassan-Kadle A. A.[3], da Costa Vieira R. F.[5]

[1]*Universidade Estadual de Londrina, Londrina, Brazil*
[2]*Universidade Federal do Paraná, Curitiba, Brazil*
[3]*Abrar University, Mogadishu, Somalia*
[4]*Universidade Federal de Lavras, Lavras, Brazil*
[5]*The University of North Carolina at Charlotte, Charlotte, USA*

*Presenting author: luiz.barros@ufla.br

Toxoplasmosis and neosporosis are parasitic diseases caused by the protozoan *Toxoplasma gondii* and *Neospora caninum*, respectively. These parasites have worldwide distribution and are associated with reproductive disorders in livestock. Additionally, toxoplasmosis is one of the most important zoonotic diseases, being a significant public health issue. In Somalia, epidemiological studies of toxoplasmosis and neosporosis in livestock are scarce. Thus, this study aimed to evaluate the molecular and serological prevalence of *T. gondii* and *N. caninum* in livestock from Somalia. For this, blood samples from 128 cattle, 184 goats, and 46 sheep were collected between December 2018 and January 2020 from Benadir and Lower Shabelle regions of Somalia. Blood samples were submitted for DNA extraction and PCR to detect the *T. gondii* and *N. caninum* DNA, targeting the repetitive fragment of 529 bp and Nc5 gene, respectively. The serum samples were submitted for the indirect fluorescent antibody test (IFAT) to detect IgG anti-*T. gondii* and anti-*N. caninum*. Overall, 29.6% (106/358) of the animals had anti-*T. gondii* antibodies, with the highest prevalence in sheep (62.2%; 30/46), followed by goats (30.4%; 56/184) and cattle (15.6%; 20/128). For *N. caninum*, 3.6% (13/358) had antibodies against the parasite, with cattle showing the highest prevalence (6.2%; 8/128), followed by goats (2.2%;

4/184) and sheep (2.2%; 1/46). DNA of *T. gondii* was detected in 2.5% of the animals (9/358), with seven positive samples from sheep (15.2%, 7/46) and two from cattle (1.6%; 2/128). No samples were positive *for N. caninum* PCR. This study described, for the first time, serological and molecular detection of T. gondii and N. caninum in livestock from Somalia, highlighting the potential implications for human health and animal production. Further studies are necessary to evaluate the potential risk of transmission to humans and animals, contributing to animal welfare, food safety, and public health in Somalia.

EEI13- *Neospora caninum* congenital transmission and abortion rates in dairy and beef cattle of central Argentina (1998–2024)

Pachiani M.[1], Sosa E.[1], Miqueo E.[1], Campero L. M.[1], Morrell E.[1], Moore D. P.[1,2], Cantón G.[1]*

[1]*Institute of Innovation for Agricultural Production and Sustainable Development (INTA Balcarce - CONICET), Argentina*
[2]*Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Balcarce, Argentina.*

*Presenting author: canton.german@inta.gob.ar

Neospora caninum is an obligate intracellular coccidian parasite that primarily affects cattle and dogs, and is a leading cause of abortion in cattle worldwide. Serologic prevalence of *N. caninum* varies significantly among regions, countries, and among beef and dairy cattle. This study aims to compare congenital transmission rates of *N. caninum* and the frequency of *N. caninum*-abortions in 1127 spontaneously aborted fetuses/stillbirths from beef (n = 921), dairy (n = 190) and no-specified (n = 16) systems of Central Argentina, registered at the Veterinary Diagnostic Service of INTA Balcarce from 1998 to 2024. *N. caninum* infection was confirmed in 314/1127 (27.9%) specimens either by PCR (brain) or IFAT (cavity fluids). *Neospora caninum* congenital transmission

was higher in dairy (79/190; 41.6%) than in beef (232/921; 25.2%) fetuses (p<0.01). Fetuses in beef operations have approximately half the risk of *N. caninum*-infection compared to those from dairy farms (OR = 0.522; CI= 0.371- 0.735; p<0.01). *Neospora caninum* was confirmed as the cause of abortion in 152/1127 fetuses (13.5%) (fetuses congenitally infected, and with inflammatory lesions compatible with protozoan abortion). *Neospora caninum*-abortion was confirmed in 98/921 (10.6%) and in 52/190 (27.3%) beef and dairy fetuses, respectively. The risk of *N. caninum*-abortion was lower in beef than in dairy fetuses (OR = 0.37; CI = 0.244-0.771; p<0.01). *Neospora caninum*-abortion occurred in 48.4% of *N. caninum*-infected fetuses (152/314). The risk of abortion in *N. caninum*-infected fetuses was higher in dairy (52/79; 65.8%) than in beef (98/232; 42.2%) cattle (p<0.01). As previous studies have showed, this data indicate that fetuses in dairy production systems have a greater likelihood of being infected and abort due to *N. caninum* compared to fetuses in beef production systems. Husbandry conditions as well as differential producing stress in these systems could influence the outcome of *N. caninum* infection.

EEI14- Equine piroplasmisis: molecular detection of *Theileria equi* and *Babesia caballi* in Corrientes, Argentina

Benitez D.[1], Ganzinelli S.[2]*, Lobayán S.[3], Schnittger L.[2, 4], Schapiro J.[2, 3]

[1]*EEA INTA Mercedes, Corrientes, Argentina*

[2]*Instituto de Patobiología Veterinaria, CICVyA, INTA, Buenos Aires, Argentina y Consejo Nacional de Investigaciones Científicas y Técnicas*

[3]*Instituto de Investigación en Veterinaria, Facultad de Ciencias Agrarias y Veterinarias, Universidad del Salvador, Buenos Aires, Argentina*

[4]*Consejo Nacional de Investigaciones Científicas y Técnicas*

*Presenting author: ganzinelli.sabrina@inta.gob.ar

Equine piroplasmosis (EP) is a tick-borne disease caused by the hemoprotozoa *Theileria equi* and *Babesia caballi* endemic to warm areas of our country and the world. Infection may affect animal welfare and has a substantial economic impact related to limitations in horse transport between endemic and non-endemic regions, reduced performance of sport or work horses, and treatment costs. In general, infected animals present as healthy, asymptomatic carriers. In the present study, the proportion of *T. equi* and *B. caballi*-infected horses belonging to 22 brick manufacturing owners of Gobernador Virasoro in the province of Corrientes was evaluated. During May 2022 to March 2023, peripheral blood was taken aseptically from healthy animals (n = 98) and direct parasite detection was carried out using a polymerase chain reaction (PCR) assay that targets a species-specific region of the 18S rRNA gene (OIE, 2021). Genomic DNA isolated from in vitro cultures of each parasite served as a positive and water as a negative control. The PCR products were visualized on a 1.5% agarose gel stained with EcoGel (INBIO Highway). Altogether 52 of the 98 samples (53.1%) reacted positive for *T. equi*. The infection rate in the different age groups was 55.6% (15 of 27) in young horses aged 1 to 3 years, 55.4% (31 of 56) in animals aged 4 to 10 years, and 40% (6 of 15) in animals older than 11 years. The same proportion of *T. equi*-infected to uninfected animals was observed in the three studied age groups (χ^2gl=2, α<0.05 = 1.21 => p=0.55). In contrast, no samples were positive for *B. caballi*. The high rate of animals affected by EP highlights the health problem in the region.

EEI15- PCR detection of cattle cyst-forming coccidian in fecal material of canids from Southeastern Buenos Aires, Argentina

Soto Cabrera A.[1]*, Moré G.[2], Dellarupe A.[3], Scioscia N.[4], Moore D. P.[1], Pedrana J.[5]

[1]*Instituto de Innovación para la Producción Agropecuaria y el Desarrollo Sostenible (INTA-CONICET), Balcarce, Argentina*
[2]*Institute of Parasitology, University of Bern, Bern, Switzerland.*
[3]*Laboratorio de Inmunoparasitología (LAINPA-FCV-UNLP); CONICET, La*

Plata, Argentina
[4]*Instituto de Investigaciones en Producción, Sanidad y Ambiente FCEyN-CONICET, Mar del Plata, Argentina*
[5]*Universidad Tecnológica Nacional-CONICET, Mar del Plata, Argentina*

*Presenting author: sotocabrera.agustina@inta.gob.ar

Neospora caninum and *Sarcocystis cruzi* have canids as definitive hosts and cattle as intermediate hosts. Infection with these protozoa causes significant reproductive and productive losses in Argentinean production systems. The aim of this study was to identify *Neospora caninum* and *Sarcocystis cruzi* oocysts in faeces of pampas fox (*Lycalopex gymnocercus*) and domestic dog (*Canis lupus familiaris*) in dairy farms in the southeast of Buenos Aires province, Argentina. We visited 24 dairy farms and collected 269 pampas fox faeces and 88 dog faeces. Microscopic analysis was performed by sucrose flotation. When oocysts or sporocysts were identified, the specific molecular diagnosis was conducted by conventional PCRs and sequencing (primers Np6+ and Np21+; and COC1-COC2) for *N. caninum* and by real-time PCR with specific Taq-man probe for *S. cruzi*. *Neospora*-like oocysts were not detected in Pampas fox samples (0/269); however, they were detected in one dog sample (1/88, 1.14%). *Sarcocystis* sp. sporocysts were found in 19% (51/269) of pampas fox faeces and in 20.5% (18/88) of dog faeces. The *Neospora*-like oocysts from a dog were only positive in PCR with COC1-COC2 primers and the consensus sequence (249 bp without primers) showed 100% identity and coverage with *Hammondia hammondi* sequences. For *S. cruzi*, based on the estimated number of sporocysts in each sample, those with the highest number of sporocysts and at least one sample per farm were considered for molecular analysis. Therefore, 15 samples from pampas foxes and 7 samples from dogs were selected, with positive results in 6/15 and 5/7 samples by real-time PCR, respectively. These results showed that in the productive systems of the southeastern of Buenos Aires province, the canids are frequently consuming muscles of cattle. Consequently, the environment

contamination with canid-transmitted cyst-forming coccidian oocysts or sporocysts is presumably high.

EEI16- Everything about neosporosis in Colombia

Rivas-López P.C.[1]*, Moore D.P. [2], Chaparro-Gutiérrez J.G.[3], Idarraga-Bedoya S.E.[4]

[1]Faculty of Agricultural Sciences, National University of Mar Del Plata, Balcarce. Argentina.
[2]Institute of Innovation for Agricultural Production and Sustainable Development (IPADS). INTA-CONICET. Balcarce. Argentina.
[3]Faculty of Agricultural Sciences, University of Antioquia, Medellín. Colombia.
[4]Faculty of Health Sciences, Veterinary Medicine and Zootechnics Program, Technological University of Pereira. Colombia.

*Presenting author: pcrmvun@gmail.com

Colombia is a South American country characterized by its diverse, isolated regions separated by high mountains or dense forest areas, resulting in varied livestock production systems across different regions. It has 28,722,536 heads of cattle distributed throughout the country, with dual-purpose production systems predominating in the lowland and midland areas that experience warm climates.
Neospora caninum was first reported in Colombia in 1999, following a survey of dairy farms with reproductive issues located in the central region. This survey revealed a 54.1% serological reactivity to the agent using the ELISA technique. Subsequent studies over the years have found seroprevalences in cattle across various regions ranging from 10.2% to 89.0%, associating it with significant economic losses due to abortions, reduced milk production, and increased culling rates. Dairy and dual- purpose cattle exhibit higher seroreactivity than beef cattle,

possibly due to greater stress and management systems that allow constant contact with dogs and other intermediate hosts. Seroprevalences have also been reported in buffaloes (45%), sheep (78.6%), and dogs (12%), among others. It is evident that this disease should be included in the differential diagnosis of various reproductive pathologies affecting cattle in Colombia and underscores the importance of implementing control and prevention measures to mitigate the economic losses caused by this condition.

EE17- Seroepidemiology of bovine neosporosis in the dairy herd of INTA Balcarce

García C.E.[1]*, Soto Cabrera A.[2], Cirone K.M.[3], Rivas López P.C.[1], Fiorani F.[1], Campero L.M.[2], Moore D.P. [1,2]

[1]UIB Facultad de Ciencias Agrarias (UNMDP), Balcarce, República Argentina
[2]CONICET, Balcarce, República Argentina
[3]INTA, Balcarce, República Argentina

*Presenting author: cristinamicaela@gmail.com

The Argentine dairy industry produces 11,113 million lts of fluid milk per year. Total exports of dairy products reach 372.9 tons, including 2,810 million lts of fluid milk, generating revenues of over $1 billion. Bovine neosporosis, caused by the protozoan parasite *Neospora caninum*, is one of the leading causes of culling worldwide, mainly on dairy farms. There are currently no vaccines available, so serological diagnosis is essential for management and control. The National Institute of Agricultural Technology (INTA) is a public institution. The first confirmed case of Neospora abortion in dairy cattle was identified by INTA researchers. Although there are 45 INTA research facilities in Argentina, only two have dairy cattle (INTA Rafaela and Balcarce). The aim of this

study was to determine the prevalence of N. caninum- seropositive cattle at the INTA Balcarce dairy farm between April and July 2022 (n=214). Blood samples without anticoagulants were collected for serum extraction. Specific antibody detection was performed with a commercial indirect ELISA kit (IDVet). Only 2 abortions were recorded during the experimental period. A generalized linear model (GLM) with a binomial distribution (seropositive vs. seronegative) was used to assess explanatory variables as potential risk factors. A mixed effects GLM with mother as a random effect was used to determine whether maternal seroprevalence influenced calf prevalence. The overall prevalence was 33.18% (71/214) with CI 95%: 31.10%-35.25%. The GLM showed no statistically significant differences for sex and category. The mixed effects GLM with 114 animals grouped into 71 clusters (AIC: 142.9, X2, 0.0033, p<0.01). showed variability between groups and an association between individual and maternal serology (OR=4.71, IC 95%: 1.57- 14.14, P=0.000779). When the mother was seropositive, the probability of her calf being seropositive was 61% (95% CI:40%-79%). This study suggests the presence of endemic neosporosis providing valuable information for implementing management strategies on the INTA Balcarce farm.

EEI18- High prevalence cluster of *Neospora caninum* identified in the Mar y Sierras dairy basin, Argentina

Soto Cabrera A.[1]*, Bernad L.[1], Moré G.[2], Betancourt Rossolli J.[3], Scioscia N.[3], Pedrana J.[4], Moore D. P.[1]

[1]*Instituto de Innovación para la Producción Agropecuaria y el Desarrollo Sostenible. INTA-CONICET, Balcarce, Argentina.*
[2]*Institute of Parasitology, University of Bern, Bern, Switzerland.*
[3]*Instituto de Investigaciones en Producción, Sanidad y Ambiente FCEyN-CONICET, Mar del Plata, Argentina.*
[4]*Universidad Tecnológica Nacional-CONICET, Mar del Plata, Argentina.*

*Presenting author: sotocabrera.agustina@inta.gob.ar

Neospora caninum is a protozoan responsible for causing abortions in cattle herds globally. In Argentina, economic losses attributed to *N. caninum* in dairy cattle exceed $40 million annually. Currently, there is no vaccine or treatment available to prevent bovine neosporosis, so control measures to reduce seroprevalence are critical and have shown promising results. This study aimed to determine the spatial distribution of high seroprevalence of *N. caninum* in dairy cattle within the Mar y Sierras basin of Buenos Aires, Argentina. A total of 720 serum samples were collected from adult cows (30 per farm) across 24 dairy farms. Serological diagnosis was performed using a commercial indirect ELISA kit (CIVTEST® BOVIS NEOSPORA, HIPRA, Girona, Spain). Geographic clustering was analyzed using SaTScan™ software (v10.2.4, August 2024) with a discrete Poisson model, where each dairy farm was treated as a unit, and its geographical position (latitude and longitude) was considered. The serological analysis revealed an overall prevalence of 24.44 % (176/720, 95 % CI: 21.37 – 27.52 %). Cluster 1 included 10 farms from Tandil and the west of Balcarce department, located at 37°48'37.32" S and 59°20'69.83" W, with a radius of 68.41 km. The expected number of cases was 73.33, while the observed number was 99, yielding an observed-to-expected (O/E) ratio of 1.35 (p=0.0029). Cluster 2 included 1 farm from General Pueyrredón department, located at 38°08'06.22" S and 57°70'31.84" W, with an expected case count of 7.33 and an observed count of 13, resulting in an O/E ratio of 1.77 (p=0.754). Identifying regions with high seroprevalence of *N. caninum* could help link specific management practices or environmental factors to the occurrence of the infection in dairy herds.

EEI20- Sarcocystis cysts burden distribution in striated muscles of lambs

Gual I.[1], Campero L. M.[2], Moré G.[3], Scioli M.[2], Lázaro F.[2], Cantón G.[2], Sander V.[4], Ángel S.[4], Clemente M.[4], Moore D. P.[1,2]*

[1]*Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Balcarce, Buenos Aires, Argentina*
[2]*Instituto de Innovación para la Producción Agropecuaria y el Desarrollo Sostenible (IPADS Balcarce), Instituto Nacional de Tecnología Agropecuaria Estación Experimental Agropecuaria Balcarce (CONICET-INTA), Balcarce, Buenos Aires, Argentina*
[3]*Institute of Parasitology, Vetsuisse Faculty, University of Bern, Bern, Switzerland*
[4]*Laboratorio de Biotecnologías en Bovinos y Ovinos, Instituto Tecnológico de Chascomús (INTECH), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)-Universidad Nacional General San Martin (UNSAM), Chascomús, Buenos Aires, Argentina*

*Presenting author: moore.dadin@inta.gob.ar

With rising global demand for lamb meat, Argentina is looking to enhance meat quality to compete in premium markets. Ovine sarcocystosis, caused by *Sarcocystis* spp., is often asymptomatic but can negatively affect meat quality. Despite high prevalence in ruminants worldwide, there is limited data on sarcocystosis in Argentine sheep. This study investigated the burden of *Sarcocystis* cysts in striated muscles of 18 Texel lambs aged 8-10 months. Tissue samples from various muscles, including the heart, tongue, diaphragm, and skeletal muscles, were examined histologically. All muscles were infected, with the heart and tongue showing significantly higher cyst counts ($p < 0.05$). Infection intensity varied from 1-5 cysts per mm^2 to over 20 cysts per mm^2. Both thin and thick-walled cysts were observed suggesting either *S. tenella* and *arieticanis* or *S. medusiformis*, respectively. Pooled cysts samples from two animals were processed by PCR targeting a 18S rRNA gene fragment and sequenced. One sequence showed a 99.7% identity with *S. tenella* sequences and the other 100% with *S. arieticanis*. Both species with canids as definitive hosts. The presence of *Sarcocystis* cysts in all lambs suggests a relatively high environment contamination with canids faeces. The significantly higher cyst counts in the heart and tongue

highlight these muscles as relevant source for completing the parasites cycle in canids. The variation in cyst densities between muscle types also suggests that infection patterns may be muscle-specific, warranting further research on muscle distribution and its impact on overall carcass quality. Potential detrimental effects of sarcocystosis on the organoleptic properties of meat, such as texture and flavor, needs further investigation. More extensive studies are needed to better understand the epidemiology of sarcocystosis in Argentina and to develop strategies for mitigating its impact on the sheep industry

SESSION 3

Diagnosis

Keynote. Serodiagnosis of Toxoplasma gondii infection: recent advances and a One Health approach

Álvarez-García G.[1]*, López-Ureña N. M.[1], Huertas-López A.[1, 2], Arranz-Solis D.[1], Calero-Bernal R.[1], Ortega-Mora L. M.[1]

[1] *SALUVET, Animal Health Department, Faculty of Veterinary Sciences, Complutense University of Madrid*
[2] *Animal Health Department, University of Murcia*

*Presenting author: gemaga@ucm.es

Toxoplasma gondii is a major foodborne pathogen with impact on animal and public health. Most human infections occur through the consumption of raw or undercooked meat of pigs and small ruminants, or the ingestion of oocysts contaminating the environment. Recent metanalyses confirmed that i) one-third of the European human population has been exposed to *T. gondii*; ii) felids, small ruminants, wild boards, and wild ruminants had a higher force of infection; iii) the One Health approach followed in the serodiagnosis of *T. gondii* still needs further integration among scientific disciplines; iv) the validation process of serological tests developed for humans and animals has limitations that hinders reproducible results.
Since the control of this zoonosis partially depends on serological monitoring in humans and animals, further progress in serodiagnosis was made to uncover relevant gaps.
First, the most widely used serological tests employed in small ruminants and pigs were compared and updated performance data were obtained to facilitate the harmonization of serological diagnosis in surveillance

studies. Moreover, a practical recommendation for diagnostic laboratories was offered to minimize false-positive reactors.
In addition, we have shown that a combination of GRA peptide homologous pairs can discriminate infections caused by *T. gondii* type II and III strains in sheep and pigs.
Thus, this serotyping method could be a promising alternative to genotyping techniques.
Finally, we have explored *T. gondii* serology as a means of attributing the source of infection. Unfortunately, despite the extensive work done to identify and screen a wide panel of oocyst-specific proteins, no suitable marker for oocyst-attributing serological diagnosis could be found leading us to even discard TgERP as a valuable marker of oocyst-derived infections.
Advances in serological diagnosis will aid the identification of effective intervention strategies.

ORAL PRESENTATIONS

D1- Development of a real-time LAMP for the specific and sensitive detection of *Cryptosporidium parvum* in water samples

de Alba P.[1]*, Garro C. [1], Mira A.[2], Dus Santos M. J.[3], Schapiro J.[1], Wigdorovitz A.[4], Florin-Christensen M.[1], Schnittger L.[1]

[1] *CONICET, Instituto de Patobiología Veterinaria (INTA-CONICET), Buenos Aires, Argentina*
[2] *Bioinnovo SA, Buenos Aires, Argentina*
[3] *Instituto de Virología e Innovaciones Tecnologicas (INTA-CONICET), Buenos Aires, Argentina*
[4] *INCUINTA (INTA), Buenos Aires, Argentina*

*Presenting author: dealba.paloma@inta.gob.ar

Cryptosporidium parvum is a zoonotic enteroprotozoan that infects newborn calves worldwide. The infection is characterized by severe diarrhea and dehydration, leading to economic losses in the dairy industry. Oocysts, the infective parasite stage, are excreted in massive quantities with calf feces contaminating the environment.
Oocysts are highly infectious, resistant to chemical disinfectants and environmental stressors, and pose a public health concern. This study aimed to develop a molecular method to detect oocysts contamination of water as an infection source for calves. A real-time loop-mediated isothermal amplification (LAMP) assay was established to facilitate rapid oocyst identification while being resilient to inhibitors. Optimal amplification temperature, reaction time, and concentration of the intercalating fluorescent were established. The detection limit was determined using a target gene- containing plasmid in a copy number ranging from 100.000.000 to 1, as well as a known concentration of genomic DNA purified from oocysts (gDNA), both evaluated in triplicate. The triplicate plasmid samples with copy number of 10.000 reacted positive as also 2 of 3 samples with a copy number of 1.000, and 1 of 3 samples with a copy number of 100. For gDNA, amplification was achieved down to 400 pg. The LAMP assay was applied to concentrated water samples originating from a dairy farm (DF, n=4) and an experimental field (EF, n=4). Three of the eight samples were positive, two of which originated from drinking troughs (DF, n=1; EF, n=1) and one from a trough-feeding water well (EF). No amplification was observed when the same samples were analyzed by real-time PCR. These preliminary results indicate the importance of using a technique suitable for detecting low numbers of oocysts in the presence of inhibitors. They also strongly suggest that water supplies play an important role in the ingestion and subsequent infection of calves and possibly humans with *C. parvum*.

D2- Modernizing diagnosis of coccidiosis: challenges faced in adding molecular species differentiation and enumeration to OPG counts from litter or fecal samples

Lindo D. P.[1]*, J. R. Barta[1]

[1] *Ontario Veterinary College, University of Guelph, Guelph ON Canada*

*Presenting author: dlindo@uoguelph.ca

The OPG (oocysts per gram) count is an important diagnostic tool for the agricultural industry that is integral to food animal medicine. It provides accurate enumeration of oocysts allowing clinicians to estimate the severity of an infection and assign appropriate treatment. Useful as it may be, morphological speciation of oocysts is unreliable and consequently the relative numbers of particular species in a sample are difficult to determine. Ultimately, molecular methods will provide accurate species identification and quantification, but these methods present their own unique challenges in the selection of appropriate methods and targets.

Molecular identification of *Eimeria* species can use polymerase chain reactions (PCR) with species-specific primers (no sequencing) or genus-specific primers and amplicon sequencing. Probe-based, quantitative-PCR and droplet digital PCR can provide both identifications and relative quantification, but such methods are only partially validated. Likewise, deep sequencing (NGS) of genus-specific PCR amplicons can provide similar information but Illumina® NGS technologies are cost-prohibitive for small sample numbers and the accuracy of Oxford Nanopore® is untested in this context.

Choosing a gene target that accurately speciates *Eimeria* remains challenging. Nuclear *18S rDNA* is too conserved for species differentiation; ribosomal ITS regions are perhaps too divergent. All nuclear rDNA loci suffer from the existence of divergent paralogous rDNA copies within their nuclear genomes. In contrast, mitochondrial *COI* and *COIII* evolve at an appropriate rate for robust species

differentiation and are found at high copy numbers in these parasites; well conserved regions upstream and downstream of these CDS regions provide convenient PCR primer sites. The mitochondrial copy number per oocyst for each species is unknown at present and the impact of the degree of sporulation is similarly unknown. We will present potential molecular targets, methodologies and associated required biological information to generate a properly validated method for simultaneous enumeration and speciation.

E-POSTERS

D3- Cryptosporidiosis in dairy cattle from Buenos Aires, Argentina

Basset C. M.[1,2]*, De Felice L.[3], Jaureguiberry M.[2,4,5], Salas M. G.[5], Bona C.[5], Konis T. P.[2,5], Gos M. L.[1,3], Unzaga J. M.[1,3]

[1] *Immunoparasitology Laboratory, Faculty of Veterinary Science-La Plata National University, La Plata, Argentina*
[2] *Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina*
[3] *Cátedra de Parasitología y Enfermedades Parasitarias-Universidad Nacional de La Plata, La Plata, Argentina*
[4] *Centro de Diagnóstico e Investigaciones Veterinarias-Universidad Nacional de La Plata, La Plata, Argentina*
[5] *Instituto de Investigaciones en Reproducción Animal (INIRA)-Universidad Nacional de La Plata, La Plata, Argentina*

*Presenting author: cmbasset@gmail.com

Bovine cryptosporidiosis has been reported worldwide as the major cause of neonatal calf diarrhea. In Argentina, *Cryptosporidium parvum* is the only species reported, with subtypes belonging to family IIa, some of them with zoonotic potential. In the present study, the aim was to

evaluate the presence of *Cryptosporidium* spp. and its association with the presence of diarrhea and the age of the calves. For this, a dairy farm located in Buenos Aires´s province was visited between July 2023 and April 2024. A total of 119 fecal samples between one to four weeks of age were randomly collected from healthy and diarrheic calves that had not been treated with antimicrobials. Samples were concentrated by sedimentation technique and stained by modified Ziehl-Neelsen technique. Samples with presence of oocysts 4-5 µm in diameter were identified as positive by light microscopy (1000×). DNA extraction from positive samples was performed using the PuriPrep SUELO DNA kit (ImbioHighway). *Cryptosporidium* spp. were identified using the *18S rRNA* gene, and 60 kDa glycoprotein gene was used to determine *C. parvum* subtype by nested PCR. Data were analyzed using a binary logistic regression model, with a significance level of $p<0,05$, in RStudio. Oocysts compatible with *Cryptosporidium* spp. were detected in a total of 39% (46/119) samples. Genus specific nPCR allowed the detection of *Cryptosporidium* DNA in 42/46 samples (91% of samples with oocyst compatible structures). The frequency of *C. parvum* was highest in the second week 50% (23/46). The proportion of positive samples was associated with the presence of diarrhea 82% (38/46) and 17% (8/46) in diarrheic and healthy calves, respectively; $p<0,05$. These preliminary results indicate the importance of this parasite as a cause of diarrhea in calves and the importance of promoting actions aimed at controlling it.

D5- Development and validation of a new IgM-ELISA to detect anti-*Toxoplasma gondii* antibodies in sheep sera

Huertas-López A.[1,2]*, Sánchez-Sánchez R.[1], Velasco-Jiménez N.[1], Calero-Bernal R.[1], Ortega-Mora L. M.[1], Álvarez-García G.[1]

[1] *SALUVET, Animal Health Department, Complutense University of Madrid, Madrid, España*
[2] *Animal Health Department, University of Murcia, Murcia, España*

*Presenting author: ana.huertas@um.es

Detection of IgM is commonly employed for early diagnosis and treatment of human toxoplasmosis. In sheep, there is a need for novel methods to provide an earlier diagnosis of the infection, that could facilitate the search of early abortions under natural conditions and that could be useful as infection markers for studies testing drugs. The aim of this study was to develop and validate a new ELISA based on lyophilized *Toxoplasma gondii* tachyzoites to detect anti-*T. gondii* IgM antibodies (IgM-ELISA) in sheep sera. Serum samples from sheep experimentally (with 10 and 1000 *T. gondii* oocysts, and non-infected, collected from 0 to 28 days post-infection - dpi-) and naturally infected were used in this study. Validation of the IgM-ELISA consisted in 1) analytical validation, 2) diagnostic performance with sera from experimentally (Mixed effect analysis) and naturally (Mann-Whitney's U test, ROC analysis and kappa) infected sheep compared to IgM-WB. The new IgM-ELISA showed excellent inter-and intra-assay precision), good accuracy and accurate analytical sensitivity. Cross-reactivity with anti-*Neospora caninum* antibodies was detected. IgM antibodies were detected by IgM-ELISA and IgM-WB from 10 and 12 dpi in sheep experimentally infected with 1000 and 10 oocysts, respectively. Results of IgM-ELISA (RIPC) decreased in most animals on 28 dpi, although they remained positive by IgM-WB. Regarding naturally infected sheep, significant differences ($p<0.0001$) were detected between positive and negative groups, ROC analysis resulted in an optimal cut-off (RIPC=24.04) that corresponded to 82% sensibility and 85% specificity, and an AUC of 0.89. Moderate agreement between IgM-ELISA and IgM-WB was found (Kappa=0.57). Detection of IgM anti-*T. gondii* could be applied to investigate ovine abortion outbreaks following exclusion of *N. caninum* infection. This method may potentially diagnose early abortions in sheep and even humans, provided that such events occur.

D6- Exploring NcSAG1 and NcSRS2: Recombinant Proteins of *Neospora caninum* and Their Role in Pathogenesis and Diagnostics

Echeverría S.[1]*, Carrion F.[2], Soñora M.[3], Ruppel F.[1], Li Calzi M.[4], Tomasina R.[1], Pritsch O.†[2], Cabrera A.[1,5], Robello C.[1,6]

[1] *Laboratorio de interacciones Hospedero-Patógeno, UBM, Institute Pasteur de Montevideo, Montevideo, Uruguay*
[2] *Laboratorio de Inmunovirología, UBP, Institute Pasteur de Montevideo, Montevideo, Uruguay*
[3] *Laboratorio de Simulaciones Biomoleculares, Institute Pasteur de Montevideo, Montevideo, Uruguay*
[4] *Laboratorio de Genómica Funcional, Institute Pasteur de Montevideo, Montevideo, Uruguay*
[5] *Departamento de Parasitología y Micología, Facultad de Medicina, UdelaR, Montevideo, Uruguay*
[6] *Departamento de Bioquímica, Facultad de Medicina, UdelaR, Montevideo, Uruguay*

*Presenting author: echeverria@pasteur.edu.uy

Neospora caninum is an obligate intracellular apicomplexan parasite that causes neosporosis, which is the leading cause of abortion in cattle worldwide. In the study of molecular mechanisms of pathogenesis, SRS proteins play a crucial role in host interaction.
SRS proteins constitute a family of coccidian-specific proteins that were first described in *Toxoplasma gondii*. They are surface proteins, most of which have a GPI anchor. Recent studies on the genomes of *T. gondii* and *N. caninum* have found that this family shows an expansion in *N. caninum*. Their functions have been associated with the adhesion of the parasite to the host cell, interaction and modulation of the immune system, and maintaining the structure of the cyst membrane, among others.

Our work aims to structurally and functionally characterize two proteins, named NcSAG1 and NcSRS2, which are orthologous to the SAG1 and SRS2 proteins of *T. gondii*, respectively. Both proteins were obtained recombinantly, soluble, and stable in *Drosophila melanogaster* cells, purified, and characterized through biochemical assays, immunofluorescence, and computational methods. Evidence of their secretion through vesicles was found, with the proteins being present in the extracellular environment in a soluble form. Dichroism assays and models obtained by AlphaFold provided the basis for creating the first structural tree of this family, opening the possibility of starting to investigate structure-function associations in the different clades obtained. Obtaining the proteins in a soluble and stable form also facilitated the development of ELISA assays for improved diagnostics and immunization studies.

D7- First report of congenital toxoplasmosis in guanacos (*Lama guanicoe*) from Los Glaciares National Park, Argentina

Campero L. M.[1]*, Rago M. V.[2], Sosa E.[1], Morsella C. G.[1], Scioli M. V.[1], García J. A.[1], Fiorentino M. A.[1], Verna A. E.[1], Subires S.V.[3], Piasentini F. J.[3], Carmanchahi P.[4], Ferreryra H. del V.[5], Uhart M.[6], Cantón G. J.[1]

[1] *Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de Innovación para la Producción Agropecuaria y el Desarrollo Sostenible (IPADS, CONICET-INTA Balcarce), Balcarce, Argentina.*
[2] *Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de Investigaciones en Biodiversidad y Medioambiente (INIBIOMA, CONICET-UNCo), Subsede Junín de los Andes, Neuquén, Argentina.*
[3] *Administración de Parques Nacionales (APN), El Calafate, Santa Cruz, Argentina.*
[4] *Grupo de Investigaciones en Ecofisiología de Fauna Silvestre, INIBIOMA - CONICET - UnCo, San Martín de los Andes, Neuquén, Argentina.*
[5] *Private Veterinary Practitioner. Gaona 360. Córdoba, Argentina.*

[6] *Karen C. Drayer Wildlife Health Center, School of Veterinary Medicine, University of California, Davis, USA.*

*Presenting author: campero.lucia@inta.gob.ar

Toxoplasma gondii is a zoonotic apicomplexan parasite associated mainly with abortion and perinatal mortality in small ruminants. South American camelids are natural hosts of *T. gondii* and variable seroprevalence were reported, however, information on clinical effects in free-living wild ruminants is scarce. In the present study we evaluated the presence of infectious agents involved in abortions from guanacos (*Lama guanicoe*) from Los Glaciares National Park, Santa Cruz, registered by park rangers between the 21st of June and the 8th of July of the 2024 (22 abortions). Full necropsies in 4 guanacos fetuses (~5 months gestation) were performed and samples assessed at the Veterinary Diagnostic Laboratories at INTA Balcarce for direct (microbiological cultures, virus isolation, PCR) and indirect routine diagnostic techniques (viral seroneutralization and indirect fluorescent antibody test). Viral (bovine viral diarrhea virus and bovine herpesvirus), bacterial (*Salmonella* spp., *Brucella* spp., *Campylobacter* spp.) and protozoan parasites (*Neospora caninum* and *T. gondii*) presence was evaluated. Histopathology on formalin fixed tissues was also performed. *Toxoplasma gondii* DNA was detected by conventional PCR in brain and placenta samples from the 4 fetuses. No other infectious agents were detected. Mild to moderate multifocal histiocytic hepatitis was recorded in all fetuses and mild focal gliosis was detected in 1 fetus. The present findings confirm for the first time transplacental transmission of *T. gondii* in guanacos. Further studies are needed to determine the cause of abortion in this episode and the impact of toxoplasmosis in guanacos needs to be clarified. In addition, *T. gondii* genotyping is being performed to provide further evidence on virulence and association with local circulating genotypes described in humans and other domestic and wild animal species.

D8- Assessment of diagnostic techniques of bovine abortion by *Neospora caninum* at INTA Balcarce

Sosa E.[1]*, Cantón G. J.[1], Morell E. L.[1], Hecker Y. P.[2], Scioli M. V.[1], Moore D. P.[1], Campero L. M.[1]

[1] *Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de Innovación para la Producción Agropecuaria y el Desarrollo Sostenible (IPADS, CONICET-INTA Balcarce), Balcarce, Argentina.*
[2] CONICET, SALUVET, Departamento de Salud Animal, Facultad de Ciencias Veterinarias, Universidad Complutense de Madrid, Madrid, España.
[3] Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Balcarce, Argentina.

*Presenting author: sosa.emiliano@inta.gob.ar

Neospora caninum is an apicomplexan parasite that causes bovine abortion and birth of congenitally infected healthy calves. Diagnosis is defined based on the presence of histopathological compatible findings and evidence of transplacental infection (DNA and/or fetal antibodies). The aim of this work was to evaluate the level of agreement between histopathology (HP), PCR and IFAT in the assessment of *N. caninum* diagnosis in spontaneously aborted bovine fetuses at INTA Balcarce (2014-2024). A total of 409 bovine fetuses (n = 350) and perinatal deaths (n = 59) were analyzed. The fetuses age ranged from 2 to 8.5 months (6.8 ± 1.49). Autopsy was performed and tissues were fixed in 10% neutral buffered formalin processed routinely. HP was considered positive when inflammatory lesions compatible with *N. caninum* such as meningoencephalitis, myocarditis and myositis were observed. IFAT was performed on cavity fluids (cut-off titer: 1/10). DNA was extracted from brain using a commercial kit (High Pure PCR Template Preparation Kit, Roche) and an adapted single-tube nested-PCR was performed for *N.*

caninum DNA detection. *Neospora caninum* was diagnosed as the cause of abortion in 22.2% of fetus (91/409), where 49.4% (45/91) were positive to the 3 techniques, 34% (31/91) to HP and PCR and 16.4% (15/91) to HP and IFAT. Substantial agreement (k = 0.61), 80.4% sensitivity and 88.5% specificity were observed when HP was compared with IFAT and PCR. Transplacental transmission with no associated lesions was detected in 11.6% (37/318), 12.3% (39/318) and 3.4% (11/318) of fetuses by PCR, IFAT and both techniques, respectively. Herein, these results highlight the importance of associating the presence of compatible lesions and parasite infection for the diagnosis of *N. caninum* abortion.

D9- A putative new *Besnoitia* species in the southern black-eared opossum *Didelphis aurita*

Arrabal J. P.[1], Orozco M. M.[2], Helman E.[3], Minatel L.[4], Notarnicola J.[1], Basso W.[5], Hartmann B. B.[1], Schapira A.[4], Moré G.[5]*

[1] *Instituto de Biología Subtropical IBS-CONICET, Universidad Nacional de Misiones- UNaM., Puerto Iguazú, Argentina*
[2] *Instituto de Ecología, Genética y Evolución de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas (IEGEBA-CONICET), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina*
[3] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ciudad Autónoma de Buenos Aires, Argentina*
[4] *Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina*
[5] *Institute of Parasitology, Vetsuisse Faculty, University of Bern, Bern, Switzerland*

*Presenting author: gaston.more@unibe.ch

Besnoitia spp. are cyst-forming coccidian parasites with a broad host range, affecting various domestic and wild animals. North American opossums (*Didelphis virginiana*) are notably impacted by *B. darlingi*. The

aim of this study was to report an infection with a putative new *Besnoitia* species in a road-killed female *Didelphis aurita* opossum from Misiones, Argentina. Numerous cysts, ranging from 0.5 to 1 mm in size, were observed in multiple muscles and visceral organs, with microscopic identification in skeletal muscles, tongue, and the heart. Histological examination revealed spherical 0.5 to 1 mm cysts containing numerous bradyzoite-like cells and a well-defined cyst wall. A small number of degenerate and ruptured cysts were also observed, surrounded by mild to moderate inflammation. Genomic DNA was extracted from an individual cyst and muscle tissue, and ITS1 marker and *18S rRNA* gene fragments from Sarcocystidae protozoa were successfully amplified by PCR and sequenced. The *18S rRNA* fragment sequence showed 100% identity with sequences of *B. darlingi* and *B. oryctofelisi*. Comparison of the complete ITS1 sequence (259 bp) revealed 99.2% identity with *B. oryctofelisi* and 97.7% with *B. darlingi*. In a bayesian inference phylogenetic tree our sequence is positioned on a branch related to *B. oryctofelisi* sequences, and as close relatives in a sister group appear *B. darlingi* and *B. akodoni* sequences. All these findings, along with phylogenetic analysis, suggest that the *Besnoitia* species identified is distinct from *B. darlingi* of North American opossums, showing a closer relationship to *B. oryctofelisi* described in rabbits from Argentina. Based on eco-epidemiological and molecular data, we propose a putative new species *Besnoitia auritai* n. sp. affecting the South American opossum *D. aurita*.

D10- Detection of *Sarcocystis capracanis* and *Sarcocystis hircicanis* in goats from Argentina

Steffen K. D.[1]*, Helman E.[1], Basset C. M.[1], Gos M. L.[1], Unzaga J. M.[1], Moré G.[2]

[1] *Immunoparasitology Laboratory, Faculty of Veterinary Science-La Plata National University, La Plata, Argentina*

[2] *Institute of Parasitology, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

*Presenting author: steffenkevin@gmail.com

Sarcocystis is a genus of apicomplexan protozoa with a worldwide distribution. Goats (*Capra aegagrus hircus*) are intermediate hosts for three species: *Sarcocystis capracanis* and *S. hircicanis*, which have canids as definitive hosts (DH), and *Sarcocystis moulei* (syn. *S. hircifelis*) that have felids as the DH. We aimed to detect and identify *Sarcocystis* spp. in goat and kid muscles utilizing optical and transmission electron microscopy and by PCR-sequencing of *18S rRNA* fragment gene. Skeletal muscle samples (masseters, base of tongue, and hamstring) were collected from 90 goats (19 adults and 71 kids aged 38 to 290 days) at slaughter or necropsy from five establishments in Buenos Aires and Salta provinces. A visual examination was performed to detect macroscopic cysts. Samples were processed as pooled muscle samples per animal (5-10 g) in a tissue homogenizer and then observed under an inverted microscope. Individual sarcocysts were placed in 1.5 ml microtubes for DNA extraction and further PCR. No macroscopic tissue cysts were found in all animals (n= 90) and no microscopic cysts in the muscle homogenate of kids (n= 71). In 89.5% (17/19) of adult goats, thin-walled (less than 1 μm) septate microscopic cysts of *Sarcocystis* spp. were found. PCR was positive in 31 cysts and 17 amplicons (from individual cysts from 17 goats) were sequenced. Homology of 99.46-100% with *S. capracanis* and 100% with *S. hircicanis* was detected in sixteen and one sequence, respectively. Four cysts showed an ultrastructure compatible with *S. capracanis* (TEM type 14b). Most of the adult goats showed sarcocysts (in a relatively high number), while none of the kids were infected with *Sarcocystis* spp. It is possible to suggest that infection occurs through ingestion of feed/water contaminated with sporocysts and gradually over time. The detected species would have canids as DH indicating a predator-prey relationship between goats and canids.

Based on molecular methods, the presence of *S. capracanis* and *S. hircicanis* was confirmed in goat muscles in Argentina for the first time.

SESSION 4

Treatment and control

Keynote. Current and potentially novel options for control of diseases caused by apicomplexans affecting farm animals

Hemphill A.

Institute of Parasitology, Vetsuisse Faculty, University of Bern, Switzerland

andrew.hemphill@vetsuisse.unibe.ch

Apicomplexan parasites such as *Toxoplasma gondii*, *Neospora caninum* and *Cryptosporidium parvum* cause substantial economic losses in livestock, with *T. gondii* and *C. parvum* also posing serious health risks to humans. However, currently marketed treatment options are limited and often associated with adverse side effects. Among those are the macrolide antibiotic spiramycin, the polyether ionophore antibiotic monesin, the quinolone decoquinate, folate inhibitors such as trimethoprim and pyrimethamine, and the triazonine toltrazuril for use against *T. gondii* and/or *N. caninum* infections. Marketed treatments of *Cryptosporidium* infection are the quinazolinone halofuginon and more recently also the aminoglycoside antibiotic paramomycin. All these treatment options have been obtained through drug repurposing, are thus unspecific inhibitors, and the emergence of drug resistance and the inherent capability of these parasites to adapt to adverse conditions warrants the development of better and more specific treatments. Multiple classes of compounds have been investigated as potential alternatives, including bumped kinase inhibitors (BKIs), endochin-like quinolones (ELQs), artemisinin derivatives, decoquinate derivatives, organometallic and organosulfur drugs, and antimicrobial peptides, through *in vitro* screening and proof-of-principle *in vivo* assays in mice.

Few compounds were advanced to ruminant models. BKIs, specifically BKI-1748 and BKI-1708 emerged as promising candidates for the treatment of congenital toxoplasmosis and neosporosis, and especially BKI-1708 for cryptosporidiosis, due to their potent efficacy and favorable safety profiles. Additionally, the combination of BKI-1748 with ELQ prodrugs, and the combination of BKI-1748 with a Listeria-based vaccine protocol, showed the potential for enhanced therapeutic and preventive efficacy in the pregnant neosporosis mouse model. Other compounds demonstrated less promising efficacy and safety profiles, especially with negative effects on pregnancy outcome.

For some compounds, we employed differential affinity chromatography coupled to mass spectrometry and proteomics (DAC-MS-proteomics), revealing drug-binding proteins, and thus potential targets, in both parasite and host cells. However, binding of a protein to a given drug does not automatically suggest that this protein is a valid drug target, and validation of potential candidate targets can be done by CRISPR-Cas9-mediated knock-out. An important feature of *Toxoplasma* and *Neospora* is that these parasites can rapidly adapt to increased drug concentrations *in vitro,* and possibly also *in vivo*. Adaptation can be permanent, although not mediated by mutations but most likely by epigenetic changes, or can be transient with parasites reverting to the original susceptibility following culture without compound. Proteomic analysis of drug-exposed parasites informs on up- and downregulation of the metabolic pathways that are affected by drug treatments, and can thus offer clues about the anticipated modes of action and also potential off-target effects of the tested compounds.

ORAL PRESENTATIONS

TC1- Effects of the novel bumped kinase inhibitor BKI-1708 against *Toxoplasma gondii, Neospora caninum* and *Besnoitia besnoiti*: differences and similarities

Ferreira de Sousa M.C.[1]*, Imhof D.[1], Hänggeli K.[1], Choi R.[2], Hulverson M.[2] Whitman G.[2], Arnold S.[2], Ojo K.[2], Van Voorhis W.[2], Fan E.[3], Sanchéz-Sanchéz R.[4], Ortega-Mora L.[4], Hemphill A.[1]

[1]*Institute of Parasitology, Vetsuisse Faculty, University of Bern, Switzerland, Graduate School for Cellular and Biomedical Sciences (GCB), University of Bern, Bern, Switzerland*
[2]*Center for Emerging and Re-emerging Infectious Diseases (CERID), Department of Medicine, University of Washington, Seattle, USA*
[3]*Department of Biochemistry, University of Washington, Seattle, WA USA Joseph Stone Doggett, Department of Biochemistry, University of Washington, Seattle, WA USA*
[4]*SALUVET, Animal Health Department, Faculty of Veterinary Sciences, Complutense University of Madrid, Madrid, Spain*

*Presenting author: maria.ferreira@unibe.ch

Apicomplexans are major morbidity-causing pathogens distributed worldwide. Among the compound classes being currently developed against these parasites, bumped kinase inhibitors (BKIs) – optimized to target the apicomplexan calcium-dependent protein kinase 1 (CDPK1) – have proven to be safe and active *in vitro* and *in vivo*. The structure of BKI-1708 is based on the same central scaffold as BKI-1748, which has exhibited promising *in vitro* and *in vivo* efficacy against both *N. caninum* and *T. gondii*. When applied *in vitro* concomitantly to infection, BKI-1708 displayed IC50 values of 120nM for *T. gondii* and 480nM for *N. caninum* and did not affect host cell viability at concentrations up to 25µM. BKI-

1708 was highly effective against *T. gondii* and *N. caninum* in experimentally infected mice by reducing parasite burden and vertical transmission. *In vitro* treatment with 2,5µM BKI-1708 induces the formation of multinucleated complexes (MNCs) - characterized by continued nuclear division and enclosing intracellular zoites lacking the outer plasma membrane, unable to finalize cytokinesis. MNC formation upon BKI treatment was reported in *T. gondii*, *N. caninum* and *B. besnoiti*. BKI-treatment has a parasitostatic effect, inhibiting proliferation under drug pressure but not clearing the infection, allowing parasites to regain infectivity. Major differences after drug removal can be noted, with *B. besnoiti* reconverting to infective tachyzoites more rapidly compared to *T. gondii* and *N. caninum*. Moreover, differences in antigen expression were observed between the three species. IFA demonstrated the presence of cyst wall components in treated *T. gondii*, but not in *N. caninum* or *B. besnoiti*. Proteomic analysis of BKI-1708 induced MNCs in *T. gondii*, *N. caninum* and *B. besnoiti* revealed differentially regulated proteins compared to untreated parasites, again with distinct differences between the three species. Further studies on the effects of BKI treatment can provide insights into the differences in stage conversion between closely related apicomplexans.

TC2- Tartrolon E: a new drug candidate for *Cryptosporidium parvum* infections. Preliminary results

Fumuso F. G.[1]*, Villarino N. F.[2], O'Connor R. M.[1]

[1]*Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota. Saint Paul, MN*
[2]*Department of Veterinary Clinical Sciences, Washington State University. Pullman, WA.*

*Presenting author: fumus003@umn.edu

Cryptosporidium parvum (CP) is considered a global endemic pathogen

in cattle and the main cause of neonatal parasitic enteric infections in calves. The animal industry is severely affected by watery diarrhea outbreaks in neonatal ruminants. Moreover, the agent is considered a major pathogen for human diarrheal disease in immunocompromised individuals and young children. Due to the lack of efficacious and safe treatment for the human and animal populations most at risk, Cryptosporidiosis remains a lethal and untreatable disease. In the last four decades, almost 35% of the novel certified antiparasitic drugs originate from natural products, being a new mean to obtain novel drug candidates. We demonstrated that the boronated polyketide Tartrolon E (trtE) has antiparasitic activity against different members of the Apicomplexa phylum, including *in vitro* and *in vivo* CP infections. The half maximal effective concentration (EC50) in CP infected human intestinal adenocarcinoma cells was determined to be 3.85 nM, with a high selectivity index over the parasite. Our *in vivo* results showed that orally infected neonatal mice ($1x10^4$ CP oocysts) had a significant lower burden of infection determined by intestinal qRT- PCR when treated with 4 doses of 4 mg/kg (every 12 h) starting 48 h postinfection. Moreover, a lower number of oocysts being shed in feces were observed when young CP infected INF knockout mice (1x105 CP oocysts) were treated similarly with 10 mg/kg starting 5 days postinfection ($p<0.05$). Our preliminary results on infected neonatal lambs, treated with 300 µg/kg of compound (5 doses) determined an average compound half-life of 6.1 h with no toxicity signs when administered. The preliminary effective antiparasitic activity of trtE in different CP rodent models of infection together with the lack of toxicity both in mice and lambs suggests that the compound could be a new therapeutic resource for Cryptosporidiosis.

TC3- *In vitro* and *in vivo* activities of a trithiolato-diRuthenium complex conjugated with sulfadoxine against the apicomplexan parasite *Toxoplasma gondii*

Boubaker G.[1], Bernal A.[1], Vigneswaran A.[1], Imhof D.[1], Ferreira de Sousa M.C.[1], Hänggeli K.P.A.[1]*, Haudenschild N.[1], Furrer J.[2], Paunescu E.[2],

Desiatkina O.[2], Hemphill A.[1]

[1]*Institute of Parasitology, University of Bern, Switzerland*
[2]*Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern, Switzerland*

*Presenting author: kai.haenggeli@unibe.ch

Recent developments in organometallic chemistry have introduced novel strategies for combating parasitic infections. A new trithiolato-diRuthenium complex conjugated with sulfadoxine (RU-SDX) has demonstrated potent antiparasitic activity against *Toxoplasma gondii*. This hybrid compound inhibited tachyzoite proliferation in human foreskin fibroblast monolayers with an IC50 of less than 150 nM, outperforming both the individual components and their equimolar mixtures. Notably, RU-SDX also showed reduced cytotoxicity in host cells, a significant improvement over the parent compounds. RU-SDX did not impair murine splenocyte proliferation at concentrations up to 0.5 μM and exhibited no embryotoxicity in zebrafish at 0.2 or 2 μM. The compound acted parasitostatically rather than parasiticidally and caused early transient mitochondrial ultrastructural changes in tachyzoites without affecting mitochondrial membrane potential, distinguishing it from other mitochondrion-targeting agents like FCCP and CCCP. In vivo efficacy studies using a murine *T. gondii* oocyst infection model showed that RU-SDX reduced parasite loads in ocular and cardiac tissues, although no significant effect was observed in cerebral parasite burden. These findings suggest that RU-SDX could be a promising candidate for targeted antiparasitic therapy, offering a balance between efficacy and host safety. Further exploration of its mechanism and therapeutic potential in other models is warranted to fully understand its capabilities and limitations.

TC4- Marine compound Tartrolon E rapidly blocks *Toxoplasma gondii* host cells invasion capacity without affecting viability, morphology, nor attachment capabilities

Fumuso F.G.[1]*, O'Connor R.M.[1]

[1]*Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota. Saint Paul, MN*

*Presenting author: fumus003@umn.edu

Toxoplasmosis is a worldwide parasitic disease caused by the Apicomplexa *Toxoplasma gondii*. The parasite is responsible of severe neurological illness in patients with immunosuppression (HIV-AIDS and organ recipients), and congenital disorders when transplacental primo infection occurs during pregnancy. New effective antiparasitic compounds to treat chronic cystic stages that are resilient to current available treatment are needed. The urgence of effective drug candidates to avoid congenital disease is a reality, since 10% of congenital infections are unresponsive to current therapeutics. Tartrolon E (trtE) is a marine secondary metabolite produced by the shipworm symbiont *Teredinibacter turnerae*. The compound has high selectivity against multiple human and animal apicomplexan parasites including *T. gondii*, *Cryptosporidium parvum* and *Plasmodium falciparum*, among others. We established that trtE exerts inhibitory activity against both extracellular and intracellular stages of *T. gondii*, blocking 80% of its infective capacity after only 30 minutes of tachyzoites exposure to compound (100 nM). Our results showed that, trtE rapid antiparasitic effect does not compromise parasite viability or host cell attachment but has a significant impact of tachyzoites invasion capacity. Prolong exposure to the compound for more than 4 h significantly affected parasite survival rate and altered its normal morphology. These effects were found to be irreversible when parasites were allowed to infect host cells for a period 24 or 48 h after treatment with trtE (100 nM). We determined that trtE has a rapid parasiticidal action on *T. gondii*, with an

irreversible effect blocking invasion capacity after short exposure and affects its normal morphology. The mechanism of action of trtE is still unknow but it would include blocking parasite invasion mechanisms thus, further research is needed it to determine the molecular target of trtE to further progress the compound as an antiparasitic candidate.

TC5- Repurposing of COVID Box and Kinetobox to discover potential drugs against *Besnoitia besnoiti* infection

Rubio-Elbal D.[1], Álvarez-García G.[1]*, Matas Aguado D.[1], Ortega- Mora L.M.[1], Ferre-Pérez I.[1]

[1]*SALUVET, Animal Health Department, Faculty of Veterinary Sciences*

*Presenting author: gemaga@ucm.es

Bovine besnoitiosis, caused by the apicomplexan parasite *Besnoitia besnoiti*, is a relevant cattle disease in sub-Saharan Africa, several parts of Asia and in Western and South Europe. Clinical manifestations range from asymptomatic to severe illness characterized by high fever, anasarca, scleroderma, and it can lead to sterility in bulls. Nowadays control relies on diagnosis and management measures since no treatment has been approved and a life vaccine is solely used in Israel. Pharmacological repositioning is a widespread approach in the search for potential candidates to combat different diseases. Indeed, a previous study identified diclazuril and decoquinate as potential therapeutic candidates against *B. besnoiti* infection. The aim of the present work was to perform an *in vitro* safety and efficacy pharmacological screening of available drugs collections against *B. besnoiti* infection: i) COVID Box provided by Medicine for Malaria Ventures (160 compounds);ii) Kinetobox, further divided into ChagasBox, HatBox and LeishBox, provided by GlaxoSmithKline (222, 192 and 192 compounds, respectively). All assays were performed in Marc-145 cell monolayers in 96 well plates. A first assessment of safety by XTT kit determined that 522 compounds didn't show cytotoxicity at 10 uM concentration. Next, 37

drugs inhibited parasite invasion by 90% when added at 0- and 6-hours post-infection. An in-silico analysis was also performed to evaluate the most important pharmacokinetic properties and 24 compounds including cyclosporine, loratadine, ivermectin and salinomycin were selected based on mutagenic, tumorigenic, irritant or reproductive effects. When the Half-Inhibitory Concentrations (IC50) were calculated seven potential drugs showed inhibition of *B. besnoiti* tachyzoites growth at nanomolar range: one belonging to ChagasBox (TCMDC-143387), five to LeishBox (TCMDC-143531, TCMDC-143568, TCMDC-143603, TCMDC-143573, TCMDC-143554) and one to COVID Box (MMV180312 known as Salinomycin). This work has identified promising compounds for the treatment of besnoitiosis, which should be considered for future *in vivo* trials.

TC6- Drug discovery against *Sarcocystis neurona* infection

Kubota R.[1]*, Hernández H.O.[2], Nihei C.[3], Igarashi M.[3], Igarashi M.[1], Nishikawa Y.[1]

[1]*National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan*
[2]*Equine Medicine Chair, Faculty of Veterinary Science, La Plata University, La Plata, Argentina*
[3]*Institute of Microbial Chemistry, Shinagawa, Tokyo, Japan*

*Presenting author: kubot1121@obihiro.ac.jp

The apicomplexan parasite *Sarcocystis neurona* is the main pathogen of equine protozoan myeloencephalitis (EPM), a serious neurological disease of horses. Many horses in North and South America are at risk of infecting *S. neurona*. The treatment success rate of EPM with pyrimethamine and sulfadiazine has been estimated to be 60%–70% and the relapse rate of 10%, making treatment with these drugs insufficient.

Identification of new drugs for EPM is needed to control the high treatment and relapse rates. In this study, we evaluated the anti-*Sarcocystis* activity of Kijimicin and Metacytofilin, which anti-*Toxoplasma* activity was confirmed in our lab, based on GFP-expressing *S. neurona*. The results showed that Kijimicin ihibited growth of *S. neurona*. This study will provide facts in the search for new therapeutic drugs against EPM and elucidate the biological pathways required for cellular infection, which *S. neurona* shares with other apicomplexan parasites.

TC7- *Cryptosporidium* specific IgY antibodies reduce severe diarrhea in an experimental calf model of infection and disease

Mira A.[1]*, Garro C.[2], de Alba P.[2], Lang, C.[1], Vivas A.[1], Medina E.[1], Franco J.C.[1], Schnittger L.[2], Parreño V.[3], Wigdorovitz A.[1], Bok M.[3]

[1]*Bioinnovo S.A. Castelar, Buenos Aires, Argentina*
[2]*IPVet, CICVyA, INTA Castelar, Buenos Aires, Argentina; Cecilia Lang; Bioinnovo S.A. Castelar, Buenos Aires, Argentina*
[3]*INCUINTA, CICVyA, INTA Castelar, Buenos Aires, Argentina*

*Presenting author: amira@bioinnovo.com

Cryptosporidium parvum is the leading cause of neonatal calf diarrhea (NCD) on many farms worldwide. As neither a vaccine nor an effective treatment is available in Argentina, this study aimed to develop an IgY treatment to control *C. parvum* NCD and test the efficacy of the product in an experimental infection model consisting of 8 newborn Holstein male calves. The IgY product was produced by immunizing intramuscularly 60 Leghorn White hens with 4 doses (T0, T15, T45 and T60) of vaccine candidates p23 and TRAP expressed in the baculovirus system and spray-dried. Four calves were provided with 2 liters of IgY-free milk twice daily, while the remaining four calves were administered 2 liters of milk supplemented with 20 grams of the product (including a p23-IgY titer of 1024 and TRAP-IgY titer of 4096 in 10 mg/ml) twice daily

for 14 days starting one day prior to infection. Fecal samples were scored (0-3) and stored daily until day 21 post-infection (PID). Oocyst excretion was quantified by qPCR (18S RNA gene). Animals of both groups became infected and developed diarrhea. Diarrhea onset was with 5.5 and 7.7 days PID non-significantly different between treated and control animals, respectively (p=0.437). The mean duration of diarrhea was 3.8 and 6.5 days PID for treated and control animals, respectively, and significantly different (p=0.032). Additionally, the severity of diarrhea as measured by the area under the curve (AUC) was highly significantly lower in treated (14.3) vs control calves (25.4) (p<0.0001). Both groups showed similar oocyst excretion patterns starting at day 5.4 and day 7.8 (p=0.564), an identical duration of 10.4 and 10.0 days (p=0.651), and a similar AUC (p=0.843) for treated and control animals, respectively. This is the first efficacy study of an IgY-based product that reduces severe *C. parvum* diarrhea in experimentally infected calves.

TC8- *In vitro* assessment of antimicrobial peptides as potential agent against *Toxoplasma gondii*

Amdouni Y.[1]*, Boubaker G.[1], Müller J.[1], Imhof D.[1], Hemphill A.[1]

[1]*Department of Infectious Diseases and Pathobiology, Institute of Parasitology, Vetsuisse Faculty, University of Bern, Switzerland*

*Presenting author: amdouniyosra.ay@gmail.com

Toxoplasmosis is a zoonotic disease caused by the protozoan parasite *Toxoplasma gondii* that afflicts humans and animals worldwide. Antimicrobial peptides (AMPs) have been characterized as potent antiprotozoal agents. This study is aiming at the *in vitro* assessment of toxicity of AMPs, particularly tyrothricin to *T. gondii* tachyzoites and host cells and the characterization of its mode of action. So far, our results showed that tyrothricin inhibited proliferation of *T. gondii* tachyzoites *in vitro* with a half inhibitory concentration (IC50) in the low-nanomolar range (< 100 nM). Furthermore, tyrothricin exhibited a non-specific

toxicity. Tyrothricin did not impair the *in vitro* proliferation of murine splenocytes at concentrations ranging from 0.1-0.5 µM but did so at 1 and 2 µM. Studies on zebrafish embryotoxicity showed that the peptide induced embryotoxicity at 2 µM, but not 0.2 µM. Tyrothricin treatment of *T. gondii*-infected cultures for 3 days did not exert parasiticidal activity, since parasites readily resumed proliferation upon drug removal, and continuous treatments of infected cultures for longer periods of time (five days, at 0.5µM) showed that *T. gondii* tachyzoites readily adapted to the continuous presence of increased tyrothricin concentrations. Study of potential changes in the ultrastructure of *T. gondii* tachyzoites treated with tyrothricin using transmission electron microscopy showed that compound can affect the mitochondria. Furthermore, *in vitro* assessment of the two components revealed that both peptides inhibited the *in vitro* proliferation of *T. gondii* tachyzoites with IC50 values of less than 0.01 µM and 0.2 µM, respectively. Comparative assessment of gramacidin and tyrocidine in the zebrafish demonstrated that gramicidin did not induce any malformations or mortality at concentrations up to 10 µM, while tyrocidine treatment resulted in 100% mortality of zebrafish embryos already at 2 µM. To get more insights into the mode of action of tyrothricin we will investigate the involvement of proteases, assess the proteomes of treated versus non-treated tachyzoites and perform affinity chromatography.

TC9- *In vitro* anticoccidial effect of naringenin and dehydrated grapefruit peel (*Citrus x paradisi*)

Pérez-Fonseca A.[1]*, Alcalá Canto Y.[2], Gutierrez Olvera L.[3], Ortega Cerrilla E.[4], Guerrero Molina C.[2], Ballesteros Rodea G. [5]

[1]*ClearVet, CDMX, Mexico*
[2]*Departamento de Parasitología, Facultad de Medicina Veterinaria y Zootecnia, UNAM, CD.MX., México*
[3]*Departamento de Fisiología y Farmacología, Facultad de Medicina Veterinaria y Zootecnia, UNAM, CD.MX., México*

[4]*Colegio de Posgraduados, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma Chapingo, Montecillo, México*
[5]*Departamento de Patología, Facultad de Agronomía y Veterinaria, Universidad Autónoma de San Luis Potosí*

*Presenting author: agustnprz@gmail.com

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, Bovine Umbilical Cord Endothelial Cells (BUVEC) were cultured and infected with *Eimeria ninakohlyakimovae* sporozoites and then exposed to different concentrations of a naringenin solution. Other variables were assessed during this study, such as pharmacokinetic variables and antioxidant properties of naringenin, oxidative stress and nitric oxide production. 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 Toltrazuril (TTZ) groups, and the invasion rate of sporozoites of *E. ninakohlyakimovae* and the number and size of schizonts/mm2 of the intestinal cells culture were reduced. These findings demonstrate the beneficial activities of naringenin and GDP on caprine coccidiosis *in vitro*.

TC10- Reduction of abortion rates in an Argentinean beef herd by culling *Neospora caninum* seropositive heifers

De Villafañe P.[1], Cirone K.[2], Campero L.M.[2], Morsella C.[2], Fiorani F.[3], Moore D.P.[2,3] *

[1]*Veterinario de la Actividad Privada*
[2]*Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Balcarce, Buenos Aires, Argentina*
[3]*Instituto de Innovación para la Producción Agropecuaria y el Desarrollo Sostenible (IPADS Balcarce), Instituto Nacional de Tecnología Agropecuaria Estación Experimental Agropecuaria Balcarce (CONICET-INTA), Balcarce, Buenos Aires, Argentina*

*Presenting author: moore.dadin@inta.gob.ar

The cattle industry is of substantial socioeconomic significance in Argentina, with a population of 45 million beef cattle. The average annual per capita consumption of beef is 50 kg, and in 2020, exports amounted to 605,845 tons, generating revenue of US$ 2.718 billion. Nearly 45% of Argentina's beef cattle are raised in the Humid Pampa ecoregion. A primary objective of beef production systems is to maximize the calves produced per breeding cow each year. While pregnancy rates are often optimal, early and late reproductive losses remain a persistent challenge within the livestock sector. Abortion and perinatal mortality rates, ranging from 5% to 12%, are common across beef production systems. This study aimed to describe the reduction of overall abortion rates in a beef herd located in the Argentinean pampas by culling *Neospora caninum* seropositive heifers. In early 2014, 10.8% of 260 pregnant heifers experienced abortion due to *N. caninum*. The diagnosis was confirmed through serological testing, which revealed a significant correlation between seropositivity and abortion, alongside characteristic histopathological findings of multifocal necrotizing non-suppurative meningoencephalitis in one fetus. In response, a control strategy was implemented annually from 2016 to 2021, involving the

serological screening of replacement heifers, with 2,801 serum samples tested for specific antibodies using a commercial indirect ELISA. By culling seropositive heifers (ranging from 20.8% of 336 heifers to 32.2% of 245 heifers), the overall abortion rate declined from 5.8% in 2016 to 2.1% in 2021. Notably, the abortion rate decreased by 1.5% for every 10% increase in culling (R^2 = 0.45; p = 0.06). Although the beef cattle industry is a cornerstone of Argentina's economy, and the diagnosis of *N. caninum* is well- established, this study represents the first documented case of a successful strategy to reduce *Neospora*-associated abortions in a beef herd within the pampas.

TC12- Efficacy of a calcium-dependent protein kinase 1 inhibitor, BKI-1708 in animal infection models of *Cryptosporidium*

Ojo K.K.[1]*, Choi R.[1], Hulverson M.A.[1], Schaefer D.A.[2], Betzer D.P.[2], Riggs M.W.[2], Huang W.[3], Sun V.[4], Whitman G.R.[1], McCloskey M.C.[1], Marsh K.[5], Buck W.R.[5], Ajiboye J.[6,7], Hemphill A.[7], Sigalapalli D.K.[3], Arnold S.M.L.[4], Barrett L.K.[1], Fan E.[3], Van Voorhis W.C.[1]

[1]Department of Medicine, Division of Allergy and Infectious Diseases, Center for Emerging and Re-emerging Infectious Disease (CERID), University of Washington, Seattle, WA 98109, USA
[2]School of Animal and Comparative Biomedical Sciences, College of Agriculture and Life Sciences, University of Arizona, Tucson, AZ 85721, USA
[3]Department of Biochemistry, University of Washington, Seattle, WA, 98195, USA
[4]Department of Pharmaceutics, University of Washington, Seattle, WA 98195, USA
[5]Research and Development, AbbVie Inc, North Chicago, IL 60064, USA
[6]Cellular, Molecular and Biomedical Sciences, University of Vermont, Burlington, VT 05405, USA
[7]Institute of Parasitology, Vetsuisse Faculty, University of Bern, Länggass-

Strasse 122, CH-3012, Berne, Switzerland

*Presenting author: ojo67kk@uw.edu

Cryptosporidium species frequently cause chronic infection in immunocompromised people and gastrointestinal injury in malnourished children, leading to wasting, stunting, and cognitive impairment. The current therapeutic of clinical cryptosporidiosis, Nitazoxanide, largely fails in these vulnerable populations, highlighting the need for new drugs. Here we report the anti-*Cryptosporidium* efficacy of bumped kinase inhibitor, BKI-1708. BKI-1708 inhibits an essential molecular target, calcium-dependent protein kinase 1(CDPK1). BKI-1708 clinical efficacy was demonstrated in the *Cryptosporidium parvum* IFNγ-KO mouse infection and calf diarrhea models. A dose response assay in the mouse model demonstrated that orally administered BKI-1708 as low as 15 mg/kg daily for 3 days completely suppressed oocyst shedding. A metabolite of BKI-1708 (M2) was identified, which retains sub-micromolar activity against *C. parvum*. Pharmacokinetic analysis of BKI-1708 and M2 in mice demonstrates good systemic compound distribution, an essential property for effective treatment of biliary- and respiratory-associated cases of cryptosporidiosis. In mice, the M2 metabolite reaches 7-fold and >3-fold higher levels over BKI-1708 in plasma and the gastrointestinal tract, respectively. Oral administration of M2 fully suppressed oocyst shedding in the mouse model at dosage as low as 8 mg/ kg for 3 days. Multi-day dosing of BKI-1708 in mice, rats, and dogs demonstrates safety at >20-fold exposures necessary for therapy. Therefore, BKI-1708 is an attractive preclinical candidate that delivers dual compound therapy of cryptosporidiosis.

SESSION 5

In vivo and *in vitro* models

Keynote. Ruminant models for tissue cysts-forming apicomplexan parasites: reflections on the parasite and the host… and a plea for consistency

Ortega-Mora L.M.[1,2] on behalf of the members of the SALUVET group

[1]*SALUVET, Animal Health Department, Faculty of Veterinary Sciences, Complutense University of Madrid, Ciudad Universitaria s/n, 28040, Madrid, Spain.*
[2]*SALUVET-Innova S.L., Faculty of Veterinary Sciences, Complutense University of Madrid, Ciudad Universitaria s/n, 28040 Madrid, Spain.*

luisucm@vet.ucm.es

Toxoplasma gondii and *Neospora caninum* are tissue cyst-forming apicomplexan parasites that suppose a relevant concern for ruminant health due to their ability to be congenitally transmitted and cause reproductive failure. In both cases, parasite interactions with the placenta determines the course of infection, leading to foetal death or parasite transmission to the offspring. No less important is the fact that congenital toxoplasmosis in humans could cause a severe infection with devastating sequelae.
In the last years, there has been a passionate debate within the scientific community about the necessity to select representative models for the diseases under study and the translationality from animal models to human beings. Regarding congenital toxoplasmosis and neosporosis there are still important gaps in knowledge on the placental host-parasite interactions that need to be addressed; in such cases, the pregnant ruminant models of infection are indispensable for providing

insights into the disease pathogenesis and for the vaccine and drug development and testing.
Particularly, in the latest, regulatory agencies require information concerning the safety and efficacy of the compound that it is going to be commercialized that originates from two types of trials: i) laboratory tests that should be performed under controlled conditions starting, wherever possible, with seronegative animals by challenge of the target animals under the recommended conditions of use (e.g., amount of challenge organisms, route of administration, etc.); and ii) field trials required to confirm the results of laboratory studies or to demonstrate efficacy when meaningful vaccination–challenge studies are not feasible. In our presentation, parasite and host components of ruminant toxoplasmosis and neosporosis congenital models will be updated and pros and cons presented. In addition, the use of sheep as a suitable alternative model for human congenital toxoplasmosis will be discussed with some examples confirming its usefulness.

ORAL PRESENTATIONS

IVIVM1- Contrary to the accepted paradigm that type III *Toxoplasma gondii* strains show remarkable diversity of virulence degrees evaluated in a harmonized mouse model

Salas-Fajardo M.Y.[1]*, Velasco-Jiménez N[1]., Huertas-López A.[1,2], Hecker Y.P.[1], Su C.[3], Schares G.[4], Álvarez-García G.[1], Ortega-Mora L.M.[1], Calero-Bernal, R.[1]

[1]Animal Health Department - Complutense University of Madrid, Madrid, Spain
[2]University of Murcia, Murcia, Spain
[3]University of Tennessee, Knoxville, United States
[4]Institute of Epidemiology, Friedrich-Loeffler-Institut - Federal Research Institute for Animal Health, Greifswald, Germany

*Presenting author: marthays@ucm.es

Toxoplasma gondii is a protist with a significant impact on public and animal health worldwide. The genotype of *T. gondii* strain along with the host's genetic background, are considered key in the infection's outcome and disease severity. Based on mouse mortality models, type III strains have been classified as non-virulent (LD_{50} >10^5).
Allelic combination of the *CS3*, *ROP18* and *ROP5* genes are proposed as highly predictive genetic markers of acute virulence in murine models. Recent observations showed the potential mouse virulence of type III strains and reported lower predictive values for such specific genetic markers. Therefore, we aimed to assess the mouse virulence of 32 *T. gondii* type III isolates using a harmonized outbred mouse model inoculated intraperitoneally with 10^3 tachyzoites. Mortality, cumulative morbidity and brain cyst loads (determined by immunolabelling with DBL-FITC) were assessed at 30 days post infection (dpi), and *CS3*, *ROP18*, and *ROP5* alleles were identified by PCR- seq. Of the 32 isolates evaluated, 28% showed high virulence, 28% were intermediately virulent, and 44% showed low virulence. All isolates caused morbidity from 7 dpi in 100% of the inoculated mice, and there was a correlation between mortality and the severity of clinical signs. Meanwhile, the number of brain cysts recorded was not associated to the degree of virulence. Finally, CS3, ROP5, and ROP18 markers expressed alleles associated with non-lethal strains. In contrast to the classical virulence paradigm, our results confirm that type III isolates exhibit a diverse range of mouse virulence profiles and highlight the limitations of the predictive value of genotyping for virulence. Future studies should focus on genotype- phenotype associations using high-throughput sequencing techniques and a broader set of phenotypic traits.

IVIVM2- Differences in virulence and oocyst shedding profiles in lambs experimentally infected with different isolates of *Cryptosporidium parvum*

Bartley P.[1]*, Thomson S.[1], Jonsson N.[2], Taroda A.[3], Innes E.[1], Katzer F.[1]

[1]*Moredun Research Institute, Edinburgh, Scotland*
[2]*University of Glasgow, Glasgow, Scotland*
[3]*Universidade Estadual de Londrina, Londrina, Brazil*

*Presenting author: paul.bartley@moredun.ac.uk

Cryptosporidiosis is a zoonotic disease known to affect humans and domestic livestock species worldwide. Infections with Cryptosporidium parvum are of particular importance, as they can cause severe and even fatal clinical disease, particularly in neonatal farm animals and immunologically vulnerable humans.
This presentation describes two experimental infections in lambs, which aimed to investigate differences in the clinical manifestations following infection with two distinct isolates of Cryptosporidium parvum at different ages.
In Exp-1, groups of naïve lambs (<1 week of age) were challenged with one of two C. parvum isolates (CP1 or CP2) and monitored for 6 weeks. In Exp-2, one group of lambs that was challenged at <1 week of age (CP1) was re-challenged with the same isolate at 6 weeks of age (CP1), while a second group was challenged for the first time at 6 weeks of age (CP1).
For both experiments, daily clinical observations were made regarding the feeding behaviour and general demeanour of the lambs. The total faecal output of each lamb was collected, and the faecal consistency of each sample was recorded. During the clinical stage of infection (0-21 days post-challenge) the number of oocysts shed by each animal was determined by microscopy.

The results from Exp-1 demonstrated differences in feeding and faecal consistency scores and significant differences in demeanour and total oocyst output when comparing lambs challenged with CP1 or CP2 at age <1 week. In Exp-2, lambs challenged at age 6 weeks (primary and re-infection) demonstrated no clinical signs of disease/diarrhoea however they still shed large numbers of oocysts.
These studies show that isolates of *C. parvum* have different pathogenicity and prior exposure to the parasite does not protect against re-infection, even with the same isolate, and apparently clinically normal lambs can shed large numbers of oocysts.

E-POSTERS

IVIVM3- Experimental infection model in calves with *C. parvum*: infectious dose, diarrhea and oocyst excretion

Garro, C.[1]*, Mira, A. [2], Franco J C. [2]; De Alba, P. [1], Wigdorovitz, A. [2], Bok, M. [2], Schnittger, L.[1]

[1] *Instituto de Patobiología Veterinaria, INTA Castelar*
[2]*INCUINTA, CICVyA, INTA Castelar, Buenos Aires, Argentina*
[3]*Bioinnovo S.A. Castelar*

*Presenting author: garro.carlos@inta.gob.ar

Cryptosporidiosis of calves is caused by the enteroprotozoan Cryptosporidium parvum. The disease manifests in severe diarrhea and massive excretion of the infective oocyst stage with feces. There are multiple studies on the prevalence of the disease, but information on the relation between the infection and the clinical response is scarce. The objective of this study was aimed to described and determine the relation between the infective dose (ID) of the oocysts, and the clinical response as determined by onset and duration of (i) diarrhea and (ii)

oocyst excretion of experimentally infected calves. Each of thirteen calves included in the study were challenged orally with an aqueous suspension of one of the following doses of C. parvum oocysts: 100, 3 x 103, 1 x 104, 2 x 104, 2 x 105, 3 x 106, 6 x 106, 2 x 107 and 5 x 107. Animals were evaluated for 21 days post infection (pi) and data analyzed by Pearson correlation and linear regression. All calves developed diarrhea and excreted oocysts with feces. The mean time to onset of diarrhea was 3.6 ± 1.3 days pi and lasted for 5.6 ± 1.1 days. The mean time to onset of fecal oocyst shedding was 5.4 ±2.5 days pi and lasted for 8.1 ± 2 days. The ID was correlated with the duration of diarrhea (r=0.72) and oocyst excretion (r=0.73). As the log-dose increased by 1, the duration of diarrhea and oocyst excretion increased by 0.2 (p=0.005) and 0.3 (p=0.003) days, respectively. The established experimental infection model allows to investigate preventive and therapeutic therapies.

SESSION 6

Parasite-host interactions and pathogenesis

Keynote. The role of dogs as definitive hosts of *Neospora caninum* and several other cyst-forming coccidian parasites.

Gondim P.

Laboratory of Coccidian Protozoa, School of Veterinary Medicine and Animal Science, Federal University of Bahia, Brazil.

pita@ufba.br

Domestic dogs have a long history in proximity to humans and are frequent companion animals in all habitable areas. Besides the emotional connection with humans, dogs serve as working animals for numerous activities and can harbor various pathogens. Dogs are definitive hosts (DH) for several cyst-forming coccidia and can shed environmentally-resistant oocysts/sporocysts through their feces. Some coccidia of livestock that may be excreted by dogs include *Neospora caninum*, *Hammondia heydorni*, *Sarcocystis cruzi, Sarcocystis tenella*, *Sarcocystis arieticanis, Sarcocystis capracanis*, *Sarcocystis hircicanis, Sarcocystis miescheriana*, *Sarcocystis levinei* and *Sarcocystis bertrami/fayeri*. When dogs act as DH of these parasites, i.e., ingesting the parasites' bradyzoites and shedding oocysts/sporocysts in the feces, they usually don't become sick. Here, we aimed to address some peculiarities of the dogs as definitive hosts of *N. caninum*, *Hammondia heydorni,* and *Sarcocystis* spp.

ORAL PRESENTATIONS

PHIP1- Identifying functional genomic variants and genes associated with *Theileria* infection in cattle

Powell J.[1]*, Connelley T.[1], Morrison L.[1], Prendergast J.[1], Hassan M.[1]

[1]*The Roslin Institute, Edinburgh, UK*

*Presenting author: jpowell2@ed.ac.uk

Theileria parva is a tickborne apicomplexan parasite that causes East Coast fever (ECF), a lymphoproliferative disease that kills over 1 million cattle annually in sub- Saharan Africa. We previously identified a 6 Mb genomic region linked with tolerance to ECF in indigenous East African cattle. Additionally, we observed that infected cells from ECF-tolerant cattle showed reduced proliferation compared to susceptible cattle. A major challenge in exploiting the ECF tolerance region to develop strategies against ECF is identifying the causal variants within the region, the genes the variants regulate, and which of those genes regulate the proliferation phenotype. To address this challenge, we are utilizing high-throughput, unbiased genomic screening approaches to identify the variants and genes underpinning ECF tolerance. Prioritizing variants within the ECF tolerance region, we are using a massively parallel reporter assay (MPRA) to identify variants that regulate the expression of an exogenous reporter gene in *Theileria*-infected cells. In parallel, we are conducting genome-wide CRISPR/Cas9-based knockout (GeCKO) screens to identify genes that regulate the transformation and proliferation of infected lymphocytes. We will then integrate data from the MPRA and GeCKO screen to pinpoint causal genetic variants and the genes they regulate. We have successfully introduced DNA into *Theileria*-infected cells, overcoming a major barrier to employing such genomic screening approaches, and we are currently developing GeCKO screens on infected lymphocytes.

PHIP2- Early dynamics of *Toxoplasma gondii* infection in pregnant sheep

Sánchez-Sánchez, R.[1]*, Horcajo, P.[1], Velasco-Jiménez, N[1], Arranz- Solís, D.[1], Re M.[1,2], Valdivia, G.[2], Alonso-Díez, A.[2], Benavides, J.[3], Fernández, M.[3,4], Calero-Bernal, R.[1], Ortega-Mora, L.M.[1].[1]

[1]*SALUVET, Animal Health Department, Faculty of Veterinary Science, Complutense University of Madrid, Ciudad Universitaria s/n, 28040, Madrid, Spain.*
[2]*Animal Medicine and Surgery Department, Faculty of Veterinary Sciences, Complutense University of Madrid, Ciudad Universitaria s/n, 28040 Madrid, Spain.*
[3]*Institute "Ganadería de Montaña" (CSIC-ULE), 24346, León, Spain.*
[4]*Animal Health Department, Faculty of Veterinary Science, University of León, Campus de Vegazana s/n, 24071, León, Spain.*

*Presenting author: robers01@ucm.es

A novel clinical presentation of ovine toxoplasmosis named "early abortion" (EA) has been frequently described in experimental infections with *Toxoplasma gondii* oocysts in pregnant sheep. EA is characterized by its occurrence within the second week post- infection (pi), lesions of placental thrombosis and fetal leukomalacia and absence of the parasite in placental and fetal tissues. The pathogenic mechanism of EA is unknown and studies addressing the early dynamics of *T. gondii* infection in pregnant sheep, that could be related to the production of EA, are lacking. Therefore, the aim of this study was to investigate the clinical signs and the parasite dissemination and lesions in target organs at early stages of infection in sheep challenged orally with 1,000 sporulated oocyst of a type II isolate on day 90 of gestation and euthanized on days 3 (n=5), 6 (n=5) and 28 pi (n=5). Uninfected animals euthanized on days 4 (n=3) and 28 pi (n=3) served as controls. Infected animals showed fever from day 5 to 8 pi and EA occurred on day 8 pi in

80% of challenged animals. In the small intestine, parasite detection and lesions (congestion, oedema and petechiae) were scarce at days 3 and 6 pi. In the mesenteric lymph nodes parasite DNA detection and lesions were sporadic at day 3 pi. At day 6 pi, mesenteric lymph nodes draining the medial and distal jejunum had the highest parasite burden, they were enlarged and/or hemorrhagic and reactive hyperplasia was found in the microscopic examination. In placentomes, parasite DNA was not detected at day 6 pi, and although lesions such as congestion or endothelial activation were found in several placentomes, thrombotic lesions were sparse. In fetuses from EA on day 8 pi, leukomalacia was widely detected while autolysis did not allow histological evaluation of the placental tissues.

E-POSTERS

PHIP3- Cytokine expression in bovine fetuses spontaneously aborted by *Neospora caninum*

Sosa, E.[1]*, Plá, N.[2], Moore, D.P.[1,2], Quintana, S.[3], Marin, M.[1], Cantón, G.[1].

[1]*Instituto de Innovación para la Producción Agropecuaria y el Desarrollo Sostenible (IPADS), INTA Balcarce-CONICET, Argentina.*
[2]*Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Argentina.*
[3]*Instituto de Investigaciones en Producción, Sanidad y Ambiente (IIPROSAM), CONICET-UNMdP, Argentina. Facultad de Ciencias Exactas y Naturales- UNMdP Centro Científico Tecnológico Mar del Plata-CONICET Centro de Asociación Simple CIC-PBA. Argentina.*

*Presenting author: sosa.emiliano@inta.gob.ar

Neospora caninum is an apicomplexan protozoan that causes bovine abortions, which can occur at any stage of pregnancy. Nevertheless,

more severe lesions are typically observed during mid-gestation. Scarce information is available on the fetal immune response in spontaneous abortions caused by *N. caninum*. The aim of this study was to evaluate the mRNA expression of cytokines in bovine fetuses spontaneously aborted due to *N. caninum* in the second and third trimesters of gestation, received at INTA Balcarce, Argentina. Fetal age was estimated according to morphometric characteristics. Fetuses between 4-6 months (n=4) and ≥7 months of gestation (n=5), in which *N. caninum* was detected by PCR and compatible lesions were observed, with no other infectious agents present, were selected. Control bovine fetuses with no inflammatory lesions and absence of infectious agents [between 4-6 months (n=2); ≥7 months of gestation (n=7)] were also included. Fetal spleen was collected, and total RNA was extracted using Trizol, followed by DNase I digestion and cDNA synthesis using MMLV reverse transcriptase. The relative mRNA levels of cytokines characterizing a Th1 (IL-8, IL-12, IFN-γ, TNF-α) and Th2 (IL-4) response were determined by RT-qPCR. Results were expressed as the mean value of the change in mRNA levels with respect to expression in control fetuses. Mid-gestation fetuses aborted due to *N. caninum* showed significant upregulation of IFN-γ and IL-8 Th1 cytokines compared to control fetuses. In infected fetuses <7 months, IL-12 and TNF- α mRNA were not detected, indicating an inhibition of these cytokines respect to control fetuses, while IL-4 was undetected in both infected and control fetuses. No significant changes in IL-4, IL-12 and TNF-α were detected in fetuses ≥7 months of gestation. These findings could be associated with an acute infection during mid- gestation and an overcome of an acute stage during late gestation in an immunocompetent fetus.

PHIP4- Immune response differences in cattle breeds revealed by differential gene expression analysis following experimental *Neospora caninum* infection

Fiorani F.[1]*, Herik H.[2], Campero L.M.[3], Cobo E.[2], Moore D.P.[1,2], Corva P.M.[1]

[1]*Faculty of Agricultural Sciences, National University of Mar Del Plata, Balcarce, Argentina*
[2]*Faculty of Veterinary Medicine, University of Calgary, Calgary, Canada*
[3]*Institute of Innovation for Agricultural Production and Sustainable Development (IPADS), INTA-CONICET, Balcarce, Argentina*

*Presenting author: fiorani.franco@inta.gob.ar

Neospora caninum (*N. caninum*) is a leading cause of bovine abortion. Currently, there are no effective vaccines or pharmacological treatments against bovine neosporosis. Host immunity plays a critical role in the outcome of the infection. Exploring the immune response of different cattle breeds against *N. caninum* could provide information to develop control alternatives. The study aimed to evaluate differential gene expression (DGE) using RNA-Seq in peripheral blood mononuclear cells (PBMC) from 14-month-old female cattle experimentally infected with *N. caninum* belonging to two contrasting breeds. Four Argentine Creole (AC) heifers (beef breed with minimal genetic selection) and four Holstein (H) heifers (dairy breed intensively selected) were intravenously infected with live tachyzoites from reference NC-1 strain (3×10^8 tachyzoites/animal). PBMC samples were collected 7 days post-infection for total RNA extraction. RNA-Seq libraries comprising 26 to 38 million sequences (2x150) were generated. The libraries were quantified against the ARS- UCD1.3 reference transcriptome by Kallisto, and DGE analysis was conducted using DESeq2. Sequences from 13,530 genes were aligned. Experimental infection activated 121 genes in H and 162 genes in AC *N. caninum*-infected heifers. In Holstein *N. caninum*-infected heifers, the infection preferentially activated metabolic pathways such as the ubiquitin-proteasome system, NLRP3 inflammasome, antigen presentation via MHC II, cytokine production via Toll-like receptors, and response to oxidative stress. Otherwise, in AC *N. caninum*-infected heifers, cell division control, endocytosis mechanisms, cytoskeleton regulation, and chemotaxis were highlighted. This study is

among the first to compare gene expression between two cattle breeds *N. caninum* infected and suggest a distinctive breed effect on the host's immune response against infection. Understanding different immunological mechanisms could help elucidate why some breeds are more susceptible to the disease and identify resistant cattle to bovine neosporosis.

PHIP5- An overexpression screen identifies interferon stimulated genes that control *Toxoplasma gondii* in porcine cells

Ungogo M.A.[1]*, Linn O.[1], Burdon T.[1], Grey F.[1], Hassan M.A.[1]

[1]*Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, United Kingdom*

*Presenting author: Marzuq.Ungogo@ed.ac.uk

Acute toxoplasmosis can cause severe morbidity and up to 57% mortality in piglets, while chronic *Toxoplasma* infection suppresses porcine immunity and presents a zoonotic risk to humans through pork consumption. Similar to all vertebrates, the cytokine interferon, through the induction of hundreds of interferon-stimulated genes (ISGs), is critical to effective immunity against *Toxoplasma* in pigs. However, compared to mice and humans, very little is known about the ISGs that control *Toxoplasma* in pigs. Here, we sought to address this knowledge gap on ISGs that control *Toxoplasma* in pigs by using a novel porcine ISG expression library. We transduced Neonatal Swine Kidney (NSK) cells with plasmids expressing individual ISGs tagged with a red fluorescent protein in a one ISG per well format in a 96-well plate. The cells were then infected with type II *Toxoplasma* PRU parasite constitutively expressing luciferase and luciferase activity used as a proxy for parasite burden in each well. We screen for a total of 432 canonical porcine ISGs. Assuredly, our screen identified ISGs known to control *Toxoplasma* in human or murine cells including IRF1. In addition, we

identified novel anti-*Toxoplasma* ISGs, such as TMEM133, EBF1, and PSMB9. We are currently functionally validating these candidate ISGs by complementing the overexpression with knockout of the corresponding endogenous ISGs. This study reports novel regulators of *Toxoplasma* in pig cells.

PHIP6- Characterization of *Toxoplasma gondii* non-archetypal isolates provide new insights into their phenotypic traits and confirm the laboratory adaptation phenomena

Arranz-Solís D.[1], Coronado-Brieva M.[1], Calero-Bernal R.[1], Hassan M.A.[2], Gennari S.M.[3], Ortega-Mora L.M.[1]*

[1]*SALUVET group, Animal Health Department, Faculty of Veterinary Science, Complutense University of Madrid, Madrid, Spain*
[2]*University of Edinburgh, Royal (Dick) School of Veterinary Studies and The Roslin*
[3]*Faculty of Veterinary Medicine, University of São Paulo, São Paulo, Brazil. Institute, Edinburgh, United Kingdomoslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, United Kingdom*

*Presenting author: luis.ortega@ucm.es

While most *Toxoplasma* research has been carried out using archetypal strains (clonal types I, II or III), little effort has been paid regarding non-archetypal strains, which are clinically more relevant as they may cause severe toxoplasmosis in immunocompetent individuals. In addition, the majority of *T. gondii* research has been traditionally performed using strains propagated in laboratory conditions for decades, which has been shown to modify their phenotype from natural conditions. In order to increase our understanding of the relatively unknown phenotypic traits in non-canonical strains, a panel of five epidemiologically representative strains maintained at low passage numbers were studied *in vitro*, and their -omic dataset obtained to perform an integrative analysis of the

molecular basis underlying potential phenotypic changes: TgRhHmBr1 (ToxoDB #13), TgHumIMTBr1 (#312), TgDogBr20 (#6, BrI), TgCatBr39 (#11, BrII), and TgCatBr74 (#8, BrIII). Our preliminary results show that the tachyzoite-to-bradyzoite conversion rate differed between isolates, with the TgHumIMTBr1 strain showing the highest level of spontaneous conversion, while TgRhHmBr1, TgDogBr20 and TgCatBr74 did not increase their conversion rates even under inducing conditions. The Principal Component Analysis of the transcriptomic data showed a separation between low and high passages in all isolates, being more pronounced for TgHumIMTBr1 and TgDogBr20. Moreover, these two isolates showed the highest number of differentially expressed genes (DEG) between low and high passage numbers (796 and 716, respectively), most upregulated, while the other three strains had a majority of downregulated DEG. In TgDogBr20 and TgHumIMTBr1, an upregulation of terms associated with intracellular motility and transport is observed in high passages, while these terms are downregulated in the other isolates. Further *in vitro* assays, as well as *in vivo* virulence in outbred mice, will be performed to complete a general picture and assess differences with previous results obtained with archetypal strains.

PHIP7- Exploring ncrop2 role in *Neospora caninum* virulence through transcriptomic analysis of bovine macrophages

Amieva R.[1], Powell J.[2], Coronado-Brieva M.[1], Arranz-Solís D.[1], Hassan M.[2], Collantes-Fernández E.[1], Ortega-Mora L.M.[1]*, Horcajo P.[1]

[1]*SALUVET, Animal Health Department, Complutense University of Madrid, Ciudad Universitaria s/n, 28040 Madrid, Spain.*
[2]*Division of Immunology, The Roslin Institute, University of Edinburgh, Edinburgh, UK*

*Presenting author: Musa.Hassan@roslin.ed.ac.uk

Bovine neosporosis is a leading cause of transmissible abortion

worldwide. Despite its significance, the molecular bases determining parasite virulence are still unknown. We previously demonstrated that deletion of the rhoptry protein NcROP2 (NcLIV_001970) in *Neospora caninum* (using CRISPR–Cas9) was associated with a reduction in its virulence in a pregnant mouse model, and its lower proliferation in bovine macrophages. However, the functions of this rhoptry protein have not been elucidated. The goal of this study was to identify how this virulence factor modulate host cell signaling pathways by performing comparative transcriptome analyses of bovine monocyte-derived macrophages infected by the mutant strain, relative to the wildtype strain and uninfected controls. To explore host transcriptomic changes, we identified genes that were differentially expressed after infection with the knockout strain but not after infection with the wildtype strain when compared to the non-infected group. Notably, genes upregulated in cells infected with the mutant strain are involved in metabolic processes and signaling through mTORC1, which has previously been described as a host resistance factor that protects macrophages and inhibits parasite proliferation. Analysis of differentially expressed parasite genes identified 38 genes unique to the NcRop2 knock-out mutant. Overall, differentially expressed parasite genes are primarily associated with pathways involving rhoptry proteins, such as catalytic activity, protein heterodimerization activity, and ATP hydrolysis activity. In summary, our findings improve the understanding *of N. caninum* pathogenesis and how NcROP2 interfere with host cell functions, which may suggest potential targets for control of this parasite.

PHIP8- Modulation of host cell membrane fluidity by *Neospora caninum*: a study using laurdan fluorescence and hyperspectral imaging

Diaz M.[1], Cabrera A.[2,3]*, Robello C.[2,4], Malacrida L.[1,5]

[1]*Unidad Bioimagenología Avanzada, Institut Pasteur de Montevideo, Uruguay*
[2]*Laboratorio de Interacciones Hospedero-Patógeno, Institut Pasteur de*

Montevideo, Uruguay
[3]*Unidad Académica de Parasitología y Micología, Facultad de Medicina, Universidad de la República, Uruguay*
[4]*Unidad Académica de Bioquímica, Facultad de Medicina, Universidad de la República, Uruguay*
[5]*Unidad Académica de Fisiopatología, Facultad de Medicina, Universidad de la República, Uruguay*

*Presenting author: cabrera@pasteur.edu.uy

Neospora caninum, the causative agent of bovine neosporosis, leads to significant economic losses in livestock production. With no effective vaccine or treatment available, understanding the parasite's biology and its interaction with host cells is crucial. As an intracellular parasite, *N. caninum* invades host cells and creates a parasitophorous vacuole (PV) where tachyzoites multiply while evading the host's immune system. The parasite hijacks host lipid resources, including sphingolipids and cholesterol, which are vital for membrane integrity and energy storage. To investigate how *N. caninum* modifies the host membrane physical properties, we used the unique solvatochromic properties of LAURDAN fluorescence in combination with hyperspectral imaging and phasor plot analysis. In Vero cells infected with the NcLiv strain of *N. caninum*, a significant increase in membrane fluidity was observed in both the plasmatic and internal host membranes after 96 hours of infection. This membrane change suggests a redistribution on cell lipids due to NcLiv infection, causing an increased fraction in liquid-disordered membranes. Furthermore, the increased membrane fluidity can be reversed by treating infected cells with cholesterol-loaded methyl-beta-cyclodextrin, restoring membrane order to those of uninfected cells. These findings offer new insights into how *N. caninum* manipulates the host cell environment to promote its survival and replication, emphasizing the role of cholesterol sequestration in altering host membrane order and dynamics.

SESSION 7

Cellular and molecular biology

Keynote. Advances in population genetic studies of *Toxoplasma gondii* and their applications in the molecular epidemiology of toxoplasmosis

Su C.

The Department of Microbiology, University of Tennessee, Knoxville, TN 37996

csu1@utk.edu

Toxoplasma gondii is genetically diverse. There are only a few genotypes of *T. gondii* dominate in the northern hemisphere, however, many genotypes co-exist in South America and there is no clear dominance of any genotypes. In addition, *T. gondii* strains from South America tend to be more virulent in mice. Recent studies suggest that the rise and expansion of farming established the domestic cat-mouse transmission cycle for *T. gondii*, which played a significant role in the selection and spread of certain linages of *T. gondii*. In South America, *T. gondii* infection rates in human populations are high and often the infection is associated with severe acute disseminated toxoplasmosis in immunocompetent individuals. With the insight of global genetic diversity and available molecular tools, it is imperative to study *T. gondii* epidemiology and ecology at a finer scale to trace local outbreaks and characterize cases of toxoplasmosis in animals and humans. Accumulation of these data will facilitate control and treatment of toxoplasmosis in the future.

ORAL PRESENTATIONS

CMB1- An ISWI-related chromatin remodeler orchestrates parasite life cycle progression by insulating gene expression in a densely packed genome

Pachano B.[1]*, Farhat D. C.[1], Shahinas M.[1], von Velsen J.[2], Corrao C.[1], Belmudes L.[3], de Bock P. J.[3], Mas C.[4], Couté Y.[3], Bowler M. W.[2], Bougdour A.[1], Swale C.[1], Hakimi M. A.[1]

1 Institute For Advanced Biosciences (Iab), Team Host- Pathogen Interactions And Immunity To Infection, Grenoble,France
2 European Molecular Biology Laboratory, Grenoble, France
3 University Grenoble Alpes, Cea, Grenoble, France
4 Integrated Structural Biology Grenoble (Isbg), Cea, Embl, Grenoble, France

*Presenting author: pachanobelen@gmail.com

In eukaryotic cells, ATP-dependent chromatin remodelers are specialized multiprotein machines that organize the genome and regulate its accessibility by repositioning, ejecting, or modifying nucleosomes. However, their role in *Toxoplasma gondii* remains poorly understood. The aim of this study is to provide the first characterization of ISWI family remodeler complexes, one of the best-studied chromatin remodeling complexes in eukaryotes. Phylogenetic analyses show that the parasite retains the commonly recognized remodeler families and has evolved two divergent proteins within the ISWI family: *Tg*SNF2h and *Tg*SNF2L. Using bioinformatic and biochemical approaches, we found that these proteins are structurally divergent and form distinct complexes. Immunoprecipitation coupled with mass spectrometry revealed that *Tg*SNF2h specifically forms a core complex with the transcription factor AP2VIII-2 and the scaffold protein *Tg*RFTS. We performed a full characterization of the role of this complex in tachyzoites by conducting ATAC-seq, RNA-seq, and ChIP-seq in

engineered lines that allow the conditional knockdown of *Tg*SNF2h and its partner *Tg*RFTS. We found that depletion of *Tg*RFTS phenocopies the knockdown of *Tg*SNF2h, restricting access to chromatin and altering local gene expression. At the genomic level, *Tg*SNF2h actively insulates highly transcribed genes from their minimally expressed or silenced neighbors. This ISWI complex establishes and maintains stage-specific gene expression by regulating chromatin accessibility to transcription factors. Using MORC as a proxy for DNA binding, we have shown that *Tg*SNF2h rules chromatin accessibility and thereby exerts an epistatic control over a key regulator of sexual commitment. In a parasite characterized by high phenotypic plasticity and a compact genome, this dedicated ISWI complex orchestrates the partitioning of developmental genes and ensures transcriptional fidelity throughout its life cycle.

CMB2- *Neospora caninum* invasion and replication are modulated by cathelicidins in mouse bone marrow-derived macrophages

Fiorani F.[1]*, Lahiri P.[2], Hannawaya R.[2], Puentes R.[2], Cirone K. M.[3], Corva P. M.[1], Bradley P. J.[4], Moore D. P.[1,3], Cobo E. R. [2]

[1] Faculty of Agricultural Sciences, National University of Mar Del Plata, Balcarce, Argentina [2] Faculty of Veterinary Medicine, University of Calgary, Calgary, Canada

[3]Institute of Innovation for Agricultural Production and Sustainable Development (IPADS). INTA-CONICET. Balcarce, Argentina

[4] Department of Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles, Los Angeles, California, USA

*Presenting author: fiorani.franco@inta.gob.ar

Cathelicidins are host defense peptides that play a role in cytokine secretion, apoptosis, phagocytosis, and inflammasome activation. *Neospora caninum* (*N. caninum*) induces the expression of cathelicidins

in human macrophages, reducing parasite internalization. Furthermore, *N. caninum* induces the release of proinflammatory cytokines such as IL-1β and pyroptosis, a form of programmed lytic cell death. While this mechanism is crucial for combating microbial infections, excessive activation can contribute to pathogenesis. This study aims to evaluate the function of cathelicidins in bone marrow-derived macrophages (BMMs) from cathelicidin deficient mice (Camp -/-) during *N. caninum* infection. Bone marrow monocytes were isolated from Camp+/+ and Camp−/− mice. These monocytes were plated in 24-well plates, cultured for 6 days, and differentiated into macrophages (BMMs) using an L929 colony-stimulating factor. The BMMs were previously stimulated with LPS (100 ng/ml) for 3 hours and infected with *N. caninum* (GFP-Nc Liverpool) at a multiplicity of infection (MOI) of 3:1 for 24 hours. Intracellular tachyzoites were recovered and counted using a hemocytometer. Supernatants were harvested for IL-1β Elisa and lactate dehydrogenase (LDH) assay. Additionally, BMMs Camp -/- and Camp +/+ pre-treated and infected were plated on coverslips in 6-well plates for examination by confocal immunofluorescence microscopy. Actin was stained with TRITC Phalloidin, and nuclei were stained with DAPI. We observed that Camp -/- BMMs showed more intracellular tachyzoites of *N. caninum* than Camp +/+ ($p>0.05$). These results are consistent with the higher levels of cytotoxicity measured by LDH ($p>0.05$). Finally, we found an increased release of IL-1β in BMMs Camp-/- compared with Camp +/+ ($p> 0.05$). Our results show that cathelicidins could modulate *N. caninum* invasion and the pro-inflammatory effects of the parasite. Further studies are needed to understand the antimicrobial and immunomodulatory properties of cathelicidins in the pathogenesis of *N. caninum*.

E-POSTERS

CMB3- Mitochondrial damage and il-1 production of monocyte by *Neospora caninum* infection is mediated by dense granule protein 7 and prohibitin

Chen Y. [1], Shimoda N.[1], Nihei C.[2], Ushio-Watanabe N.[1], Nishikawa Y. [1]*

[1]*National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan*
[2]*Institute of Microbial Chemistry, Shinagawa, Tokyo, Japan*

*Presenting author: nisikawa@obihiro.ac.jp

The intracellular proliferation of *N. caninum* tachyzoites and the host immune response against the infection are key steps in the pathogenesis of neosporosis. In this study, we first demonstrated that the NLRP3 inflammasome in monocytes can be activated by NcGRA7 and mediates IL-1β release. The NcGRA7 knock-out parasite decreased host mitochondria damage and apoptosis in THP1 cells compared with the parental strain of *N. caninum*, suggesting NcGRA7 has a crucial role in host mitochondria and apoptosis process during infection. Furthermore, we identified NcGRA7- binding proteins, NcGRA7 formed a complex with PHB1 and PHB2 (which are related to host mitochondria). The localization of NcGRA7 was also confirmed, the endogenous NcGRA7 was detected in the fraction of mitochondria, and the sub- mitochondrial localization of the NcGRA7 indicated the distribution of NcGRA7 in the inner mitochondrial membrane to the matrix of host mitochondria. Using inhibitors of PHB1 and PHB2, or transfection of *N. caninum*-infected THP 1 cells with both PHB1 siRNA and PHB2 siRNA, significantly decreased IL-1β production. Understanding the role of NLRP3 inflammasome activation in *N. caninum* infection may contribute new insights into the development of therapeutic options or vaccine strategies to combat this pathogen.

CMB4- Innate immune response in *Neospora caninum* and BoAHV-1 co-infection: the interaction of TLRs and cathelicidins

Plá N.[1] *, Burucúa M. M.[2], Moore D. P.[1, 3], Odeón A. C.[1], Cobo E. R.[4], Quintana S.[5], Marin M. S.[2]

[1] *Unidad Integrada Balcarce (Facultad de Ciencias Agrarias, UNMDP/Estación Experimental Agropecuaria Balcarce, INTA), Balcarce, Buenos Aires, Argentina*
[2] *Departamento de Biología, Facultad de Ciencias Exactas y Naturales, UNMdP, Mar del Plata, Buenos Aires - Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina*
[3]*Instituto de Innovación para la Producción Agropecuaria y el Desarrollo Sostenible (IPADS) INTA-CONICET, Balcarce, Buenos Aires, Argentina;*
[4] *Production Animal Health, Faculty of Veterinary Medicine, University of Calgary, Canada* [5]*Instituto de Investigaciones en Producción, Sanidad y Ambiente (IIPROSAM), Facultad de Ciencias Exactas y Naturales, UNMdP, Centro Científico Tecnológico Mar del Plata CONICET, Centro de Asociación Simple CIC-PBA, Mar del Plata, Buenos Aires, Argentina - Instituto de Biología Molecular Aplicada, Mar del Plata, Buenos Aires, Argentina*

*Presenting author: nataliapla996@gmail.com

Neospora caninum causes significant global economic losses due to abortions in cattle. Immunosuppression induced by viral infection worsens neosporosis. However, the innate immune mechanisms in *N. caninum* and BoAHV-1 co-infections remain unknown. We previously showed an overexpression of toll-like receptor (TLR) 7 and LL-37 human cathelicidin in this co-infection. This study investigated in vitro effects of bovine alpha-herpesvirus-1 (BoAHV-1) infection on TLRs and cathelicidin antimicrobial peptides signaling, and *N. caninum* replication in co-infected neuronal cells. Human neuroblastoma SH-SY5Y cells were pretreated with chloroquine (inhibitor of endosomal TLRs, 10 µg/ml), imiquimod (agonist of TLR7, 5 µg/ml),sodium butyrate (promoter of

endogenous cathelicidin, 4 mM), or subjected to silencing of LL-37 (shLL-37), followed by infection with the reference strains *N. caninum* NC-1 (MOI: 0.2) and BoAHV-1 Cooper (MOI: 0.1). At 24 h post-infection, gene expression levels of key immune molecules involved in TLR mediated signaling pathways (NF-κβ, MyD88, IRF3, and IRF7) were assessed by RT-qPCR and tachyzoite replication was examined microscopically. The results indicated that *N. caninum* replication in co-infection with BoAHV-1 was significantly decreased both intracellularly and extracellularly after chloroquine pretreatment and shLL-37 compared to imiquimod and sodium butyrate treatments. This reduction was accompanied in co-infection by a significant increase, with respect to the control, in MyD88 and IRF3 expression (3.9-fold and 5.5-fold, respectively, $p<0.05$) after chloroquine treatment, and a decrease in IRF3 expression after shLL-37 (0.409-fold, $p<0.05$). While treatments with imiquimod and sodium butyrate resulted in a significant decrease, with respect to the control, in the expression of three of the four molecules analyzed (NF-κβ: 0.126 and 0.074; MyD88: 0.365 and 0.508; IRF7: 0.078 and 0.01, respectively, $p<0.05$). These findings highlight the role of TLR7 and LL-37 in co-infection, emphasizing their impact on dynamics.
TLR7 and LL-37 regulate BoAHV- 1 replication, promoting *N. caninum* replication when both pathogens are present.

CMB5- Virulence studies of *Neospora caninum* in goat trophoblast primary culture

Alvarez, B.E. [1,2]*, Rambeaud, M. [1], Dellarupe, A. [1,2], Campero, L.M. [2,3], Venturini, M.C. [1]

[1]*Laboratorio de Inmunoparasitología (LAINPA), FCV, UNLP, La Plata.*
[2]*CONICET.*
[3]*Instituto de Innovación para la Producción Agropecuaria y el Desarrollo Sostenible (IPADS) (CONICET-INTA Balcarce).*

*Presenting author: alvarezbetianaelizabeth@gmail.com

Neospora caninum is a well-documented cause of abortion in cattle worldwide. The relevance of the abortifacient role of this parasite in small ruminants remains under investigation, especially in areas with high seroprevalence. The objective of this study was to evaluate the virulence (tachyzoite yield [TY] and invasion rate [IR]) of a locally isolated *N. caninum* strain (*NC-Argentina LP1*) compared to a reference strain (*NC-1*) in goat trophoblast cells. Primary culture was obtained from cotyledons of a goat seronegative for *N. caninum* and *Toxoplasma gondii*, and negative by PCR, using the explant method. A total of 10^5placental cells/well (passage 4) were seeded into 24-well plates and grown to confluence (48 hours). For TY and IR studies, cultures were infected with $2x10^5$tachyzoites/well and 10^4tachyzoites/well of each strain, respectively, in 3 independent assays. At 48 h post infection, TY was quantified by qPCR, and IR was evaluated by immunolabeling of parasitophorous vacuoles (PVs) with anti-*N. caninum* hyperimmune serum and its conjugate (Alexa Fluor 488, Life Technologies) and DAPI staining. Fifty fields per replicate were counted and PV size was measured. Regarding TY, significant differences were found between *NC-Argentina LP1* and *NC-1*, with lower values for *NC-Argentina LP1* compared to *NC-1* ($p<0.01$). In addition, *NC-Argentina LP1* showed a lower IR than the reference strain ($p<0.0001$). However, the size of the PVs was larger for *NC-Argentina LP1* with respect to *NC-1* ($p<0.0001$), which could be related to its lower capacity to reinvade. These results suggest that *NC-Argentina LP1* can invade and replicate in primary cultures of goat trophoblast, although with lower virulence than the reference strain *NC-1*, and may aid in predicting *in vivo* behavior.

SESSION 8

Immunology and vaccines

Keynote. Modulating type and localization of adaptive immunity to improve protection against *Neospora caninum* and *Toxoplasma gondii*

Leitão, A.[1,2]

[1]*Centro de Investigação Interdisciplinar em Sanidade Animal (CIISA), Faculdade de Medicina Veterinária, Universidade de Lisboa, 1300-477 Lisboa, Portugal.*
[2]*Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), 1300-477 Lisboa, Portugal.*

*Presenting author: alexandre@fmv.ulisboa.pt

Toxoplasma gondii and *Neospora caninum* are tissue cyst-forming coccidian parasites with complex life cycles that include both prey-predator transmission and vertical transmission, presenting substantial challenges for vaccine development. Key pathogenic events occur in specific tissues: the gut, where the parasites initially invade the host; the brain and muscles, where bradyzoite reactivation happens; and the fetal-maternal interface, where excessive inflammation can cause abortion. Effective vaccines must target these critical sites and induce a properly polarized and regulated CD4 T cell response localized to the relevant tissues. Current subunit vaccines, however, struggle to elicit both the precise immune profile and the durable, tissue-specific protection needed.
Our research focuses on exploring TLR2 unique role in promoting balanced immune responses and gut-tropic T cell activation. In a pregnant mouse model, vaccination with antigens fused with a TLR2 ligand induced a mixed Th1/Th2 immune response in adult mice, led to significantly increased protection against cerebral infection and reduced

vertical transmission. We are now exploring TLR2 activation associated with other innate immune pathways to block parasite entry at the gut. Our studies show that combined activation of dendritic cells (DCs) through distinct toll-like receptors (TLRs) – TLR2, which alone does not induce clear Th1/Th2 polarization, and TLR4, which does not imprint mucosal tropism – enables non-mucosal DCs to prime gut-homing CD4+ T cells with enhanced Th1 polarization. This finding suggests that targeting DCs with defined combinations of innate stimuli is a promising strategy for directing T cell polarization and tissue localization and, therefore, provide a rational framework for designing future immunization strategies aimed at enhancing protection against *T. gondii* and *N. caninum* through precise modulation of adaptive immunity.

ORAL PRESENTATIONS

IV1- Evaluation of the humoral immune response of pigs immunized by intradermal and intramuscular routes with a multigene DNA vaccine (ROP18 + SAG1) against *Toxoplasma gondii*

Candeias A.P.M.[1], Silva A.C.S.[1], Jurkevicz R.M.B.[1], Cavallieri A.C.[1], Sasse J.P.[1], Nino B.S.L.[1], Rodrigues F.S., Barros L.D.[2], Garcia J.L.[1]*

[1]*Universidade Estadual de Londrina (UEL), Londrina, Paraná, Brasil*
[2]*Universidade Federal de Lavras (UFLA), Lavras, Minas Gerais, Brasil.*

*Presenting author: joaoluisgarcia10@gmail.com

The objective of the present study was to evaluate the humoral immune response against *T. gondii* from pigs immunized with a multigene DNA vaccine produced with partial sequences of the ROP18 and SAG1 genes. Fourteen pigs seronegative in Indirect Immunofluorescence Assay (IFA) for T. gondii (<1:64) were divided into four groups (G), animals from G1 (n=4) were vaccinated intradermally (ID); and G2 (n=3) intramuscularly

(IM) with 300 μg of the vaccine (ROP18+pcDNA3.1 and SAG1+pcDNA3.1); animals from G3 (n=4) received pure pcDNA 3.1 (300 μg) by IM route; and animals from G4 (n=3) received only saline solution (0.9%) by IM route. All groups received levamisole 2.0% (v/v) as adjuvant. The vaccines were performed on days (D): D0, D14, D35 and D56. The challenge was performed on D70, orally with sporulated oocysts of the ME49 strain (~4x103), and the slaughtered on D100.
Regarding the results obtained through ELISA-IgG, G2 (IM) presented a statistically significant difference in the immune response after the third dose of vaccine (OD = 0.287; $p < 0.0001$), in contrast, G1 (ID) only after the fourth dose (OD = 0.219).
Regarding the ELISA-IgM, G2 presented a statistically significant difference after the first dose of the vaccine (OD = 0.268; $p < 0.0001$), while G1 presented a response only after the third dose of the vaccine (OD = 0.205). These results contribute to the advancement of research, since pigs immunized with the partial gene segment of the *T. gondi*i ROP18 and SAG1 proteins in combination with Levamisole (2%) showed a constant increase in antibody levels starting before the challenge. The vaccine by IM route produced higher humoral immune response than ID route.

IV2- Development of next generation vaccine against *Toxoplasma gondii*

Nishikawa Y. *

National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan

*Presenting author: nisikawa@obihiro.ac.jp

Background and aims: Toxoplasma gondii infects almost all warm-blooded animals including humans. Because of the high incidence and severe consequences of T. gondii infection, a safe and suitable vaccine

is needed. Currently, T. gondii effector is one of the targets for development of subunit vaccine. Additionally, lipid nanoparticles (LNPs) are promising new platform to develop next-generation DNA/mRNA vaccines. In this study, we aimed at generation of Toxoplasma DNA vaccine with the LNPs.
Methods: A luciferase assay focused on nuclear factor-kappa B (NFκB) signaling was conducted for screening of effector proteins including rhoptry and dense granule proteins (GRAs). The LNPs consisting of a series of functional materials prepared with vitamin E, such as SS-cleavable and pH-activated lipid-like materials (ssPalmE), were prepared to encapsulate pCpGfree-T. gondii DNA.
Results: GRA7 and GRA14 were partially involved in the activation of NFκB, whereas GRA15 was essential for NFκB activation. We prepared ssPalmE-LNP to encapsulate pCpGfree-T. gondii dense granule protein 15 DNA (ssPalmE-LNPTgGRA15). Following a challenge infection with T. gondii, the mice immunized with ssPalmE-LNPTgGRA15 had a significantly higher survival rate and lower clinical scores compared with the control mice. Furthermore, mix administration of mice with three antigens significantly enhanced protective effects against the challenge infection.
Conclusions: This study provides new evidence that combination of the ssPalmE-LNP with DNA of T. gondii effector will induce host protective immunity for future vaccine development. Currently, we are performing vaccine and challenge experiment using sheep.

E-POSTERS

IV3- Identification of conserved putative surface antigens expressed in infective stages of *Eimeria tenella* and *E. acervulina* through reverse vaccinology

Britez J.D.[1,2]; Pisón-Martínez M.L.[1]; Cuba F.G.[3], Poklepovich T.J.[3]; Marugan-Hernandez V.[4]; Rodriguez A.E.[5] Tomazic M.L.[1,6]*

[1]*Instituto de Patobiología Veterinaria (IPVET), INTA-CONICET, Hurlingham, Argentina*
[2]*Universidad de Buenos Aires, Facultad de Veterinaria, Cátedra de Parasitología y enfermedades parasitarias, CABA, Argentina*
[3]*Unidad Operativa Centro Nacional de Genómica y Bioinformática - ANLIS "Dr. Carlos G. Malbrán", CABA, Argentina*
[4]*The Royal Veterinary College, University of London, London, UK*
[5]*Instituto de Patobiología (IP), IPVET, INTA-CONICET, Hurlingham Argentina*
[6]*Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Biotecnología, CABA Argentina*

*Presenting author: tomazic.mariela@inta.gob.ar

E. tenella (Et) and *E. acervulina* (Ea) are two of the most frequent species causing chicken coccidiosis, which has a huge economic impact on the poultry industry worldwide. Next-generation vaccines against coccidiosis are scarce and the identification of antigens that could effectively elicit host immunoprotection is needed. Glycosylphosphatidylinositol (GPI)-anchored proteins, displayed on the parasite's surface, are considered relevant vaccine candidates. This study aimed to study the conservation between isolates of GPI-anchored proteins identified by reverse vaccinology and to evaluate their gene transcription in sporulated oocysts and sporozoites.
A bioinformatic pipeline identified 15 Ea and 89 Et putative genes encoding GPI- anchored proteins from which easag2, easag4, eahyp, etsag, etsag1, ethyp, and etsag10 were selected based on: i. Prediction of GPI-anchor; ii. Surface localization; iii. Evidence of transcription in sporozoites, and iii. No. of strong/weak binders in MHC-I predicted sites. Specific PCRs amplified these selected genes from 10 Eimeria- DNAs extracted from Argentinian isolates that were then subjected to NGT-sequencing. Sequence analyses were performed by BLAST (NCBI),

Clustal Omega (EBI) and MEGA11 for identity, multiple sequence alignments and phylogenetic analysis. Gene transcription was analysed by RT-PCR from RNA isolated of sporulated oocysts and sporozoites of Et-W and Ea-H reference strains.
Results showed high conservation among isolates and the reference strains, being the most conserved etsag1 (99.97%, n=10), etsag4 (100.00%, n=10), ethyp (100.00%, n=5) and easag4 (99.99%, n=10); and etsag10 (99.16, n=5) the less. All genes but eahyp showed transcription in oocysts and sporozoites, but in a differential manner. Interestingly, etsag1 from one isolate contained two mutations and the phylogenetic analysis branched them apart from Argentinean (n=10), Indian (n=2), Chinese (n=20) and Korean (n=74) sequences, suggesting a geographic distribution.
In conclusion, using reverse vaccinology conserved potential vaccine antigens of two frequent Eimeria sp. that are expressed in infective stages could be identified.

IV4- Immunization with plant-based vaccine expressing *Toxoplasma gondii* SAG1 fused to plant hsp90 elicits protective immune response in lambs

Campero L.M.[1]*, Gual I.[1,2], Sander V.[3], Mendoza Morales L.[3], Ramos Duarte V.[4], Formigo P.[5], Sosa E.[1], Lázaro F.[1], Miqueo E.[1,2], Scioli M.V.[1], Morsella C.[1], Atela A.[4], Legarralde A.[4], Hozbor F.[1], Cantón G.J.[1], Angel S.[5], Moore D.P.[1,2], Clemente M.[4]

[1]Instituto de Innovación para la Producción Agropecuaria y el Desarrollo Sostenible (IPADS), INTA Balcarce-CONICET, Balcarce, Argentina eterinario de la Actividad Privada
[2]Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Balcarce, Buenos Aires, Argentina
[3]Laboratorio de Biotecnologías en Bovinos y Ovinos, Instituto Tecnológio de Chascomús (INTECH), Consejo Nacional de Investigaciones

Científicas y Técnicas (CONICET)-Universidad Nacional General San Martin (UNSAM), Chascomús, Argentina
[4]Laboratorio de Molecular Farming y Vacunas, INTECH, EByN, CONICET-UNSAM, Chascomús, Argentina
[5]Laboratorio de Parasitología Molecular, INTECH, EByN, CONICET-UNSAM, Chascomús, Argentina

*Presenting author: campero.lucia@inta.gob.ar

Toxoplasma gondii is a protozoan parasite causing toxoplasmosis, a principal concern for public health and livestock industries. Effective vaccination strategies are crucial for controlling this infection, particularly in sheep, which are natural reservoirs of *T. gondii*. In addition, ovine toxoplasmosis also causes economic losses due to abortions and reproductive complications in sheep. In this study, we evaluated two immunization strategies to elucidate the immune protective potential of SAG1 fused to the plant Hsp90 adjuvant against acquired toxoplasmosis in sheep. We performed an oral administration (plant vaccine) of fresh leaves infiltrated with heat shock protein 90-kDa chaperone of *Arabidopsis thaliana* (AtHsp81.2) fused to B- and T-cell antigenic epitope-containing surface protein SAG1 (SAG1HC) and a subcutaneous administration of recombinant *Nicotiana benthamiana* Hsp90.3 (NbHsp90.3) fused to SAG1HC produced *in Escherichia coli* (recombinant vaccine). We evaluated the humoral response and analyzed the protective value of both vaccine formulations. Only the recombinant vaccine showed a significant increase in the anti-rSAG1 IgG values. However, post-challenge, plant and recombinant vaccine groups showed IgG values significantly higher than the corresponding controls. We also observed a significant increase in brain lesions from lambs from vehicle and control groups compared to vaccinated groups. *Toxoplasma gondii* genomic DNA (gDNA) presence in brain tissue was evaluated by PCR, but only one *T. gondii* gDNA sample was detected. However, when we evaluated the presence of *T. gondii* gDNA in muscles with high commercial value (N= 8 muscles/lamb), the plant vaccine

group had the lowest *T. gondii* detection (56.25%) compared to the rest of the groups (>75% detection). Plant vaccine group reduced the probability of muscle infection by 90% compared to control group ($p < 0.01$). However, recombinant vaccine and vehicle groups showed non-significant reductions ($p = 0.65$ and $p = 0.31$, respectively). Finally, we carried out a serological analysis to follow the infectious process. We choose the chimera rGra4-Gra7 as an acute phase marker. All lambs from control and vehicle groups showed higher rates of serological reactivity, correlating with the lesions and inflammatory processes of the animals. Altogether results suggest that the plant-based vaccine is a promising candidate for controlling *T. gondii* infection in sheep, with potential benefits for enhancing public health and animal welfare.

SESSION 9

Biological Systems (Genomics, Proteomics) and Evolution

Keynote. From the bench to the field: translating functional genomics discoveries into practical solutions for the productive sectors

Francia M. E.

Laboratory of Apicomplexan Biology, Institut Pasteur de Montevideo, Uruguay.

mfrancia@pasteur.edu.uy

South America harbors significant genetic diversity among apicomplexan strains responsible for abortion in livestock, particularly cows and sheep. In this study, we utilized long-read sequencing technologies to reassemble the genomes of *Toxoplasma gondii* and *Neospora caninum*, providing a refined description of their karyotypes

and genomic architectures. We genetically characterized *T. gondii* strains associated with abortion in sheep, isolating and analyzing autochthonous strains. Our analysis revealed extensive genomic, transcriptomic, and phenotypic diversity within these strains, highlighting the rich variability found across the region. Despite this diversity, we found that the fundamental mechanisms of host cell invasion are conserved across different strains and species. Leveraging these insights, we developed and tested a synthetic vaccine based on the functional domains of invasion-critical proteins. Our results demonstrate that this vaccine provides protection against the vertical transmission of *T. gondii*.

ORAL PRESENTATIONS

BSE1- The tiny, but diverse, mitochondrial genomes of eimeriid and adeleorinid coccidia

Barta J. R.1*, Rotolo J. L.1, Zeldenrust E. G.1, Slawnych N.1, Leveille A.1, Kruth P. S.1

[1]Ontario Veterinary College, University of Guelph, Guelph ON Canada

*Presenting author: jbarta@uoguelph.ca

Coccidia retaining intact mitochondrial (mt) genomes possess compact (6-12kb), typically circular-mapping, mt genomes encoding a reduced complement of protein and rRNA products. Adeleid and eimeriid coccidia have mt genomes with 3 CDS regions encoding cytochrome coxidase subunits I (mtCOI) and III (mtCOIII) and cytochrome B (mtCytB). Interspersed among the 3 CDS are a varying number of small and large subunit ribosomal DNA fragments ranging from 16 to >180 bp in length that are highly conserved compared with CDS sequences. These conserved rDNA fragments suggest that they remain 'functional' in some way; NGS of small 'non coding' ncRNA fragments from sporozoites of

Eimeria tenella have demonstrated that these fragmented rDNAs are indeed transcribed into poly-adenylated RNA fragments.
Members of the Sarcocystidae (tissue coccidia such as Toxoplasma or Sarcocystis spp.) have complicated and fragmented mtDNA with multiple functional and non-functional/partial CDS copies interspersed into the nuclear genome. For eimeriid coccidia such as Eimeria, Isospora, Caryospora, and Lankesterella, the organization of the mt genome is well conserved but becomes widely varied in non-eimeriid coccidia of amphibia, fish or reptiles, and even more diversely organized in heteroxenous and monoxenous adeleid coccidia such as species of Haemogregarina, Hepatozoon, Klossiella and Klossia.
The high copy number and PCR-friendly nature of CDS regions make these genetic targets highly suited for species-level diagnostics (e.g. ddPCR or amplicon NGS for mixed parasite infections) and for molecular phylogenetics. Site saturation of CDS sequences likely impacts utility of CDS sequences beyond the familial level but other loci, such as nu 18S rDNA, can resolve these more ancient relationships. MtCOI and mtCOIII sequences are near-ideal species-level DNA barcode targets that should complement morphometric descriptions in all new species descriptions or re descriptions. Sequence diversity can be augmented with organizational innovations (content and arrangement) in various groups of coccidia for inferring relationships.

BSE2- Genomic analyses of *Goussia degiustii* and *Goussia leucisci*, coccidian parasites infecting fish

Slawnych N.[1], Cohen B.[1] *, Kruth P. S.[1], Barta J. R.[1]

[1]*Ontario Veterinary College, University of Guelph, Guelph ON Canada*

*Presenting author: ecohen02@uoguelph.ca

Coccidia are an incredibly diverse group of parasites that have been

found in virtually all terrestrial and aquatic food animals. Research conducted on coccidia infecting terrestrial food animals is extensive; however, coccidia infecting fish, including members of the genus *Goussia* (Labbé, 1896) are largely understudied. Historically, differences in the morphology of specific life stages were used to infer the evolutionary relationships among coccidia and classify them. Although now most morphological data from coccidia infecting terrestrial food animals are accompanied by genomic data, these data from fish are lacking. To date, no complete genomes are available from any *Goussia* species and the molecular data that are available from them are extremely limited. Due to the lack of molecular data from this genus, phylogenetic relationships among coccidia parasitizing fish, including bait fish and species consumed by humans, remain unclear. Including coccidia infecting fish in phylogenetic studies may improve our understanding of the taxonomic diversity and phylogenetic relationships of coccidia infecting more economically relevant hosts. To explore the evolutionary history of *Goussia* species, molecular techniques were used to generate complete mitochondrial (mt) genomes from two *Goussia* species as well as nuclear and plastid genomic sequences from one of these species. Complete mt genomes were generated using PCR and Sanger sequencing. Plastid and nuclear sequences were generated using both short- and long-read NGS technologies following the purification of oocysts from fish splenic tissue involving a DNase treatment. Comparison of the mt genome structure between the two *Goussia* species revealed considerable differences in both genome organization and content, suggesting that *Goussia* may be paraphyletic and in need of taxonomic revision.

Phylogenetic analyses of mt COIII and nuclear 18S rDNA sequences were conducted to help further clarify evolutionary relationships among coccidia infecting various hosts including food animal species.


ALOJAMOS
HISTORIAS

TABLE OF CONTENTS