APICOWPLEXA 2017

4th International Meeting on Apicomplexa in Farm Animals, 11-14 October 2017 - Madrid, Spain

UNIVERSIDAD COMPLUTENSE MADRID

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PROCEEDINGS

Complutense University of Madrid

Euroforum, El Escorial (Madrid, Spain)

Scientific committee

Alessandra Torina (Istituto Zooprofilattico Sperimentale della Sicilia "A.Mirri", Italy)

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Welcome to ApiCowplexa 2017

ApiCOWplexa-APICOMPLEXA IN FARM ANIMALS
4th International meeting-San Lorenzo de El Escorial, Madrid, Spain

Dear colleagues,

It is our great pleasure to extend a warm welcome to all participants to the fourth edition of "ApiCOWplexa: Apicomplexa Parasites in Farm Animals" which is being held at the Palacio de los Infantes in San Lorenzo de El Escorial, Madrid, Spain. We are delighted to be hosting this meeting following on from the three highly successful and enjoyable ApiCOWplexa meetings held in Portugal, Turkey and Scotland.

In the present edition, more than 120 delegates from all over the world, working in different fields related to apicomplexans in food animals, are going to be present, making this venue an excellent setting for networking activities in this area. A special emphasis has been placed this time in increasing the awareness on the diseases caused by this very special group of protozoan parasites from the point of view of Animal Health and Public Health, and thus ApiCOWplexa 2017 is hosting a special session on public engagement and knowledge exchange. In addition, during the meeting ten key-note lectures, thirty oral communications and sixty-one posters are going to be presented in seven different scientific sessions along three days.

This event would not be possible without the excellent collaboration of the different invited speakers, partners and sponsors, our special thanks to all of them, particularly to the International Journal for Parasitology team for their great effort editing a special issue of the journal at the time point concomitant to the meeting.

In summary, we wish you a productive and stimulating conference and hope that you have a very enjoyable time in El Escorial.

¡Bienvenidos!

The Organizing Committee

UNIVERSIDAD COMPLUTENSE MADRID

ApiCOWplexa-APICOMPLEXA IN FARM ANIMALS
4th International meeting-San Lorenzo de El Escorial, Madrid, Spain

WEDNESDAY, 11th October 2017 - Euroforum (San Lorenzo de El Escorial, Madrid)

14:00 **Registration**

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

Opening Session

Session Chairs: **Andrew Hemphill** and **Alexandre Leitão**

17:15 - 18:15
Key note (K) 01
Apicomplexan parasites: Their relevance for livestock production and veterinary public health. **Franz Conraths,** Friederich Loeffler Institute, Germany

18:15 - 19:15
K 02
"Prison Break". The natural egress of *Toxoplasma* from infected cells is a tightly programmed event. **Dominique Soldati**, University of Geneva, Switzerland

20:00 **Welcome reception**

THURSDAY, 12th October 2017 - Euroforum (San Lorenzo de El Escorial, Madrid)

Systems biology, 8:30 - 10:00

Session Chairs: **Dominique Soldati** and **Jeroen Saeij**

Oral communications (O)

8:30 - 8:45 **O 01** An integrated model of host-parasite interactions in Coccidian parasites, **Dong Xia**

8:45 - 9:00 **O 02** The three-dimensional ultrastructure of *Eimeria tenella* sporozoites, **Alana Burrell**

9:00 - 9:15 **O 03** Transcriptome modulation of bovine trophoblast cells *in vitro* by *Neospora caninum*, **Pilar Horcajo**

9:15 - 9:30 **O 04** Evolution of the micropyle of *Eimeria acervulina* young oocyst and its assumed role, **Jean Michel Répérant**

9:30 - 9:45 **O 05** Generation of population genomics data for an intracellular parasite of nucleated mammalian cells, **Richard Bishop**

9:45 - 10:00 **O 06** Differential gene expression in *Neospora caninum*, **John Ellis**

10:00 - 10:30 Coffee Break

Host-parasite interactions I, 10:30 - 12:30

Session Chairs: **Jon Boyle** and **Fiona Tomley**

Invited Speaker

10:30 - 11:15 **K 03** *Toxoplasma* proteins that modulate the host cell. **Jeroen Saeij**, University of California-Davis, United States of America

Oral communications

11:15 - 11:30 **O 07** A new class of apically confined, transmembrane micronemal proteins play a pivotal role in host cell invasion by *Toxoplasma gondii* tachyzoites, **Furio Spano**

11:30 - 11:45 **O 08** *Toxoplasma gondii* aspartyl protease 3: a microneme and rhoptry protein maturase essential for host plasma membrane lysis during egress and rhoptry discharge during invasion, **Sunil Kumar Dogga**

11:45 - 12:00
O 09
miR-126-5p by direct targeting of JNK-interacting protein-2 (JIP-2) plays a key role in *Theileria*-transformed macrophage virulence, **Malak Haidar**

12:00 - 12:15
O 10
In vitro characterization of bovine macrophages infection by *Neospora caninum* isolates of high and low virulence, **Marta García-Sánchez**

12:15 - 12:30
O 11
Virulence and attenuation of *Theileria annulata*-infected macrophages are stable parasite-associated traits transferable to fresh macrophages of a different MHC type, **Gordon Langsley**

12:30 – 14:00 Lunch

Host-parasite interactions II, 14:00 - 15:00

Session Chairs: **Furio Spano** and **Julio Benavides**

Oral communications

14:00 - 14:15
O 12
Cultivation of *Hammondia hammondi in vitro* reveals new insights into the timing of parasite development that can be exploited for genetic manipulation, **Jon Boyle**

14:15 - 14:30
O 13
Experimental *Toxoplasma gondii* and *Eimeria tenella* co-infection in chickens, **Lysanne Hiob**

14:30 - 14:45
O 14
Neospora caninum infection in a pregnant bovine model at mid gestation: comparison of early infection dynamics between high- (Nc-Spain7) and low- (Nc-Spain1H) virulence isolates, **Laura Jiménez-Pelayo**

14:45 - 15:00
O 15
Development of the first in vivo bovine experimental model of chronic besnoitiosis, **Carlos Diezma-Díaz**

Epidemiology and diagnostics I, 15:00 - 16:15

Session Chairs: **Luis Gondim** and **Frank Katzer**

Invited Speaker

15:00 - 15:45
K 04
Extensive review on potential risk and protective factors for *Toxoplasma gondii* infection in farm animals: What is remaining after excluding all presumably confounding or effect modifying factors?
Gereon Schares, Friederich Loeffler Institute, Germany

Oral communications

15:45 - 16:00
O 16
Analysis of *Toxoplasma gondii* clonal type-specific antibody reactions in experimentally infected turkeys and chickens by peptide microarray, **Pavlo Maksimov**

16:00 - 16:15
O 17
Serotyping of *Toxoplasma gondii* infections using strain-specific peptides, **David Arranz-Solís**

16:15 - 16:45 Coffee Break

Epidemiology and diagnostics II, 16:45 - 18:15

Session Chairs: **Franz Conraths** and **Michael Reichel**

Invited Speaker

16:45 – 17:15
K 05
Advances in the diagnosis of bovine besnoitiosis: current options and applications for control. **Gema Álvarez-García,** Complutense University of Madrid, Spain

Oral communications

17:15 - 17:30
O 18
Deciphering 'cryptic' *Eimeria* isolated from the domestic chicken, **Fiona Tomley**

17:30 - 17:45
O 19
Multigenome sequence based genotyping of *Eimeria* species causing coccidiosis in Ontario sheep and goats, **Evelin Rejman**

Invited Speaker

17:45 - 18:15 **K 06**	An overview on Theileriosis: new diagnostic tools and their limits **Alessandra Torina,** Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Italy

18:15 - 19:30	**Poster session**

20:00	**Dinner**

FRIDAY, 13th October 2017 - Euroforum (San Lorenzo de El Escorial, Madrid)

Vaccination and immune responses, 8:30 - 10:00

Session Chairs: **John Ellis** and **Ivan Morrison**

Invited Speaker

8:30 - 9:15
K 07
DC-complexa: species-specific adaptation of dendritic cells to pathogen diversity. **Artur Summerfield,** University of Berne, Switzerland

Oral communications

9:15 - 9:30
O 20
Analysis of local and peripheral immune response developed in sheep experimentally infected with *Toxoplasma gondii* at different times of gestation, **Julio Benavides**

9:30 - 9:45
O 21
CD103+CD11b- intestinal dendritic cells are critical players for controlling the different steps of *C. parvum* infection, **Fabrice Laurent**

9:45 - 10:00
O 22
Coccidiosis in poultry: development of *Eimeria* as vaccine vectors to streamline anticoccidial vaccination, **Iván Pastor-Fernández**

10:00 - 10:30 Coffee Break

Biosafety and treatment, 10:30 - 12:30

Session Chairs: **Kayode Ojo** and **Ronald Kaminsky**

Invited Speaker

10:30 - 11:00
K 08
Drugs and drug targets in pregnant models for *Toxoplasma* and *Neospora* infection. **Andrew Hemphill**, University of Berne, Switzerland

Oral communications

11:00 - 11:15
O 23
Bumped kinase inhibitors and their effects on the host-parasite relationship during experimental *N. caninum* infection *in vitro* and in mice, **Pablo Winzer**

11:15 - 11:30 **O 24** Characterisation of drug-resistance to buparvaquone in *Theileria annulata* populations in Turkey, **Tulin Karagen**

11:30 - 11:45 **O 25** *In vitro* screening of commercially available anti-coccidials identifies diclazuril and decoquinate as potential therapeutic candidates against *Besnoitia besnoiti* infection, **Alejandro Jiménez-Meléndez**

11:45 - 12:00 **O 26** Variations in anticoccidial efficacy on different *Eimeria* spp. in lambs based on oocyst excretion, **Ane Odden**

Invited Speaker

12:00 - 12:30 **K 09** Advances in bumped-kinase inhibitors for human and animal therapy of cryptosporidiosis. **Wes VanVoorhis,** University of Washington, United States of America

12:30 - 14:00 Lunch

Food and waterborne zoonosis, 14:00 - 15:45

Session Chairs: **Gereon Schares** and **Lee Innes**

Invited Speaker

14:00 - 14:45 **K 10** Food and Waterborne Protozoa: a veterinary and public health perspective, **Frank Katzer,** Moredun Research Institute, United Kingdom

Oral communications

14:45 - 15:00 **O 27** The impact of vegetation on the environmental transfer of *Cryptosporidium* oocysts from faeces to soil, **Claire E. Paton**

15:00 - 15:15 **O 28** *In vitro* host cell viability as putative correlate of virulence of *Cryptosporidium parvum* field isolates in Eastern Germany, **Ivette Holzhausen**

15:15 - 15:30 **O 29** Impact of confinement housing on study end-points in the calf model of cryptosporidiosis, **Jennifer Zambriski**

15:30 - 15:45 **O 30** Host-pathogen interactions in neonatal calves naturally and experimentally infected with *Cryptosporidium parvum*, **Sarah Thomson**

15:45 - 16:15 Coffee Break

3 minutes poster presentations (P), 16:15 – 17:15

Session Chairs: **Alexandre Leitão** and **Tulin Karagen**

P 1. Comparative study between pregnant mouse interference test and zebrafish embryo acute toxicity test: a possible replacement in anti-parasitic chemotherapy trials?, **Nicoleta Anghel**

P 2. Investigating the virulence of *Toxoplasma gondii* isolates from Brazil and Saint Kitts in mice, **Lauren E. Black**

P 3. Retrospective molecular diagnosis of *Neospora caninum* in bovine aborted fetus in Uruguay: Preliminary results, **Briano Carolina**

P 4. Isolation of a *Neospora caninum* goat strain from Southern Minas Gerais, Brazil, **Rafael C. Costa**

P 5. A component of the core kinase module of MEN and Hippo pathways, Mob1 is a critical factor in *Toxoplasma gondii* replication, **Inês Delgado**

P 6. Cytokine response to *Cystoisospora suis* infections in immune competent pigs, **Barbara Freudenschuss**

P 7. Transcriptional analysis of bovine monocyte-derived macrophages infected with high and low virulent isolates of *Neospora caninum*, **Marta García-Sánchez**

P 8. Phylogenetic diversity of *Eimeria* spp. in different genotypes of house mice (*Mus musculus*) from the European Hybrid Zone using a multiple marker approach, **Víctor Hugo Jarquín-Díaz**

P 9. Host-parasite interactions of *N. caninum* isolates of different virulence in bovine fetal and maternal placental cell lines, **Laura Jiménez-Pelayo**

P 10. HSP81.2 from *Arabidopsis thaliana* enhances the immune response against NcSAG1 from *Neospora caninum* protein and partially protects mice from congenital neosporosis, **Sofia A. Bengoa-Luoni**

P 11. Effects of *Eimeria tenella* infection on chicken caecal microbiome diversity, exploring variation associated with severity of pathology, **Sarah Macdonald**

P 12. Dose-titration of virulent *Neospora caninum* isolate Nc-Spain7 in pregnant sheep at 90 days of gestation, **Roberto Sánchez-Sánchez**

P 13. A reduction in weight gain in beef calves with clinical cryptosporidiosis, **Hannah Jade Shaw**

Public engagement and knowledge exchange, 17:15 – until finishing the discussion

How to raise awareness for ruminant apicomplexan (open round table)

Chairman: Ronald Kaminsky, Consulting for Parasitology and Drug Discovery
Participants: Fiona Tomley, RVC, **Wes VanVoorhis,** U-Washington, **Peter Opdam**, MSD and **Darrell Klug,** Brakke Consulting, Inc.

20:00 Conference dinner

Poster session

P 14. Microgametes – just motile nuclei or pivotal stages of the life cycle of *Cystoisospora suis*?, **Anja Joachim**

P 15. Demonstration of the presence of *T. gondii* in bio pigs intended for human consumption in Belgium, **Ignacio Gisbert-Algaba**

P 16. Analysis of allelic diversity of two immunodominant antigen genes of *T. annulata* and *T. lestoquardi* in Oman, **Salama Al-Hamidhi**

P 17. Genetics differentiation of *T. lestoquradi* in Africa and Asia, **Hamza Babiker**

P 18. Role of wildlife in the transmission of *Cryptosporidium parvum* to humans and livestock, **Ross Bacchetti**

P 19. Anti-cancer drugs affecting apicomplexan parasites: characterization of novel ruthenium-based compounds and their effects on *Toxoplasma gondii*, **Vreni Balmer**

P 20. Semi-high throughput screening of the Pathogen Box for inhibitors with dual efficacy against *Giardia lamblia* and *Cryptosporidium parvum*, **Lynn Barrett**

P 21. Evaluating the impact of pregnancy-associated immunomodulation on specific immune mechanisms against *Neospora caninum* infection in mice, **Afonso P. Basto**

P 22. Can multi-copy genes be an alternative for the diagnosis of TBDs in cattle? **Huseyin Bilgic**

P 23. Differential modulation of the Golgi and endosomal system in host cells infected with *Besnoitia besnoiti*, *Toxoplasma gondii* and *Neospora caninum*, **Rita Cardoso**

P 24. A new indirect ELISA for the detection of *Besnoitia besnoiti* antibodies in individual and bulk milk samples, **Loic Comtet**

P 25. The IDScreen® *Besnoitia* indirect 2.0 serum ELISA perfectly correlates with confirmatory techniques, **Loic Comtet**

P 26. Experimental infection with *Besnoitia besnoiti* tachyzoites in calves and young bulls, **Carlos Diezma-Díaz**

P 27. A new ELISA test to diagnose *Besnoitia* spp. infection in bovids and wild ruminants with improved specificity avoiding the use of a confirmatory test, **Carlos Diezma-Díaz**

P 28. Development and characterization of monoclonal antibodies against *Besnoitia besnoiti* tachyzoites, **Alejandro Jiménez-Meléndez**

P 29. Serological and molecular epidemiology of *Toxoplasma gondii* infection in intensive pig farms in Northern Italy, **Alessia Libera Gazzonis**

P 30. *Sarcocystis neurona* and *Neospora caninum* in Brazilian opossums (*Didelphis* spp.): Molecular investigation and *in vitro* isolation of *Sarcocystis* spp., **Luis Gondim**

P 31. Development of an alternative assay to study the infectivity of *T. gondii*, **Tina Goroll**

P 32. Integrative transcriptome and proteome analyses define marked differences between *N. caninum* isolates throughout the tachyzoite lytic cycle, **Javier Regidor-Cerrillo**

P 33. *Toxoplasma gondii* infections in stranded marine mammals in France and Romania, **Radu Blaga**

P 34. Immune response induced by the Mic1-3 Knockout *Toxoplasma gondii* vaccine strain in the parasite definitive feline host, **Radu Blaga**

P 35. Genus-specific antibodies for the diagnosis of *Neospora caninum* and *Toxoplasma gondii* using Immunohistochemistry in abortion cases of ruminants, **Tanja Lepore**

P 36. Characterization of *Theileria equi* antigen infecting donkeys in Egypt, **Olfat A. Mahdy**

P 37. Coccidian in *Oryctolagus cunniculus* from Tenerife, Canary Islands, Spain, **Natalia Martín Carrillo**

P 38. Carotenoid-enriched corn protects poultry against coccidiosis, **Beatriz Serrano-Pérez**

P 39. Toxoplasma inhibitor of STAT1 transcription effector protein IST and its relevance in virulence for closely related *Neospora caninum* and *Hammondia hammondi*, **Philipp Olias**

P 40. Anticoccidial effect of naringenin and grape fruit peel extract in growing lambs naturally-infected with *Eimeria* spp, **María Cristina Guerrero Molina**

P 41. Microsatellite analysis reveals high diversity among geographically close isolates of *Cystoisospora suis*, **Baerbel Ruttkowski**

P 42. Chicken line-dependent mortality after experimental infection with three type IIxIII recombinant *Toxoplasma gondii* clones, **Gereon Schares**

P 43. Immune-endocrine patterns in dairy cattle experimentally infected with *N. caninum* in the second trimester of gestation, **Beatriz Serrano**

P 44. Development of a recombinant protein based indirect ELISA for the detection of serum antibodies against *Cystoisospora suis* in swine, **Aruna Shrestha**

P 45. Deciphering the host cues responsive circadian transcriptome of apicomplexan parasite *Plasmodium chabaudi*, **Amit Kumar Subudhi**

P 46. Analysis of *Babesia bigemina* Ap+C7:C67ical Membrane Antigen-1 immunogenicity and its characterization in Apicomplexa, **Alessandra Torina**

P 47. Distribution of cattle babesiosis in Palermo province (Sicily), **Alessandra Torina**

P 48. Serological study of *Toxoplasma gondii* and *Neospora caninum* in a wildlife conservation area in southern Portugal, **Alexandre Leitão**

P 49. Evaluation of *Eimeria* sp. infection and predictors of oocysts excretion in newly introduced beef cattle in northern Italy, **Maria Teresa Manfredi**

P 50. Mutual influences of the apicomplexan parasites *Toxoplasma gondii* and *Eimeria tenella* in poultry macrophages, **Runhui Zhang**

P 51. Efficacy of VFO-IS-01, a live attenuated immunostimulant against *Salmonella enteritidis*, *Eimeria acervulina* and *Influenza* H7N1 infections in chicks, **Mehdi Ellouze**

P 52. Effects of challenge dose and inoculation route of virulent *Neospora caninum* Nc-Spain7 isolate in pregnant cattle at mid gestation, **Patricia Vázquez**

P 53. Significant reduction of *Neospora caninum* vertical transmission and postnatal mortality by a toll-like-receptor 2-targeting vaccine formulation in the pregnant mouse model of neosporosis, **Afonso P. Basto**

P 54. Evidence that transfer of *Theileria annulata* parasites from infected to uninfected leukocytes occurs by spontaneous cell fusion, **Ivan Morrison**

P 55. Placental lesions associated with abortions and stillbirths in goats naturally infected with *Neospora caninum* from Southern Minas Gerais, Brazil, **Rafael C. Costa**

P 56. *Toxoplasma gondii* tubulin-binding cofactor B a polarity factor required for host cell invasion and replication, **Inês Delgado**

P 57. Pharmacokinetics, safety and efficacy of Bumped Kinase Inhibitor (BKI) 1553 in a pregnant sheep model of neosporosis, **Roberto Sánchez-Sánchez**

P 58. *Cryptosporidium* infections among animals and humans in Greece, **Smaragda Sotiraki**

P 59. Cryptosporidium parvum increases intestinal permeability through interaction with epithelial cells and IL-1? and TNF? released by inflammatory monocytes, **Fabrice Laurent**

P 60. Investigation of tick-borne disease in Indian bovines: breed resistance and transmission blocking as strategies for improved productivity, **Stephen Larcombe**

P 61. The relationship between presence of antibodies and direct detection of *Toxoplasma gondii* in slaughtered cattle from four European countries, **M. Opsteegh**

Opening session

Apicomplexan parasites: Their relevance for livestock production and veterinary public health

K 01

F.J. Conraths[1], L.M. Ortega-Mora[2], P. Maksimov[1], B. Bangoura[3], A. Daugschies[3], G. Schares[1]

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[2] SALUVET, Animal Health Department, Faculty of Veterinary Sciences, Complutense University of Madrid, Ciudad Universitaria s/n, 28040 Madrid, Spain
[3] Institute of Parasitology, Faculty of Veterinary Medicine, University Leipzig, An den Tierkliniken 35, 04103 Leipzig, Germany

While coccidia of the genus *Eimeria* play the most important role for birds among apicomplexan parasites, the spectrum affecting mammals is much wider, although not all apicomplexan parasites of livestock mammals are economically important or of veterinary public health concern. The most relevant apicomplexan parasites for livestock mammals include *Eimeria* spp. in cattle, sheep, goats, horses, donkeys, pigs and rabbits, and *Isospora* spp. in pigs. *Toxoplasma gondii* causes economic damage as an abortifacient in sheep and goats and represents a major veterinary public health concern because of its zoonotic potential and its wide intermediate host range, which also includes birds. Infections of poultry with *T. gondii* are increasingly discussed as a veterinary public health problem. *Neospora caninum* is considered as one of the most important cause of abortion in cattle worldwide. *Neospora hughesi* has been described in horses. Bovine besnoitiosis reduces the productivity and fertility in affected herds. The disease continues its expansion in Europe. Some *Sarcocystis* spp. can cause disease in cattle, sheep, goats and pigs, which serve as intermediate hosts for the parasite. Humans represent the definitive hosts for *S. hominis* and *S. suihominis*. These parasites therefore have zoonotic potential, although disease in humans is usually not severe (nausea, abdominal pain, diarrhea) and transient. Cryptosporidia can cause severe disease, particularly in cattle and poultry, and some taxa, particularly the bovine genotype of *Cryptosporidium parvum*, have substantial zoonotic potential. *Babesia* spp. and *Theileria* spp. play a major role as tick-borne diseases in ruminants and equids, mainly in tropical and subtropical countries.

The economic importance of apicomplexan parasites for livestock production, veterinary public health concerns and options for control will be discussed.

"Prison Break"
The natural egress of *Toxoplasma* from infected cells is a tightly programmed event

K 02

H. Bisio, M. Lunghi, **D. Soldati-Favre**

Department of Microbiology and Molecular Medicine, University of Geneva, 1 rue Michel Servet, 1211 Geneva, Switzerland

The phylum Apicomplexa groups obligate intracellular parasites responsible for severe veterinary and human diseases. Gliding motility, powered by an actomyosin system, assists invasion and egress from the infected cells, two key steps in the lytic cycle of the Apicomplexa. Exit from the host cells is recognized as a complex and temporally orchestrated process. Underpinning this process is the release of apical secretory organelle termed micronemes. Activation of the cGMP dependent protein kinase (PKG) and an increase in intracellular calcium level, likely resulting from parasite phosphatidylinositol phospholipase C (PI-PLC) activation at the plasma membrane, activate calcium-dependent protein kinases (CDPKs) that subsequently phosphorylate specific substrates critical for micronemes exocytosis. Downstream of PI-PLC activation, diacylglycerol kinase 1 (DGK1) produces phosphatidic acid (PA) from diacylglycerol (DAG) and contributes to microneme exocytosis in concert with the apical pleckstrin homology domain containing protein (APH), an acylated protein at the surface of micronemes that acts as PA sensor. The signaling cascade leading to egress responds to environmental changes in K^+, Ca^{2+} and H^+. Additionally*, Toxoplasma gondii* and other members of the coccidian subgroup of Apicomplexa possess a second diacylglycerol kinase (DGK2) that is secreted into the parasitophorous vacuole (PV). Genetic depletion of *DGK2* severely delays natural egress from the host cell without impacting on parasite intracellular growth or its capacity to respond to various triggers of induced egress. The mechanism by which PA produced in the PV serves as molecular clock and governs natural egress is under investigation.

Systems biology

An integrated model of host-parasite interactions in Coccidian parasites

O 01

N. Randle[1*], V. Marugán-Hernandez[2*], **D. Xia**[2*], D. Blake[2], F. Tomley[2], J. Wastling[3]

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[2] Department of Pathobiology and Population Sciences, Royal Veterinary College, Hawkshead Lane, Hertfordshire, AL9 7TA, United Kingdom.
[3] Faculty of Natural Sciences, Keele University, Keele, Staffordshire, ST5 5BG, United Kingdom.
[*] Authors contributed equally

Coccidian parasites display a range of life cycles and adaptations to their hosts. Whilst some are promiscuous and important zoonotic pathogens, others are highly host restricted. Despite these differences, all Coccidia share significant common biological characteristics: they undergo sexual reproduction and form environmentally resistant oocysts which, when ingested by a host, excyst to release sporozoites that invade and colonise epithelial cells of the gastrointestinal tract.

Using quantitative proteomics and transcriptomics we have simultaneously analysed gene expression, protein expression and protein phosphorylation from both the host and three coccidian parasites (*Toxoplasma gondii*, *Cryptosporidium parvum* and *Eimeria tenella*) during the process of cell invasion. These data were used to perform a systems analysis of the complex molecular events occurring in both host and parasite during a single round of sporozoite invasion and parasite asexual replication. We observed that both host and parasites underwent wide-ranging transcriptional and protein expression changes within the first 24 hours of invasion. Functional enrichment tests and network analysis identified several host genes that were consistently modulated between the parasites and which were key regulators of host functions such as cell cycle and protein folding. The functional role of these candidate host genes are currently being validated *in vitro* using RNAi. Functional studies of specific parasite genes will also be carried out using *in vitro* invasion blocking assays.

This study was funded by the BBSRC through grants BB/L002477/1 and BB/L00299X/1.

The three-dimensional ultrastructure of *Eimeria tenella* sporozoites

O 02

A. Burrell[1,2], S. Vaughan[1], V. Marugan-Hernandez[2], F. Tomley[2]

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Eimeria tenella is a key agent of poultry coccidiosis, a disease which involves the destruction of intestinal epithelial cells and has significant impacts on poultry welfare and agricultural economics worldwide. Despite numerous microscopic and chemical investigations since the start of the last century, many questions regarding the complex biology and life cycles of this genus are still unanswered. By using three dimensional electron microscopy techniques (serial block face – scanning electron microscopy and transmission electron tomography) we were able to quantify organelle numbers and volumes for the sporozoite of *E. tenella.* In addition, we have used light microscopy to investigate the dynamics of the poorly understood retractile body organelles; these were shown to reduce in number from two to one per sporozoite within the first few hours following the invasion of mammalian cells in vitro. We hope that increasing our knowledge of the natural biology of *E. tenella* will help to inform future researchers looking for novel ways to combat this pathogen.

This PhD project is funded by the Royal Veterinary College research fund and Oxford Brookes 150 anniversary research fund.

Transcriptome modulation of bovine trophoblast cells *in vitro* by *Neospora caninum*

O 03

P. Horcajo[1], L. Jiménez-Pelayo[1], M. García-Sánchez[1], J. Regidor-Cerrillo[1], E. Collantes-Fernández[1], D. Rozas[1], N. Hambruch[2], C. Pfarrer[2], L.M. Ortega-Mora[1]

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Neospora caninum is one of the most efficiently transplacentally transmitted pathogens in cattle. The invasion and proliferation of *N. caninum* in the placenta and its dissemination to the foetus are crucial events in the outcome of an infection. Maternal and foetal epithelia in placentomes form a physical barrier against foetal infection. Furthermore, trophoblast cells act as an innate immune defence at the foetal-maternal interface. *N. caninum* proliferates in trophoblast cells *in vitro*, but it is unknown whether host cell modulation events occur. In this work, we investigated the transcriptome modulation by *N. caninum* infection in the bovine trophoblast cell line F3. With this purpose, two *N. caninum* isolates with marked differences in virulence, Nc-Spain1H and the Nc-Spain7, were used. The results showed a clear influence of *N. caninum* infection on the expression of genes involved in extracellular matrix reorganization, cholesterol biosynthesis and the transcription factor AP-1 network. Interestingly, although differences in the transcriptome profiles induced by each isolate were observed, specific-isolate modulated processes were not identified, suggesting similar regulation by both isolates. Interestingly, expression of *N. caninum* genes involved in host-cell attachment and invasion, glideosome, roptries, metabolic processes, cell cycle and stress responses varied between isolates.

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Evolution of the micropyle of *Eimeria acervulina* young oocyst and its assumed role

O 04

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Eimeria acervulina is a protozoan parasite belonging to coccidia. This species only infects Gallus gallus and develops in duodenum and jejunum of infected birds. It has a major economic impact, being responsible for subclinical coccidiosis. It is also one of the most frequent species encountered in broiler farms.

The life cycle is monoxenous and oocysts are released at the end of this life cycle in the environment into bird feces. When excreted, these oocysts do not exhibit a visible micropyle, but when they are newly produced locally in the duodenum and jejunum of infected birds, they harbour a well defined micropyle. After experimental infection and collection of young oocysts in duodenum 96 to 97 hours post infection, we observed that this micropyle evolved rapidly within a few hours, concomitant to changes of the oocyst content. Videos have been taken that show these changes. The micropyle seemed to seal and the protoplasm which was close by, receded and retracted.

Trying to understand the ground of these modifications, we assumed that fertilization could take place at the oocyst level, and the evolution of the micropyle could be an event occurring after fertilization in order to avoid polyfertilization. However, since we could not observe fertilization directly, this hypothesis remains to be further investigated.

Generation of population genomics data for an intracellular parasite of nucleated mammalian cells

O 05

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Obligate intracellular pathogens present a significant challenge to reverse vaccinology approaches based on population genomics data because it is often financially or technically unfeasible to generate genome sequence data for those organisms. The problem is particularly acute when the pathogen parasitizes nucleated host cells, resulting in very low ratios of parasite to host DNA. *Theileria parva* is a tick-transmitted apicomplexan parasite that causes East Coast fever (ECF), an acute fatal disease that kills over 1 million cattle each year in sub-Saharan Africa. The proliferation of *T. parva* occurs within bovine lymphocytes, and susceptible animals die in 3 to 4 weeks post-infection from severe damage to the lymphatic system and pulmonary edema. In late stages of infection *T. parva* infects erythrocytes and DNA extracted from the tick-infective piroplasm stage can be obtained from bovine blood in sufficient quantity for genome sequencing but this results in the sacrifice of the animal. In order to generate population genomics data we customized a DNA sequence capture approach to obtain *T. parva* genomic DNA from *T. parva*-infected bovine lymphocyte cell lines. *T. parva* DNA represents <1% of the total starting DNA material. We successfully captured sequence reads that map to 95-99% of the reference genome, from several independent samples. When assembled, the reads produce high quality *de novo* draft genome assemblies that map to >98% of the reference genome. Sequencing error is negligible, at $\leq 10^{-5}$/bp. The SNP density among cattle-derived parasites is remarkably high, at ~10 SNPs/Kb, and over 10-fold higher than that observed in intra-species comparisons in *Plasmodium*. SNP density between *T. parva* isolates sampled from cattle and those obtained from the African buffalo (*Syncerus caffer*), the natural reservoir, is twice as high as that observed between cattle-derived parasites, suggesting that the most recent common ancestor of all *T. parva* isolates is relatively ancient. The distribution of polymorphisms across loci is highly variable, with ~3% of the loci (among which are known antigens) with polymorphism levels >>100 SNPs/Kb and negligible levels of polymorphism at most other loci. These results demonstrate the feasibility of parasite whole-genome capture from a mix of parasite and host DNA, and that the quality of the data is very high. This approach is directly applicable to a variety of human intracellular pathogens of similar genome size and complexity.

Differential gene expression in *Neospora caninum*

O 06

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Illumina RNAseq was used to compare the tachyzoite transcriptomes of NC-Liverpool and NC-Nowra through alignment to the *N. caninum* genome. The existence of differentially expressed genes occurring in tachyzoites was investigated *in silico* using a variety of different models, including Cufflinks and CuffDiff or HTSeq, in order to determine how the two tachyzoite populations differ.

Several methods for analyses of differential expression were used to develop a consensus set of genes, exons and isoforms that were found to be differentially expressed across multiple models. While this produces a conservative set of differentially expressed genes, it is much more likely that the set will be robust. The results identified as many as 700 genes may be differentially expressed between the two populations of tachyzoites, which represents 1/9 to 1/10 of the total number of genes that are predicted to exist in *N. caninum*. Annotation of these genes using gene ontology shows that “ribosome” and “ATP binding” are highly represented terms in the list of differentially expressed genes, suggesting that protein synthesis and kinase activity by a tachyzoite is an important contributor to virulence in *N. caninum*. A small group of genes were also identified that were not previously known and are described for the first time.

The emphasis, within the lists of differentially expressed genes generated by the various models, on ribosomal proteins, translation initiation factors and kinases as being differentially expressed between tachyzoites of NC-Liverpool and NC-Nowra provides identities on new potential virulence factors of *N. caninum*, which hitherto have not been studied. The study also highlights the need for improved annotation of parasite genes.

Host-parasite interactions I

Toxoplasma proteins that modulate the host cell

K 03

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Many intracellular pathogens secrete proteins that manipulate host cells to create the niche in which the pathogen can replicate. In turn, host cells have intricate mechanisms to detect and combat invading pathogens. As a consequence pathogens and their hosts co-evolve, leading to variation in both host and pathogen genomes and to the emergence of differences in pathogen virulence and host resistance. Compared to bacteria and viruses, relatively little is known about how more complex eukaryotic pathogens co-evolve with and manipulate their hosts.

We are interested in host-parasite interactions between the obligate intracellular eukaryotic parasite *Toxoplasma gondii* and its mammalian hosts. *Toxoplasma* virulence differs, often quite dramatically, depending on the infecting strain and the host. The focus of the Saeij laboratory over the last years has been to identify genes of *Toxoplasma* that modulate the host cell and/or determine virulence, host genes and pathways that determine resistance/susceptibility, and to characterize their specific interactions.

In this talk I will discuss how what we learned from how *Toxoplasma* interacts with its hosts can be used to understand how other apicomplexan parasites interact with their hosts. I will also present our lab's recent results on *Toxoplasma*-inflammasome interactions.

A new class of apically confined, transmembrane micronemal proteins play a pivotal role in host cell invasion by *Toxoplasma gondii* tachyzoites

O 07

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The *T. gondii* genome encodes two thrombospondin-related paralogs, MIC14 and MIC15, showing the hallmarks of TM micronemal proteins. While MIC14 is not expressed in tachyzoites, MIC15 is present in the micronemes of tachyzoites, bradyzoites and sporozoites. MIC15 is relocated onto the parasite extreme apical surface, where it is peculiarly confined during gliding, invasion and egress. Immunofluorecence showed that MIC15 is apically located with the respect to the inner membrane complex membrane and follows conoid extrusion, suggesting a possible interaction with the MyoH apical motor in the early phases of invasion and egress. Consistent with the lack of retrograde transport and a bona fide TM rhomboid cleavage site, the ectodomain of MIC15 is not recovered in the soluble fraction during secretion assays. Following unsuccessful attempts to knockout the *MIC15* gene, we generated a tetracycline-regulatable conditional knockdown (*MIC15-iKD*) and showed that MIC15-depleted tachyzoites are significantly impaired in invasion (80%) and 50% less efficient during egress. In addition, the MIC15-iKD invasion defect is attenuated in parasites spontaneously upregulating MIC14 after prolonged growth in presence of anhydrotetracycline, further supporting the concept that in *T. gondii* functional redundancy underlies crucial invasion-related molecular mechanisms.

Toxoplasma gondii aspartyl protease 3: a microneme and rhoptry protein maturase essential for host plasma membrane lysis during egress and rhoptry discharge during invasion

O 08

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The micronemes and rhoptries are specialized organelles that secrete their contents at the apical tip of apicomplexan parasites in a sequential and regulated fashion. Exocytosis of adhesins (MICs), perforins and proteases by the micronemes critically participate in motility, invasion and egress from infected cells. Concomitantly, the discharge by the rhoptries of RONs and ROPs ensures circular junction formation during invasion and subversion of host cellular functions during intracellular growth, respectively. These secreted proteins traffic first through an endosomal-like compartment (ELC) and are subjected to proteolytic maturation prior to organellar storage and discharge. The *Toxoplasma gondii* aspartyl protease 3 (ASP3) resides in the ELC and is essential for invasion and egress from infected cells. A comparison of the N-terminome, by terminal amine isotopic labelling of substrates (TAILS) in wild type and ASP3-depleted parasites identified microneme and rhoptry proteins as plausible substrates. ASP3 was confirmed to act as maturase for known as well as newly identified secreted proteins and to indirectly affect the post-exocytosis processing of some MICs. The crucial role of ASP3 during invasion is associated to rhoptry discharge and host plasma membrane lysis during egress. Furthermore, derivatives from a series of antimalarial compounds based on a hydroxyethylamine scaffold interrupt the lytic cycle of *T. gondii* at submicromolar range by selectively targeting ASP3.

This work was supported by Carigest SA, the Swiss National Science Foundation (FN3100A0-116722 to D.S.-F. and CRSII3_16002 to A. H. and D.S.-F). D.S.-F is an HHMI senior international research scholar. BM is recipient of long-term EMBO fellowships. SKD was supported from the Sir Jules Thorn Charitable Overseas Trust reg., Schaan (Dr. Karine Frenal) and MalarX (SystemsX.ch).

miR-126-5p by direct targeting of JNK-interacting protein-2 (JIP-2) plays a key role in *Theileria*-transformed macrophage virulence

O 09

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Theileria parasites transform bovine leukocytes, but remarkably transformation is reversible, as upon parasite death transformed leukocytes revert to quiescent cells and die. *T. annulata*-transformed monocytes/macrophages lose their hyper-disseminating virulent phenotype during long-term cell culture and are used as attenuated live vaccines to fight tropical theileriosis. Here, deep microRNome sequencing revealed that infection of B cells and macrophages alters the expression of a large number host cell microRNAs (miRs). We focused on miRs whose expression diminished in attenuated macrophages to identify miR-126-5p and show that it plays a key role in *Theileria*-induced tumour dissemination. miR-126-5p directly targets and suppresses JIP2 liberating JNK to phosphorylate nuclear c-Jun and drive transcription of mmp9, so promoting dissemination. The tyrosine phosphatase PTP1B associates with AGO2 in virulent but not in attenuated macrophages, leading to increased phosphorylation of AGO2 and decreased miR-126-5p levels. Therefore, variations in miR-126-5p levels underpin dissemination and attenuation of *T. annulata*-transformed macrophages.

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In vitro characterization of bovine macrophages infection by *Neospora caninum* isolates of high and low virulence

O 10

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Neospora caninum represents one of the main causes of abortion in cattle. It has been suggested that the immune response generated by the infection contributes greatly to the pathogenesis of neosporosis. Macrophages are mediators of the innate immune response against infection and likely one of the first cells found by the parasite. We investigated how the parasite interacts with bovine macrophages and the influence of the isolate virulence in the subsequent response.

Monocyte derived macrophages were generated in vitro from bovine peripheral blood and characterized by determining phagocytosis, secretion of proinflammatory (IL-12) and regulatory (IL-10) cytokines, and antigen presentation capacity. Macrophages were infected by a high or a low virulent isolate of *N. caninum* (Nc-Spain7 and Nc-Spain1H respectively). Both isolates showed very similar invasion rates and were able to survive and replicate into the cells. Nc-Spain7 proliferation was significantly higher following an exponential growth model, whereas Nc-Spain1H presented a delay on the beginning of duplication and did not adjust to an exponential pattern. The cellular response of macrophages to infection was also determined, evaluating the expression of surface markers and its capacity to stimulate lymphocytes, by means of quantification of IFN-γ release. Infected macrophages exhibited lower expression of CD86 and CD1b molecules that not infected ones, but differences were not observed between isolates. Nc-Spain1H infected macrophages were able to induce a higher production of IFN-γ by lymphocytes than Nc-Spain7 infected cells, in which levels of this cytokine were similar to those produced by not stimulated lymphocytes.

Our results suggest that *N. caninum* isolates of diverse virulence can modulate target innate immune cells in a different manner, and represent an important step towards the knowledge of the strategies followed by this parasite to subvert or induce protective innate responses.

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Virulence and Attenuation of *Theileria annulata*-infected macrophages are stable parasite-associated traits transferable to fresh macrophages of a different MHC type

O 11

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**This study is dedicated to the memory of Niall McHugh*

Live attenuated vaccines are generated by long-term culture of *Theileria annulata*-infected macrophages and are widely used in endemic regions to combat tropical theileriosis. At different time points in the attenuation process vaccine lines are empirically tested for their reduced ability to induce clinical disease in calves. As disease-causing *T. annulata* schizonts are *stricto senso* intracellular, the pathogenic entity is the infected macrophage. The virulence of transformed macrophages is associated with heightened dissemination throughout the animal and this has been linked to upregulation of infected host cell virulence traits such as AP-1-driven *mmp9* expression. In live attenuated vaccines, these virulent host cell traits are diminished. To directly test the parasite's ability to induce these virulence traits schizonts from virulent and attenuated macrophages were transferred *in vitro* to fresh macrophages of a different MHC type. These freshly established lines were tested both *in vitro* and in mice for maintenance versus loss of virulence traits. Parasites transferred to fresh macrophages conferred on them the corresponding virulence or attenuated phenotypes of the donor lines. These virulence-associated properties of infected macrophage are therefore clearly parasite-encoded and are ablated by multiple passage *in vitro*. The availability of transferred parasites in fresh macrophages should facilitate identification of *Theileria*-encoded virulence traits and our progress in this endeavor will be reported.

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Host-parasite interactions II

Cultivation of *Hammondia hammondi in vitro* reveals new insights into the timing of parasite development that can be exploited for genetic manipulation

O 12

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Hammondia hammondi is the closest extant relative of the human pathogen, *Toxoplasma gondii,* but they have critical life cycle and host range differences. The molecular and genetic mechanisms for these differences are unknown. We have thoroughly characterized the *in vitro* development of *H. hammondi* sporozoites compared to *T. gondii*, confirming previous observations and identifying new life cycle features that distinguish these species. *H. hammondi* vacuoles robustly and predictably converted to *Dolichos bifluorous* lectin (DBA)-positive cysts between 12 and 23 days post infection in the absence of any externally applied stressors. In contrast, *T. gondii* maintained a lower (~0-20%) DBA-positive population and continued to replicate over the entire cultivation period. Prior to spontaneous cyst conversion we found that *H. hammondi* could be used to infect new host cells and mice for up to 8 days post-excystation, representing the first time that *H. hammondi* has been successfully subcultured *in vivo*. We exploited this critical period to successfully generate the first transgenic *H. hammondi* line with a disrupted *UPRT* locus and a stable *dsRED* transgene. Overall these data show that *H. hammondi* undergoes a stringently regulated progression through its life cycle that is lacking and/or flexible in *T. gondii*, but that an early time period of cultivability can be exploited to perform genetic manipulations in this organism.

Experimental *Toxoplasma gondii* and *Eimeria tenella* co-infection in chickens

O 13

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Toxoplasma gondii (T. gondii) and *Eimeria tenella* (*E. tenella)* are regarded as widespread important pathogens with high prevalence in chickens. Our study investigated mutual influences in co-infected chickens, focusing on immune response and course of infection. In two separate trials, a total of 96 one-day-old chicks were divided in four study groups: group NC (negative control, uninfected); group PC-T (oral or intramuscularly infection with *T. gondii* oocysts or tachyzoites, respectively), group PC-E (oral infection with *E. tenella*) and group TE (co-infection). Examinations of body weight gain, oocyst counts, *T. gondii*-specific antibodies and parasite distribution in tissues were performed. Two necropsies were carried out for lesion scoring of the ceca and cytokine messenger RNA (mRNA) expression measurement in gut and spleen tissue. We detected *T. gondii*- specific antibodies earliest 4 days post infection (p.i.) by immunoblot, i.e. antibodies to antigens of 20, 30 and 43 kDa Mr. 22.1 % of all tissue samples from *T. gondii* infected chicken were tested positive by nested PCR or magnetic capture PCR. Microscopical scoring suggested a *T. gondii*- related promotion of *E. tenella* merogony in the gut without enhancement of total oocyst excretion. An increased mRNA expression in Th1- (IFN-γ, IL-12, TNF-α) as well as Th2- related cytokines (IL-10) was mainly detected in groups PC-E and TE. In conclusion, changes in cytokine mRNA expression could be attributed mainly to the *E. tenella* infection and was not affected by the co-infection with *T. gondii*. This is the first report of a co-infection of chickens with *E. tenella* and *T. gondii*.

Neospora caninum infection in a pregnant bovine model at mid gestation: comparison of early infection dinamics between high- (Nc-Spain7) and low- (Nc-Spain1H) virulence isolates

O 14

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The present work studies the effect during early infection (clinical outcome, parasite distribution and burden and lesion development) of inoculation with high (Nc-Spain7) or low (Nc-Spain1H) virulence isolates of *N. caninum* in pregnant heifers inoculated at 110 days of gestation. Pregnant Asturiana heifers (n=24) were distributed into three experimental groups: 6 in group 1 (G1) inoculated intravenously (IV) with phosphate buffered saline (PBS), 9 in group 2 (G2) and 9 in group 3 (G3) inoculated IV with 10^7 tachyzoites of Nc-Spain7 and Nc-Spain1H, respectively. Three animals from G1, 4 from G2 and 4 from G3 were culled at 10 days post-infection (dpi) and the rest of animals at 20 dpi. Animals were monitored daily until euthanasia. A peak of fever was detected at 1 dpi in both infected groups ($P < 0.0001$), and a second peak was detected at 3 dpi in G2 but not in G3 ($P < 0.0001$). Foetal mortality was detected in two heifers from G2 culled at 20 dpi, whereas foetuses from G3 remained viable throughtout the experiment. An earlier detection of *N. caninum* specific antibodies in G2, starting at day 9 pi, than in G3, starting at day 13 pi was observed. In addition, all animals (5/5) from G2 culled at 20 dpi presented specific antibodies while only 3 (3/5) animals from G3 culled at 20 dpi had seroconverted. One heifer from G2, culled at 10 dpi, showed mild lesions in the placenta, while moderate lesions were seen in fetuses and placentas from all animals from the same group and culled at 20 dpi. No lesions were found in any heifer from G1. Similarly, parasite DNA was more frequently detected in caruncles and cotyledons from those animals infected with Nc-Spain7 and culled at 20 dpi. In conclusion, evident clinical, parasitological and lesional differences between two different virulence isolates of *N. caninum* have been confirmed during early infection in a pregnant bovine model infected at mid gestation.

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Development of the first *in vivo* bovine experimental model of chronic besnoitiosis

O 15

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We have refined a previously developed bovine experimental model of *Besnoitia besnoiti* infection. Herein, we have evaluated 2 new variables: parasite stage and inoculation route. Twelve Holstein Friesian 3-month old male calves were randomly divided into 4 groups of 3 animals each. Three groups were inoculated with 10^6 bradyzoites by intravenous (G1); subcutaneous (G2) and intradermal (G3) routes, respectively, along with PBS in a non-infected control group G4). The trial lasted 90 days that included a daily clinical monitoring and weekly blood collection from all animals for antibodies and parasitemia detection. Once the calves were euthanized, tissues from skin, eyes, respiratory and reproductive tracts among others, were collected for histological and PCR determinations.

Remarkably, both acute and chronic stages of the disease were successfully reproduced. The outcome of the infection was classified as mild-moderate for the acute stage and moderate-severe for the chronic stage. All infected calves showed lymphadenopathy from 4 days post-infection (pi). G1 developed a sporadic febrile response around the second week pi, fever was observed in G2 from 1 week pi until 2 weeks pi and in G3 from 17 days pi until 24 days pi. Parasitemia was detected sporadically in calves from G1 at 15 and 61 days pi (n=1), G2 at 12, 22 and 41 days pi (n=1) and G3 at 2 (n=1), 15 (n=1), 19 (n=3), and 22 (n=2) days pi. Animals from G1, G2 and G3 showed clinical signs characteristic of the chronic stage such as pathognomonic conjunctival cysts. Tissue cysts were more abundant in G3, where one calf also developed skin lesions. G2 seroconverted earlier at 19 days pi, followed by G1 at 22 days pi and G3 at 25 days pi. *Besnoitia*-DNA was identified in 82 tissues and G3 showed the highest number of PCR positive tissues (n=57). In conclusion, we have successfully developed the first *in vivo* bovine experimental model for the chronic stage of *B. besnoiti* infection. The parasite stage and the inoculation route are key factors that influence on the outcome of the infection.

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Epidemiology and diagnostics I

Extensive review on potential risk and protective factors for *Toxoplasma gondii* infection in farm animals: What is remaining after excluding all presumably confounding or effect modifying factors?

K 04

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An extensive review was conducted to identify studies which looked at risk factors of *T. gondii* infection in production animals. Studies published in English, French, Spanish, Dutch, German and Italian were considered. The review was restricted to the most important domestic food-producing animals in Europe (cattle, pigs, sheep, goats, horses and chickens), European husbandry systems and peer reviewed papers published since 1994. More than 100 studies were included and provided information on various putative risk and protective factors for *T. gondii* infections in farm animals including definitive host related factors, management practices, specialization and factors characterizing the likelihood of fodder or water contamination. The presence of cats increased the risk of infection as revealed in a number of studies in pigs and small ruminants. Most studies assessing the potential role of rodents identified a risk effect. The majority of reports suggested that outdoor access increased the risk of infection in pigs. A low level of management intensity in small ruminants revealed conflicting results. Degree of specialization played a role in chickens. Surprisingly, studies assessing a potential role of contaminated water as a risk factor for infection did not reveal a consistent association. The review showed that further studies are necessary to solve conflicting findings and to complete knowledge especially in cattle, equids and in poultry. More efforts are necessary to address the biases in studies caused by confounding variables and to include important effect modifying variables into epidemiological modelling.

Analysis of *Toxoplasma gondii* clonal type-specific antibody reactions in experimentally infected turkeys and chickens by peptide microarray

O 16

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Knowledge on *T. gondii* genotypes in infected animals and humans is important for understanding the epidemiology of *T. gondii* infections.

The aim of the present study was to analyse the ability of experimentally infected turkeys and chickens to develop *T. gondii* clonal type-specific antibody responses (IgY).

We used a peptide microarray with a panel of 101 different peptides derived from polymorphic regions of 16 *T. gondii* proteins and 120 sera collected at different times post infection (p.i.) from experimentally infected chickens and turkeys inoculated with different doses of *T. gondii* tachyzoites representing clonal types I (RH), II (ME49) or III (NED) and uninfected controls.

After screening the peptides with reference sera from chickens and turkeys, 30 and 37 peptides were identified, respectively, that showed type-specific reactions.

Differences in recognized peptide patterns were observed between individual animals as well as between different weeks p.i. With selected peptides it was possible to determine until 7 weeks p.i. the *T. gondii* type used for experimental infection of chickens and turkeys on the group level.

Our results demonstrate that experimentally infected chickens and turkeys are able to develop a clonal type-specific IgY antibody response: time p.i. and dose of infection seems to influence the peptide patterns recognized.

Serotyping of *Toxoplasma gondii* infections using strain-specific peptides

O 17

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The intracellular parasite *Toxoplasma gondii* can cause chronic infection in most warm-blooded animals, including humans. In the USA, four different *Toxoplasma* strains (types I, II, III and XII) are commonly isolated from animals, whereas atypical strains not belonging to these lineages are predominant in other continents such as South America. Strain type is a key factor in determining the outcome of *Toxoplasma* infection. Therefore, our aim is to develop a non-invasive assay that can distinguish infections by the main genotypes and the atypical strains. For that end, we selected 61 genes encoding polymorphic or differentially expressed proteins, synthetized 850 peptides that were predicted to be antigenic directly on a cellulose membrane (peptide array), and analyzed them for the ability to distinguish strain type. From the initial 850 peptides tested, 180 were antigenic and 26 were able to discriminate among type I, II and III infections in sera samples from mice, rabbits and humans. Those that displayed the highest strain specificity were synthesized as soluble peptides, coupled to the carrier protein KLH (keyhole limpet hemocyanin) and analyzed by ELISA. Preliminary results suggest that antibodies in mice experimentally infected with a particular strain are able to react specifically to some peptides. Importantly, our results indicate that a single antigenic peptide is not able to determine strain type, but instead, the reactivity of the serum of an infected animal/person will provide a characteristic “fingerprint” of the strain they are infected with. We will test the most promising peptides in panels of sera from different species (cats, horses, marine mammals and birds, among others), as well as for humans. The development of this assay would enable an inexpensive and easy method of diagnosing the strain involved in *Toxoplasma* infections and correlate *Toxoplasma* genotype to disease outcome.

Epidemiology and diagnostics II

Advances in the diagnosis of bovine besnoitiosis: current options and applications for control

K 05

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Bovine besnoitiosis, which is caused by the cyst-forming intracellular parasite *Besnoitia besnoiti,* is a chronic and debilitating disease that is responsible for severe economic losses in the cattle raised under extensive husbandry systems. The absence of vaccines, treatments or a health scheme at local, national, and international levels has led to a rapid spread of bovine besnoitiosis from Western Europe towards Eastern countries and northwards. Moreover, this parasitic disease is widely present in many sub-Saharan countries. Thus, bovine besnoitiosis should be included in the animal health scheme of beef cattle herds. Accurate diagnostic tools and common diagnostic procedures are mandatory in any control programme. Relevant advances have been made in this field for the last decade. The success of an accurate diagnosis will rely on the employed technique and the antibody and parasite kinetics of the infection stage, which may notably influence control programmes and surveillance. Moreover, control programmes should be adapted to the epidemiological status of the disease, as the disease presentation in a herd has important implications in the perspectives for control. Herein, we review the clinical disease presentation of bovine besnoitiosis and the correlation between its clinical course and laboratory parameters. We also provide an update on the available diagnostic tools, discussing their strengths and pitfalls, and provide guidelines for their use in control, surveillance and epidemiological studies. A rationale control strategy is also recommended.

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Deciphering 'cryptic' *Eimeria* isolated from the domestic chicken

O 18

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There are seven well-defined species of *Eimeria* that parasitise domestic chickens, causing coccidiosis (enteritis) with varying degrees and types of pathology. *Eimeria* parasites are globally distributed and most formulations of live coccidiosis vaccines contain many or all of these known species. Recently three highly divergent Operational Taxonomic Units (OTUs x, y and z), originally identified as novel ITS2 sequences in mixed populations of *Eimeria* oocysts collected from chickens in Australia, have been variously detected in countries of sub-Saharan Africa, southern India and Venezuala. The overall global occurrence, pathology and precise relationship of these OTUs to the seven known species is not fully understood. Potentially they may represent divergent genotypes of known species, genetic hybrids between one or more known species, or 'emerging' cryptic species that have been hitherto undetected.

Mixed field isolates from Nigerian broiler chickens were shown to contain OTU x, y and z parasites by sequencing of ITS1-5.8S-ITS2. Three isolates were independently passaged through chickens that had been super-vaccinated with the live-attenuated vaccine Paracox®8, which protects against all seven of the known *Eimeria* species. Recovered parasite populations were shown by PCR to contain OTUs x, y or z and were negative for the seven known species. By light microscopy oocyst length (L), width (W) and L/W ratios were determined as 30.5 µm, 23.4 µm, 1.30 (OTUx); 26.8 µm, 22.9 µm, 1.17 (OTUy) and 17.6 µm, 15.3 µm, 1.15 (OTUz), values that were corroborated for OTUx and z by comparison with reference isolates collected in Australia.

Total genomic next generation DNA sequences for Australian OTUx, y and z isolates have been generated using a Nextera XT protocol, resulting in assemblies of 42.9 Mb (16,071 contigs) for OTUx, 58.0 Mb (40,800 contigs) for OTUy, and 50.6 Mb (27,925 contigs) for OTUz. Phylogenetic inference using a range of reference genes as well as mitochondrial and antigen coding sequences shows significant divergence from the seven recognised *Eimeria* species, with OTUz being the most distinct. Additional analysis of these new genomes is underway to determine in more detail their taxonomic position; at the moment the risk to poultry posed by these novel 'cryptic' genotypes is unknown. It is now essential to understand their basic pathogenic potential, to assess in detail their capacity to escape from current anticoccidial vaccines, and to examine the impact they have on poultry production in those parts of the world where they are currently circulating.

Multigenome sequence-based genotyping of *Eimeria* species causing coccidiosis in Ontario sheep and goats

O 19

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The Ontario Animal Health Network in Canada identifies coccidiosis, caused by *Eimeria* spp., as a top clinical issue causing morbidity and mortality in lambs and kids. Despite infrequent published case reports, coccidiosis in small ruminants has not been studied systematically since 1984 in Ontario, Canada. There are ~11 named *Eimeria* spp. that infect sheep and at least two, *E. crandallis* and *E. ovinoidalis* are considered pathogenic. There are ~9 *Eimeria* spp. that infect goats and there has been considerably less work done on determining the species that are pathogenic. The identification of individual species, particularly those that are most pathogenic, is crucial information for effective and targeted anticoccidial treatment. Large numbers of oocysts from less pathogenic species can be excreted without apparent clinical effects; therefore, it is difficult to determine the clinical significance of infections using conventional oocyst enumeration methods that provide only aggregate counts. The objective of this study is to develop and validate an economical molecular assay for the identification of *Eimeria* species present in feces. This requires identifying species-specific sequences from the mtCOI and 18S rDNA loci. Field samples from sheep and goat producer farms across Ontario supplemented with samples from small ruminant producers globally were obtained. Oocyst morphometrics were linked to species-specific mtCOI and 18S rDNA genotypes using sequences obtained by PCR from single-species infections. NGS of PCR amplicons of a region of the mtCOI locus appear suitable for simultaneous identification and enumeration of multiple *Eimeria* species in mixed samples. Such an enhanced diagnostic tool can help development of management, feed and treatment options that have the greatest potential to decrease the impact of coccidiosis.

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An overview on Theileriosis: new diagnostic tools and their limits

K 06

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Theileria species infect a wide range of domestic and wild animals and are transmitted by ixodid ticks of Amblyomma, Haemaphysalis, Hyalomma and Rhipicephalus genera.

Theileriae include host-cell transforming and non-transforming species. New *Theileria* species and genotypes were discovered in the last decade and mixed infections were reported even in domestic animals. Subclinical *Theileria* infection in cattle in endemic regions produces chronic carrier state and serves as sources of infection for ticks. Therefore, latent infections are important in theileriosis epidemiology. Discrimination of species responsible for disease outbreaks is important for diagnostics and epidemiology.

Theileria diagnostics ranges from microscopic examination to serological and molecular assays; Giemsa stained blood smear is used for rapid diagnosis of acute cases, but it requires expertise in microscopy in subclinical or chronic infections. IFAT is the OIE gold standard assay widely used, its low throughput and cross-reactivity lead development of ELISA tests using specific Ag allowing a higher sensitivity, as well as the implementation of LFD useful in the field.

As concerning molecular methods, development of diagnostic assays from conventional to nested to real-time PCR or LAMP allowed improvement in sensitivity, quantification and detection speed, while RLB, bead arrays, pan-FRET assays and HRMa permit multiple species or genotypes detection at the same time.

A critical analysis of different methods used, joint to clinical and epidemiological data, is needed for Theileriosis diagnosis while many lacks are still present in diagnostic tools for this complex disease.

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Vaccination and immune responses

DC-complexa: species-specific adaptation of dendritic cells to pathogen diversity

K 07

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Dendritic cells (DC) play a central role not only in antigen presentation to T cells but also in sensing invading pathogens to alarm the immune system. This pathogen-DC interaction also directs the immune response and thereby plays an important role in determining if for instance type 1, type 2 or type 3 immunity will be induced. This may shift the balance between protective immunity and immune response-mediated disease. Considering the complexity of pathogens, it is not surprising that several subsets of DC with specialized functions have been described in mammals. These are conventional DC1 particularly potent in activation MHC class I-dependent CD8 T cell responses, conventional DC2 specialized in the induction of MHC class II-depended CD4 T cell responses, and plasmacytoid DC specialized in the production of large quantities of interferon type I in response to nucleic acids. In addition to these bona fide DC subsets, monocyte-derived DC have been described and these cells can be prominent during pathogen-induced inflammation. Noteworthy, all DC subsets also have a differential impact on polarizing the immune response towards Th1, Th2 or Th17. Considering the importance of these processes we have identified and characterized DC subsets in pigs and cattle, in particular focusing on their response to toll-like receptor ligands. The comparison of porcine, bovine, human and mouse DC subsets enabled the identification of conserved phenotype and functions as well as species-specific differences. These could have evolved as a consequence of evolutionary pressure due to unequal environmental exposures to microbes and for instance different digestive systems impacting the microbiome.

Analysis of local and peripheral immune response developed in sheep experimentally infected with *Toxoplasma gondii* at different times of gestation

O 20

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Toxoplasmosis is one the main infectious causes of reproductive failure in sheep where the time of gestation when sheep are infected affects the clinical and lesional outcome of the disease. In order to investigate the variations of local and peripheral immune responses during gestation, pregnant sheep were infected at early, mid and late gestation and subsequently culled at 2, 3 and 4 weeks post infection. In those sheep inoculated during the second term, serological antibodies were detected earlier and the increase in serological γ-IFN was higher than in the other infected animals. Regarding the local immune response at the placenta, infiltration of inflammatory cells was mainly found in the maternal septa, although it also invaded foetal mesenchyme adjacent to the lesions. The increase in the number of T lymphocytes was observed only in ewes infected during the second and last terms of gestation while the increase of B cells occurred in sheep infected at the first and second terms. The expression of iba-1 antigen by macrophages was more frequent after infection during the first term whereas macrophages expressing lysozyme, CD163 or calprotectin were more frequent in infections at mid-gestation. A significant increase in the transcription of γ-IFN, when compared to control animals, occurred after infections in the first and second term, while TNF-α and IL-10 transcription increased only in the second and last term, respectively. There were no differences when comparing transcription of cytokines between animals infected at different terms of gestation. This study shows that the time of gestation when infection occurs has a clear influence over the pathogenesis of ovine toxoplasmosis as a greater inflammatory response was found after inoculating sheep at the second term of gestation. This finding may explain the later invasion of the placenta by the parasite at early and mid gestation described in previous studies.

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CD103+CD11b- intestinal dendritic cells are critical players for controlling the different steps of *C. parvum* infection

O 21

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Intestinal epithelial cells play a key role in maintaining homeostasis. We investigated the factors contributing to the alteration of the epithelial barrier function during *Cryptosporidium parvum* infection which represent the second most common cause of diarrheal diseases in infants in developing countries. Polarized epithelial cell monolayers infected by the parasite exhibit a drop in transepithelial resistance associated with a delocalization of E-cadherin and β-catenin from their intercellular area of contact, the adherens junction complex. In neonatal mice infected by *C. parvum*, the increased permeability is correlated with parasite development and with an important recruitment of $Ly6c^{+}$ inflammatory monocytes to the subepithelial space. $CCR2^{-/-}$ neonatal mice which have few circulating inflammatory monocytes were infected at similar level than conventional mice but with a lower increase in intestinal permeability suggesting a deleterious role of inflammatory monocytes during cryptosporidiosis. We next demonstrated that TNFα and IL-1β known to modulate tight junctions proteins are produced by inflammatory monocytes in the lamina propria of infected neonates and therefore can play a key role in the loss of barrier function. Our findings demonstrate for the first time that both the parasite and inflammatory monocytes contribute to the loss of intestinal barrier function during cryptosporidiosis.

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Coccidiosis in poultry: development of *Eimeria* as vaccine vectors to streamline anticoccidial vaccination

O 22

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Coccidiosis, caused by *Eimeria* species parasites, has been ranked among the ten most economically significant enzootic livestock diseases with associated costs predicted to exceed £2.5 billion worldwide every year in farmed poultry. Both routine chemoprophylaxis and/or vaccination with live parasite vaccines are effective to control *Eimeria* parasites, although the emergence of drug resistance and the relative cost and production capacity of the vaccine strains can prove limiting. Recently, the availability of protocols supporting genetic complementation of *Eimeria tenella* has raised the prospect of generating parasite lines that could be used as vaccine vectors. Complementation with sequences encoding immunoprotective antigens from other *Eimeria* species offers an opportunity to reduce the complexity of species/strains currently included in anticoccidial vaccines, and consequently increase their cost effectiveness in the market. Here, we describe the ongoing development of *E. tenella*-vectored vaccines expressing the *Eimeria maxima* apical membrane antigen 1 (EmAMA1), previously described as immunoprotective, and fused with two different delivery signals – the signal peptide (SP) from *E. tenella* microneme protein 2 (EtMIC2) and the glycophosphatidylinositol (GPI) anchor from *E. tenella* surface antigen 1 (EtSAG1) – in an attempt to modify transprotein trafficking and thus improve antigen exposure to the host immune system. Vaccination of chickens using these parasites conferred protection against *E. maxima* challenge, with levels of efficacy comparable or better than those obtained using recombinant protein or DNA vaccines. This proof of concept supports the use of *E. tenella* as a vaccine vector, not only against the different *Eimeria* species, but also against other pathogens affecting poultry.

Biosafety and treatment

Drugs and drug targets in pregnant models for *Toxoplasma* and *Neospora* infection

K 08

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Neospora caninum and *Toxoplasma gondii* are closely related apicomplexan parasites, which can cause abortion and fetal defects in many animal species. While *N. caninum* is a veterinary problem and is most relevant in cattle, *Toxoplasma* induced abortion in sheep is a major problem, and *T. gondii* is also an important human pathogen, most notably when infection takes place during pregnancy, or in immune compromised patients. There is no approved treatment for neosporosis. The current treatments of toxoplasmosis are based on a combination therapy comprising sulfonamides and pyrimethamine or other antimicrobials including inhibitors of apicoplast division. In many countries, there is no approved treatment for maternal and fetal *T. gondii* infections. Therefore, current therapies are not optimal and novel treatment options are required. Most drug efficacy studies for neosporosis and toxoplasmosis have been carried in non-pregnant models, although it is imperative for any drug to be considered for the treatment of these infections that no interference in pregnancy occurs. For neosporosis in cattle, the major mode of transmission is thought to occur via endogenous transplacental transmission, but there is no mouse model that can mimic this process; thus, pregnant neosporosis mouse models rely on inoculation of culture-derived tachyzoites during pregnancy. For *T. gondii*, a pregnant oocyst infection model has been recently established. The anti-coccidial toltrazuril has been earlier assessed in pregnant mice and cattle, with clear readout in the mouse model, but ambiguous results produced in bovines. More recently bumped kinase inhibitors, which target calcium-dependent protein kinase 1, have emerged as novel treatment options *in vivo.* The screening of open-source drug libraries such as the MMV Malaria Box and Pathogen Box has revealed several compounds that could be potentially repurposed for the treatment of neosporosis and toxoplasmosis in pregnant animals.

Bumped kinase inhibitors and their effects on the host-parasite relationship during experimental *N. caninum* infection *in vitro* and in mice

O 23

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The bumped kinase inhibitor BKI-1294, targets *N. caninum* calcium-dependent protein kinase 1 (*Nc*CDPK1), interferes in tachyzoites proliferation *in vitro*, and blocks its transplacental transmission and fetal infection in pregnant mice. We have studied the localization of *Nc*CDPK1 in *N. caninum* tachyzoites, by immunofluorescence and immunogold-electron microscopy. In intracellular tachyzoites, *Nc*CDPK1 is localized within the cytoplasm. Localization shifts towards the apical part once parasites are maintained extracellularly, and *Nc*CDPK1 is associated with the supellicular membrane, few micronemes, and the conoid. However, even at high concentrations, BKI-1294 does not exert parasiticidal effects *in vitro*. Thus, it is not fully understood whether the widely demonstrated *in vivo* efficacy of BKI-1294 therapy is associated with other undefined host factors. We have earlier shown that BKI-1294 treatment leads to the build-up of large schizont-like multinucleated complexes (MNCs). We now show that these MNCs remain viable for extended periods of time. While parasites are blocked during cytokinesis, newly formed tachyzoites emerge from these MNCs 6-8 days after the drug is removed. During treatment, MNCs exhibit a deregulated mRNA and antigen expression pattern, and we hypothesize that this could cause the exposure of new, potentially protective epitopes that might not be otherwise accessible during infection with *N. caninum* tachyzoites. We hypothesize the increased antigen expression during BKI-1294 therapy leads to protective immunity that leads to clearance of the parasites. This hypothesis has been followed-up in an *in vivo* study, where protection in chronically infected and BKI-1294 treated mice against re-challenge during pregnancy is compared to non-treated chronically infected mice. In addition, the therapeutic potential of BKI-1294 and other BKIs for the management or treatment of chronic *N. caninum* infection is being investigated. The results of this study could lead to a better understanding of the cross-talk between chemotherapy and immunity in experimental murine neosporosis.

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Characterisation of drug-resistance to buparvaquone in *Theileria annulata* populations in Turkey

O 24

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Buparvaquone is the only drug for the treatment of tropical theileriosis since the 1980s. We previously demonstrated the presence of two different mutations at two drug-binding sites of the cytochrome b gene. We further demonstrated that 11/105 *T. annulata* isolates are phenotypically resistant to buparvaquone. Sequence analyses indicated that 6/21 non-synonym mutations are determined to be at the drug-binding sites at the cytochrome b gene. It was demonstrated that fitness of resistant parasite populations are high and that the transmission rate to ticks and the proliferation capacity in culture are similar among resistant and susceptive parasite populations. A causative relationship between copy number of the cytochrome b gene and resistance to buparvaquone could not be established. Response of two resistant isolates of *T. annulata* to buparvaquone treatment was compared against an isolate known to be susceptible in vivo. Resistant isolates caused more severe clinical symptoms and higher levels of parasitemia. Plasma concentrations of buparvaquone did not differ between animals infected with sensitive and resistant isolates. Therefore, there is a need for an immediate action to replace buparvaquone with more potent drugs against *T. annulata.*

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In vitro screening of commercially available anti-coccidials identifies diclazuril and decoquinate as potential therapeutic candidates against *Besnoitia besnoiti* infection

O 25

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Toltrazuril, diclazuril, imidocarb, decoquinate, sulfadiazine and trimethoprim (alone or in combination with sulfadiazine) are currently marketed compounds with proven efficacy against apicomplexan parasites. These drugs were screened for activitiy against *Besnoitia besnoiti* tachyzoites *in vitro* using a previously established infection model in Marc-145 cells. This included firstly an assessment of safety using a XTT kit for cell proliferation, secondly a primary drug screening at 0 and 6 hours post-infection (hpi) by direct immunofluorescence, and thirdly, those compounds displaying promising efficacy were selected for IC_{50} and IC_{99} determination by qPCR. In addition, the impact of drugs on the tachyzoite ultrastructure was assessed by TEM and long-term assays were performed. Cytotoxicity assays confirmed that all compounds were safe at the highest concentration employed in the drug screening. Diclazuril and decoquinate administered at 0 hpi and at concentrations of 30 µM and 240 nM, respectively, inhibited parasite invasion by 83 and 90%, and when administred at 6 hpi, by 73 and 72%, respectively. The remaining drugs showed lower invasion and proliferation inhibition rates, and were not further studied. Diclazuril and decoquinate exhibited IC99 values of 29.9 µM and 100 nM, respectively. TEM showed that decoquinate primarily affected the parasite mitochondrium, whilst diclazuril interfered in cytokinesis of daughter zoites. The present study demonstrates proof of concept for the efficacy of diclazuril and decoquinate against *B. besnoiti in vitro* and further assessments of safety and efficacy of both drugs should be performed in the target species.

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Variations in anticoccidial efficacy on different *Eimeria* spp. in lambs based on oocyst excretion

O 26

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Coccidiosis in lambs, caused by *Eimeria* spp., is a common cause of clinical disease and reduced growth rates, which is associated with substantial economic losses. Anecdotal reports of reduced anticoccidial efficacy in Norwegian sheep production systems, probably related to the extensive use of anticoccidials, necessitates investigation. The main aim of this study was to assess anticoccidial efficacy in Norwegian lambs, and here we present the effect of anticoccidials on different *Eimeria* spp. A total of 41 flocks were recruited based on questionnaire data indicating signs of coccidiosis in lambs treated with anticoccidials. Two faecal samples were collected from 8 twin pairs (≥14 days old at turnout) in each flock. Sample 1 was taken 6-8 days after turn out, and one twin was treated with 20 mg/kg toltrazuril (Baycox® Sheep vet, Bayer Animal Health) and sample 2 was taken 7-11 days post treatment. Oocyst excretion (McMaster with a sensitivity of 5 oocysts per gram (OPG)), faecal score and weight gain were measured. Speciation was performed in samples ≥ 1.000 OPG, based on morphology with *E. crandallis* and *E. weybridgensis* not being differentiated due to their similarities. From a total of 11 *Eimeria* spp identified, the average number of species per positive sample was 4.6 ± 0.1 in sample 1 and 5.5 ± 0.1 in sample 2. The most frequently found species in both sample 1 and 2 were *E. weybridgensis/crandallis, E. ovinodalis* and *E. parva.* When looking at all 41 flocks as a whole, there were significantly fewer oocysts of *E. ovinoidalis* and *E. crandallis/weybridgensis* in the treated group, compared with the control group, in sample 2 (post treatment). This could indicate a higher efficacy of toltrazuril against the pathogenic species compared with the non-pathogenic. Our finding highlights the difference between ovine *Eimeria* spp. and the importance of speciation, especially when looking at anticoccidial efficacy.

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Advances in bumped-kinase inhibitors for human and animal therapy of cryptosporidiosis

K 09

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Bumped kinase inhibitors (BKIs) have shown promise in animal models of cryptosporidiosis, toxoplasmosis, neosporiasis, and sarcocystosis. Improvements have been made to the safety and efficacy of BKIs, and they are advancing toward human and animal use for treatment of cryptosporidiosis. As the understanding of BKI pharmacodynamics for cryptosporidiosis therapy has increased, it has become clear that better compounds for efficacy do not necessarily require substantial systemic exposure. We now have a BKI with reduced systemic exposure, acceptable safety parameters, and efficacy in both the mouse and newborn calf models of cryptosporidiosis. This compound is a promising pre-clinical lead for cryptosporidiosis therapy in animals and humans.

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Food and waterborne zoonoses

Food and Waterborne Protozoa: a veterinary and public health perspective

K 10

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Protozoan parasites, such as *Toxoplasma gondii* and *Cryptosporidium parvum*, are well known for their ability to cause foodborne or waterborne outbreaks in humans and this knowledge is well supported by the scientific and public health literature. For example, globally there were 285 waterborne outbreaks of cryptosporidiosis reported up until 2010. While in the United Kingdom another study has shown that there were 66 drinking waterborne outbreaks of cryptosporidiosis between 1992 and 2015. However, infection by *T. gondii* tissue cysts from raw or undercooked meat is recognised as the main infection route for people. For these well studied transmission routes the emphasis is now on intervention strategies.

This is not the whole story. There are vital gaps in our understanding of the transmission of protozoan parasites that will be discussed in this presentation. One area of research that has received more interest in recent years is the potential of *T. gondii* oocysts contaminating catchments and water sources, which could lead to contaminated drinking water supplies. Only a few waterborne outbreaks of toxoplasmosis have been described worldwide; does that mean they are rare or are they just not noticed? Another neglected area of research for the zoonotic protozoan parasites is the role that fresh fruit and vegetables play in the transmission of oocysts to humans. There have been a very few reported outbreaks of cryptosporidiosis where vegetables have been implicated. Does this mean that this route is rare or do we not recognise these transmission routes? The same is also true for *T. gondii*, where oocysts could contaminate vegetables or fresh fruit but why do we have so little scientific evidence for this potential transmission route? Generally, we know so much more about how these parasites are transmitted to humans but there are many evidence gaps in our knowledge when it comes to infection routes for other animals. For example how do neonatal calves become infected with *Cryptosporidium*? Do they get it from other calves, their mothers or the environment? All of these questions will be addressed in this presentation.

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The impact of vegetation on the environmental transfer of *Cryptosporidium* oocysts from faeces into soil

O 27

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Environmental transfer of the parasite *Cryptosporidium* has major economic, animal welfare and public health implications. Disease transmission is associated with ingestion of infective oocysts which are robust and can survive in the environment for extended periods, ultimately transferring to watercourses where additional transmission routes are opened.

Investigation into the movement of *Cryptosporidium* from land to water, and the possible impacts of land management on such transmission, has to date been limited. The aim of this study was to use intact soil and vegetation cores to analyse oocyst movement from bovine faecal deposits into soil, with particular focus on the role of vegetation type and density in hindering transfer following rainfall. Concurrent studies involve analysis of vegetation, soil, water and faecal samples throughout the year from two similar glens at SRUC Hill & Mountain Research Centre (Crianlarich); one heavily grazed, the other rested for 17yrs. This comparison enlightens us to the interplay of environmental and agricultural factors involved in *Cryptosporidium* transmission and may lead to the development of models, which can be applied to wider catchment areas.

In vitro host cell viability as putative correlate of virulence of *Cryptosporidium parvum* field isolates in Eastern Germany

O 28

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Cryptosporidium (C.) parvum is a widely distributed protozoan parasite in suckling calves. The variable degree of clinical disease may partly influenced by keeping conditions and immune status of the host, but diversity of isolate virulence may also contribute.

A total of 60 farms in Eastern Germany were visited three times each at weekly interval. Faecal samples of 571 calves were collected. Faecal consistency and overall clinical condition was scored and faeces were examined microscopically for oocysts using Heine-staining. We applied the MTT-assay to evaluate effects of several *C. parvum* field isolates on cell viability of human ileocecal adenocarcinoma monolayers. To assess whether virulence of isolates can be estimated from *in vitro* host cell viability, clinical and parasitological data of the sampled calves were considered. Furthermore, isolates were subgenotyped at GP60 locus to evaluate whether subgenotypes are suitable to predict virulence of field isolates.

Altogether cell viability of monolayers inoculated with oocysts of *C. parvum* field isolates varied considerably between isolates with values of 8.5% (±1.2%) to 99.5% (±7.1%). Oocyst excretion was significantly higher for isolates that induced low cytotoxicity ($P < 0.01$) and highest oocyst excretion displayed a significant positive correlation with cell viability ($r_s = 0.699$, $P < 0.01$).

Subgenotyping was performed for 47 isolates. A15G2R1 was the most common subtype detected in 66% of the farms, followed by A16G3R1 (6 isolates). Subtypes A16G1R1, A16G2R1, A17G2R1 or A14G2R1, A17G1R1, A17G4R1, A19G2R1 were less prevalent and found in one or two farms. Calves infected with GP60 subtype A16G2R1 or A17G2R1 had a significantly ($P < 0.05$) higher peak of oocyst excretion as compared to the other subgenotypes. Logistic regression analysis showed that infections with *C. parvum* GP60 subtype A15G2R1 were associated with diarrhea ($P = 0.01$) and were related to dehydration ($P = 0.02$).

Impact of confinement housing on study end-points in the calf model of cryptosporidiosis

O 29

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Diarrhea is the second leading cause of death in children < 5 years globally and *Cryptosporidium* is a leading cause of that diarrhea. Currently, there are no consistently effective treatments. Drug development is dependent on the calf model of cryptosporidiosis, which is the best approximation of human disease. However, the model is not consistently executed. The two most common methods used are Complete Fecal Collection (CFC), which requires use of confinement housing, and Interval Collection (IC), which permits use of box stalls. CFC mimics the human challenge model but the impact of confinement on study end-points is unknown. We compared CFC and IC and evaluated the impact of housing on study end-points. Calves were randomized to confinement (n = 14) or box stall housing (n = 9) and challenged with 5 x 10^7 *C. parvum* oocysts. There were no significant differences in mean log oocysts enumerated per gram of fecal dry matter between CFC and IC samples ($P = 0.6$), nor were there diurnal variations in oocyst shedding ($P = 0.1$). Calves in confinement shed more oocysts ($P = 0.05$) and had higher plasma cortisol ($P = 0.001$), and required more supportive care ($P = 0.0009$) than calves in box stalls. We conclude that housing method confounds study end-points in the calf model of cryptosporidiosis. Due to increased stress data collected from calves in confinement housing may not accurately estimate the efficacy of chemotherapeutics targeting *C. parvum*.

Host-pathogen interactions in neonatal calves naturally and experimentally infected with *Cryptosporidium parvum*

O 30

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Calves infected with *C. parvum* can suffer from profuse watery diarrhoea, dehydration and in severe cases death may occur. Neonatal calves are highly susceptible to infection however, older calves become infected but do not show clinical signs. Our understanding of the host-pathogen interactions that determine disease outcome and host resistance in cattle is very limited. This study aims to examine the *in vivo* host response to infection with *C. parvum* in naturally and experimentally infected neonatal calves.

The study involved four uninfected age-matched calves as negative controls, ten naturally infected calves and fifteen calves which were infected at 3 or 4 days of age with 2.3E7 *C. parvum*. Regular blood and faecal samples were taken from all calves and groups of calves were culled at defined time points (days 3, 6, 9, 12 and 18 post-infection).

Ileum, lymph nodes, faeces and blood were collected at each time point. Sections of ileum and lymph node were collected for histological examination, *in-situ* hybridisation and immunohistochemistry. Clinical data was recorded and sera were used to measure specific antibody responses throughout.

Preliminary results from the ongoing study showed that calves experimentally and naturally infected with *C. parvum* displayed inappetence, diarrhoea and lethargy from day 3 to 15 post-infection. Severe clinical disease occurred in some experimentally infected animals. Histological examination of ileum sections showed mild to moderate changes to the villi and infiltration of eosinophils in experimentally infected calves. In the Peyer's patches large numbers of apoptotic and mitotic figures were present. Pathological findings from time points throughout the study will be presented.

3 Minute Oral Poster Presentations

Comparative study between pregnant mouse interference test and zebrafish embryo acute toxicity test: a possible replacement in anti-parasitic chemotherapy trials?

P 01

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Anti-parasitic drugs require toxicity assessment *in vivo.* Bumped kinase inhibitors (BKIs) have proven activities against apicomplexan parasites such as *Plasmodium, Cryptosporidium, Toxoplasma* and *Neospora*. For treatment of all clinical cases resulting from these parasitic infections, it is important to know whether chemotherapy could have potential interference with pregnancy outcome. Such assays are usually run using rodents as a model organism, but alternative tests that comply with the 3R concept (Replacement, Reduction & Refinement) are of high interest. We have assessed a set of 9 BKIs in pregnant mice. All compounds were well tolerated in single dose, and some had been shown to lack toxicity after multiple dose administration in non-pregnant mice. These BKI analogues exhibited selective toxicity against *C. parvum, N. caninum* and *T. gondii*. Experimental set-up included 6-7 mice per group, each receiving the compounds (60mg/kg/day) emulsified in corn oil for 5 days, starting at day 9 of pregnancy. Three BKIs, did not interfere with pregnancy outcome while 3 showed strong interference resulting in very high number of spontaneous abortions and/or still births. The last 3 BKIs exhibited intermediate effects, as reflected by 15-20% lost to stillbirth. Here, we present the comparison between the mouse pregnancy test and an assessment carried out with embryos from the zebrafish (*Danio rerio)*. Zebrafish embryos were exposed to the same compounds as used in mice. Following the OECD Test Guideline 236, the development of zebrafish embryos was followed light microscopically from the first hour post-fertilization, and then at 24h, 48h, 72h and 96h. This study will provide information on whether the zebrafish embryo test is a useful alternative to the use of mice in the pregnancy interference assessment.

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Investigating the virulence of *Toxoplasma gondii* isolates from Brazil and Saint Kitts in mice

P 02

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The species, *Toxoplasma gondii* (*T. gondii*) consists of many strains that cause varying levels of pathology in different hosts. A majority of European and North American *T. gondii* strains belong to one of three distinct lineages, type I, II or III. In South America, diverse atypical strains are more prevalent and in Brazil, BrI, BrII, BrIII and BrIV have been described. Currently, minimal literature regarding the virulence of Brazilian strains after oral ingestion of oocysts exists, but generally they are reported to be more virulent than clonal strains.

Here, we present the findings from two separate studies. In study one, 3 groups of Swiss Webster mice were orally inoculated with 50 *T. gondii* oocysts from the Moredun, U.K., isolate (M4), and two isolates from Brazil, BrI (Toxo DataBase genotype #6) and BrIII (#8) (one isolate per group). Mice were culled at different time points post inoculation and several tissues were collected for histological analysis.

In study two, 200 tachyzoites from 6 atypical field isolates from Saint Kitts, Caribbean, the M4 isolate and one Brazilian isolate (BrI) were inoculated intraperitoneally into 8 groups of Swiss Webster mice (one group per isolate). Mice were culled at different time points post inoculation and several tissues were collected for histological analysis.

Histology and immunohistochemistry were used to identify pathological changes and parasite distribution in the tissues of infected mice. A scoring system was established to allow statistical analysis of results.

Preliminary results show some strains are more likely to cause tissue cysts and mild pathology, while other strains are more virulent with a large number of tachyzoites associated with severe pathology. This research will provide a better understanding of the *T. gondii* genotypes located in different geographical areas.

Retrospective molecular diagnosis of *Neospora caninum* in bovine aborted fetus in Uruguay: Preliminary results

P 03

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Neospora caninum is a protozoan parasite considered one of the main causes of bovine abortion worldwide. In Uruguay, the estimated seroprevalence in beef cattle is 13 % and 22% in dairy cattle. Overall, 37% of cattle abortion in Uruguay is due to *N. caninum*. The routine diagnosis of *N. caninum* abortion is based upon histopathologic changes in fetal tissues and the identification of parasites by immunohistochemistry.

The aim of this study was to set up a PCR to detect *N. caninum* infection in fetal tissues from spontaneous bovine abortion. For this purpose, frozen samples of archived, diagnosed as positive to *N. caninum* through histopathology and immunohistochemistry test, were used. The results showed that 26 out of 31 (84%) analyzed samples were *N. caninum*-positive by PCR. This preliminary result demonstrates that when interpreted in conjunction with significant histopathologic changes in aborted fetal tissues, PCR is a valuable confirmatory tool to diagnose *N. caninum* in cattle. With this technique, it makes possible to perform retrospective analyses of archived samples and improves routine diagnosis of *N. caninum* abortion.

This is the first step to conduct further genetic studies in order to identify the *N. caninum* biovars available in Uruguay.

Isolation of a *Neospora caninum* goat strain from Southern Minas Gerais, Brazil

P 04

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Neospora caninum is sporadically reported as a cause of abortion and encephalitis in goats. However, little is known about the pathogenicity and behavior of this parasite in caprine species. Studies from our research group showed some aspects of the pathogenesis of neosporosis in naturally infected goats, and in the present study, we describe the first isolation of a *N. caninum* strain from a naturally infected animal. An one-year-old male goat with a *N. caninum* IFAT titer of 1:800 was euthanatized and the brain aseptically collected. Approximately, 100 grams of brain was liquefied in a mixer, and then digested with Tripsin-EDTA 0.5%. Aliquots of 20 ml of the final solution were collected and mixed with Percoll to a final concentration of 35%, centrifuged at 2200 g for 15 min without break, for the removal of myelin. The pellets of all samples were collected, washed twice in PBS and resuspended with PBS in a final solution of 20 ml. Samples were collected for qPCR of the *N. caninum* NC5 gene, and a parasite load of $5x10^4$ were intraperitoneally inoculated in four five-week-old gerbils. None of the gerbils showed clinical signs at 15 days post-inoculation (dpi) or 30 dpi. On 15 and 30 dpi the brain of two gerbils were collected, processed and intraperitoneally inoculated in C57BL/6 IFNg KO mice, as previously described for the goat brain. In IFNg KO mice on 15 dpi, there was a severe peritonitis with a large number of macrophages and tachyzoites. The peritoneal fluid of mice infected with *N. caninum* was inoculated in Vero cell monolayers. The isolated *N. caninum* strain presented low growth rate on Vero cells but could be maintained and multiplied on IFNg KO mice. This is the first report of *N. caninum* isolation from a goat. More studies will be performed to evaluate the genetic identity of this goat strain and its biological behavior, which will help to understand aspects of caprine neosporosis.

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A component of the core kinase module of MEN and Hippo pathways, Mob1 is a critical factor in *Toxoplasma gondii* replication

P 05

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The Hippo pathway and the mitotic exit network are pathways characterized, respectively, in drosophila/mammals and yeast that are involved in the control of cell cycle, cell proliferation and apoptosis. Mob1 is a component of the core kinase module of both these signaling cascades, thus an excellent candidate for the control of *T. gondii* replication. We have characterized a *mob1* gene in the *T. gondii* genome (TgMob1) and studied its expression through real time PCR. TgMob1 mRNA levels dramatically decrease (94%) during parasite replication inside the host cell. In agreement, a transgenic strain overexpressing TgMob1 shows delayed replication. Using an in-house mouse immune serum produced against the TgMob1 we observed that the protein mostly localizes at the parasite's posterior pole, where the basal complex is localized. Additionally Mob1 staining also shows a specific central punctate localization. At both regions of the cell parasite it co-localizes with the *T. gondii* MORN1, a protein shown by others to be involved in cytokinesis, being required for basal complex assembly and daughter cells segregation. The central localization of TgMob1 also presents a close association with both kinetochore (Ndc80) and centromere (CenH3) markers. Additionally, conditional knockout (KO) strains, using the CRISPR/Cas9 system, show that the depletion of Mob1 causes abnormal replication of *T. gondii* inside the host cell. KO strains originate large cellular masses with abnormal ultrastructure and enlarged and multiple nuclei. Overall, these results strongly suggest that TgMob1 is a critical factor in *T. gondii* replication.

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Cytoquine response to *Cystoisospora suis* infections in immune competent pigs

P 06

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The cellular immune system is thought to play a major role in the control of coccidiosis. In suckling piglets, the age group most prone to *Cystoisospora suis* infections, studies on local and systemic cellular immune responses have shown an involvement of TcR-Yδ$^+$ T cells and an antigen-specific IFN-γ production by lymphocytes. During infection, some peripheral cell populations significantly decrease with an increase in the gut, indicating their migration to the site of infection. However, only limited data are available on cellular and cytokine responses of immunocompetent pigs. Yet, they are of particular interest in terms of developing a passive immunization strategy. Therefore, 40 immunocompetent weaners were infected with *C. suis*, and blood was taken weekly until slaughter to compare their cytokine responses to that of non-infected pigs. Relative-quantitative real-time PCR was performed on white blood cells, spleen and mesenteric lymph node (MLN) cells to measure mRNA expression of TNF-α, IFN-γ, TGF-β, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12 and IL-27. Expressions did not differ in spleen or MLN while in white blood cells, most differences were observed within the first two weeks post infection. Expression of IL-2, TNF-α, IFN-γ and TGF-β was significantly lower in infected pigs. They may play a relevant role only at the site of infection as they are known to be involved in coccidial infections. These cytokines are also produced by TcR-Yδ$^+$ T cells which, just as in suckling pigs, might have migrated to the gut upon infection. The significant increase of IL-4 and IL-10 might reflect a shift towards a humoral immune response; *C. suis* is known to lead to increased serum antibody titers. IL-1b, IL-6 and IL-12 were significantly increased in infected pigs. We conclude that infections with *C. suis* induce a systemic cytokine response in immunocompetent pigs.

Transcriptional analysis of bovine monocyte-derived macrophages infected with high and low virulent isolates of *Neospora caninum*

P 07

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Neospora caninum is an obligate intracellular parasite, and its ability to survive inside host immune cells may be one of the key mechanisms responsible for the establishment of infection. Macrophages are key effectors in the innate immune response and one of the main target cells for this parasite. Recent *in vitro* studies carried out by our group have shown the capacity of *Neospora* to grow into bovine macrophages and differences in behavior dependent on the isolate. To investigate how *Neospora* manipulates its host cell environment, the transcriptional profile of bovine monocyte-derived macrophages infected with high (Nc-Spain7) or low (Nc-Spain1H) virulent *N. caninum* isolates was investigated. The activation pattern of infected cells was very similar between isolates. It was characterized by the induction of a M1 phenotype with expression of recognition receptors TLR2 and TLR3, which lead to a high expression of iNOS, proinflammatory cytokines (IL-1β, IL12p40, TNFα, IL-6) and the regulatory cytokine IL-10. Functional enrichment revealed over expression of genes implicated in chemokine signaling, inflammation, cell survival and the inhibition of genes related with the lysosome. Enriched pathways were the same for both isolates, but Nc-Spain1H-infected cells exhibited a greater response. Heat-inactivated tachyzoites induced a lower inflammatory response, and failed on altering the lysosomal pathways. Expression profile of *N. caninum* showed a higher expression in Nc-Spain7 of genes related with replication and metabolism. By contrast, cell signaling and response to stress were the main processes enriched among Nc-Spain1H over-expressed genes. Further, bradyzoite-related gene expression was increased in Nc-Spain1H. These results are consistent with those observed in our previous studies, which showed a greater capacity to survive and proliferate of the Nc-Spain7 isolate into macrophages. Concerning the low-virulent Nc-Spain1H, these findings suggest an increased cell modulation ability by this isolate, where the stronger antimicrobial response induced may be rationalized as a parasite strategy to achieve latency.

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Phylogenetic diversity of *Eimeria* spp. in different genotypes of house mice (*Mus musculus*) from the European Hybrid Zone using a multiple marker approach

P 08

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Eimeria is the most diverse genus of apicomplexans and several species cause problems for agriculture. Evolution of host-parasite interactions and parasite diversification has not been investigated using closely related host species. We study *Eimeria* spp. in the European house mouse Hybrid Zone (between *Mus musculus domesticus, M. m. musculus*) as a model for host-parasite interactions with the aim to distinguish phylogenetic diversity of *Eimeria* in the different genotypes of host. Markers from the three different genomes (nuclear [18S rRNA], mitochondrial [COI] and apicoplast [tRNA, 16S rRNA, 23S rRNA and ORF470]) were used to gain insight into interspecific relationships or even at subspecies level. Our results show that *Eimeria* strains are clustered in two groups; one specialist clade closely related to *E. falciformis* found preferentially in the pure *M. m. domesticus* host genotype and one generalist clade that infects not only *M. m. domesticus* but also pure *M. m. musculus* and hybrids between them. Moreover, the primer pair "Ap5" designed in our project targeting the apicoplast genome from different species of *Eimeria* shows higher diagnostic sensitivity in comparison with primers targeting the small ribosomal subunit (18S) and cytochrome C oxidase (COI). These universal primers might be suitable for detection of coccidians in other hosts.

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Host-parasite interactions of *N. caninum* isolates of different virulence in bovine fetal and maternal placental cell lines

P 09

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Neospora caninum, one of the main causes of abortion in cattle, is very effective at crossing the placental barrier. Bovine trophoblast and caruncular cell layers play a fundamental role in maintenance and immune regulation during pregnancy. In this work, tachyzoite invasion and growth kinetics of high- (Nc-Spain 7) and low- (Nc-Spain 1H) virulence isolates as well as mRNA expression of relevant host-cell factors (IFN-γ, TNF-α, TGF-β1, IL-12p40, IL-6 and IL-12 cytokines, TLR2, ICAM and VCAM), were investigated in established cultures of bovine caruncular epithelial (BCEC-1) and trophoblast (F3) cells. The parasite invasion (pIR) and cell infection (cIR) rates were higher in F3 than in BCEC-1 ($P < 0.05$). In addition, the isolate Nc-Spain7 showed higher pIR and cIR than Nc-Spain1H in F3. Nc-Spain7 showed shorter doubling times and higher tachyzoite yield than the Nc-Spain1H in F3 cells. Invasion and proliferation mechanisms were similar for both isolates in BCEC-1, although the tachyzoite proliferation of Nc-Spain1H showed a non-exponential growth pattern. The mRNA expression levels of IL-6, IL-12p40 and TLR-2 in F3 and ICAM, TGF-β1 and IL-8 in BCEC-1 varied significantly in infected cells respect to the uninfected cells at 24 hours post-infection. Differences between isolates were not found in the expression of any of the host-cell factors studied. Our findings confirm that *N. caninum* is able to proliferate in trophoblast and caruncular cells *in vitro,* and give important clues on the role of trophoblast and caruncular cells in the immune responses and pathogenesis of neosporosis.

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HSP81.2 from *Arabidopsis thaliana* enhances the immune response against NcSAG1 from *Neospora caninum* protein and partially protects mice from congenital neosporosis

P 10

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Neosporosis is caused by *Neospora caninum*, the main pathogen agent responsible for economic losses in the cattle industry. Currently, there are not cost-effective control options for neosporosis, and the development of a vaccine appears to be the best approach. In this study we administrated a novel vaccine formulation including the well characterized major surface antigen of *N. caninum* (NcSAG1) and as adjuvant the 81 KDa heat shock protein (HSP81.2) from *Arabidopsis thaliana* in a murine model of congenital neosporosis. Both proteins were expressed in *Escherichia coli*. BALB/c female mice were i.p. immunized on 0 and 15 dpi with a combination of equimolar quantities of rNcSAG1(10 μg) and rAtHSP81.2(30 μg) or each protein alone. Control group was administered 200μl of PBS. Mice were bled on 0, 15, 30, 60 and 90 dpi to determine total Immunoglobulin G (IgGt), IgG1 and IgG2a. On 60 dpi, mice were mated. Five pregnant mice per group were s.c. challenged with 2.10^6 NC-1 *N. caninum* tachyzoites on day 7.5-10.5 after vaginal plug was observed. Five pregnant mice from the control group were not challenged. The offspring were euthanized 60d of age. High IgGt, IgG1 and IgG2a specific antibody levels directed against rNcSAG1 protein were developed by the group that received the combined vaccine formulation and their offspring showed improved survival rates. In conclusion, the rNcSAG1+rAtHSP81.2 vaccine was able to induce an important humoral response in immunized mice and confers partial protection against *N. caninum* to their offspring encouraging us to further study its effectiveness against congenital neosporosis.

Effects of *Eimeria tenella* infection on chicken caecal microbiome diversity, exploring variation associated with severity of pathology

P 11

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Eimeria species cause coccidiosis, most notably in poultry, which impacts on animal health and welfare. When combined with *Clostridium perfringens*, *Eimeria* also contributes to necrotic enteritis, but there is a paucity of information regarding the impact of infection on the broader intestinal microbiome. Parasite-associated microbiome variation may exacerbate outcomes of infection, or predispose towards secondary infections. The severity of disease caused by *Eimeria* can be quantified by lesion score; of interest was an analysis of microbiome variation associated with different lesion scores.

Caecal microbiome structure and variation were assessed using 16S rRNA amplicon sequencing. Following *Eimeria tenella* infection the diversity of taxa within the caecal microbiome remained largely stable, however infection induced significant changes in the abundance of some taxa. The greatest changes were observed in samples collected from birds displaying severe caecal pathology; taxa belonging to the order Enterobacteriaceae were increased, while taxa from Bacillales and Lactobacillales were decreased. Many differential taxa from the order Clostridiales were identified, with some increasing and others decreasing in abundance. Quantification by lesion score revealed differential taxa were most abundant comparing samples from either end of the lesion score scale and identified clustering based on lesion score.

Greater understanding of caecal microbiome dysbiosis associated with *Eimeria* induced caecal tissue damage could aid in the development of in-feed probiotics with the aim of reducing the most severe effects of these ubiquitous parasites. Moreover, this work highlights the importance of accounting for differences in caecal lesions when investigating the relationship between *E. tenella* and the poultry intestinal microbiome.

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Dose-titration of virulent *Neospora caninum* isolate Nc-Spain7 in pregnant sheep at 90 days of gestation

P 12

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Neospora caninum is considered one of the main causes of abortion in cattle and recent studies showed its relevance as abortifacient in small ruminants. Recently, the well-characterized Nc-Spain7 isolate has been tested in a pregnant sheep model of *Neospora* infection at mid gestation (90 days of gestation) using 10^6 tachyzoites intravenously and resulting in 100% abortion and highly parasite detection in target fetal tissues. This isolate has also been titrated in a pregnant mice model suggesting that a dose of 10^5 tachyzoites would be suitable for testing drugs or vaccine candidates. Thus, a refinement and standardization of the pregnant sheep model of neosporosis is also recommendable. Whit this purpose, pregnant sheep were inoculated intravenously at 90 days of gestation with 10^5 (n=6) (G1), 10^4 (n=5) (G2), 10^3 (n=5) (G4), 10^2 (n=4) (G5) tachyzoites; and subcutaneously with 10^4 tachyzoites (n=4) (G3) of the Nc-Spain7 or with PBS (n=3) (G6). Clinical outcome and lesions, parasite detection, parasite burden in target tissues and humoral and cellular immune responses were evaluated. Fetal mortality was detected between 32-44 days post infection in G1 (6 out of 6 pregnant ewes), G2 (4 out of 5 pregnant ewes), G3 (3 out of 4 pregnant ewes), G4 (3 out of 5 pregnant ewes), and G5 (2 out of 4 pregnant ewes). Pregnant sheep in G6 gave birth to healthy lambs. Significant differences were found in the fetal survival rate between G1 and G6 ($P < 0.05$). Serological analysis showed IFAT titers higher than 1:32 and 1:200 in thoracic liquid of all aborted fetuses and precolostral sera of all lambs, respectively. *N. caninum* DNA was widely detected in placentomes/cotyledons investigated from all animals in the different infected groups. In fetal brain, parasite DNA was detected in 81.8%, 91.7%, 87.5%, 66.7% and 90.5% of samples examined in G1, G2, G3, G4 and G5 respectively. Significant lower parasite detection was found in G4 compared to G2 ($P < 0.05$). Parasite load in fetal brain was lower in G2 ($P < 0.05$) and G4 ($P < 0.001$) compared to G1 and G5. In addition, G4 showed lower brain parasite burden compared to G2 ($P < 0.05$). In conclusion, clear differences were observed concerning abortion between pregnant sheep inoculated with different parasite doses while less pronounced differences related to vertical transmission to the fetus were found between doses or routes of administration.

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A reduction in weight gain in beef calves with clinical cryptosporidiosis

P 13

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Cryptosporidium parvum is one of the main causes of enteric disease in young calves and lambs. It has been shown that this parasite has a long-term effect on the growth of children who are affected at a young age, although little research has been done to explore the economic impact of this parasite in livestock. Thirty-four calves on a Scottish beef farm have been scored for clinical signs of cryptosporidiosis every second day between birth and 16 days of age. Scores of clinical signs included both demeanour and faecal consistency. An overall score was assigned to each animal that also included duration of symptoms. These animals were split into three groups based on their scores: severe clinical cryptosporidiosis, mildly affected and seemingly unaffected. Animals were weighed periodically during 6 months up until they went to market. Animal weight at 6 months was compared between the three groups.

Results show that there is a statistically significant difference in the mean weight of animals with clinical cryptosporidiosis and those, which had no signs of disease, although there is no significant difference in the weight of severely affected and mildly affected animals. Calves with severe clinical signs of infection were on average 34 kg lighter than calves with no clinical disease. This, along with other associated costs, shows that cryptosporidiosis has considerable economic impact.

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Posters

Microgametes – just motile nuclei or pivotal stages of the life cycle of *Cystoisospora suis*?

P 14

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The apicomplexan life cycle encompasses gamogony which results in two morphologically very different stages, the micro- and the macrogamont, that mature and form micro- and macrogametes that fuse to form a zygote. In the Coccidia, this is followed by the formation of oocysts. Earlier works have described the attachment of the microgamete to and its presence in the macrogamete ultrastructural studies; however there is some debate over the frequency and necessity of the fusion of these two stages in Coccidia. In *Toxoplasma gondii* the number of microgametes appears to be insufficient to fertilise most of the macrogametes, so it was assumed that, at least in this species, gamete fusion is not a prerequisite for oocyst sporulation. On the other hand, microgametes in *Eimeria* spp. are more numerous, and in some species of this genus, inhibition of microgamete formation or attachment inhibition of microgametes to the macrogamete by anti-microgamete antibodies is prohibitive for sporogony. *Cystoisospora suis* is phylogenetically most closely related to *T. gondii* but biologically more similar to *Eimeria* spp. as it appears to multiply primarily (if not exclusively) in the intestinal epithelium. What sets this species apart from both is the availability of a simple cell culture system which brings forth all endogenous stages of the parasite after infection with sporozoites. Gamonts can be seen from day 8 of cultivation with a peak on day 11, with numerous motile microgametes found both intra-and extracellularly. The high number of microgametes and the exposed position outside the host cell raise the question whether these stages may play a decisive role in the development of *C. suis* and may be accessible to host antibodies.

Demonstration of the presence of *T. gondii* in bio pigs intended for human consumption in Belgium

P 15

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Various indirect and direct methods can be used for the diagnosis of toxoplasmosis. Serological tests are useful to proof that a contact with the parasite has occurred. Although easy to perform, the actual presence of the parasite in the tissues of a seropositive animal is not demonstrated. For this, mouse bio-assay is still today competing with molecular techniques to be the reference method.

In this study we present a sensitive, efficient and ISO 17025 validated Magnetic Capture qPCR method for the detection of archetypal *T. gondii* strains in animal tissues with higher sensitivity than mouse bioassay (mouse Bioassay:86.49%; MC-qPCR:94.12%). In order to proof if the method is strong enough to implement for screening purposes, a pilot study was designed with visits to a Belgian slaughterhouse where bio pigs are slaughtered. A total of 92 pigs representing 17 different farms were tested first with an indirect serological method (MAT) and in case of positive results, the presence of the parasite was demonstrated by testing hearts with 3 different direct methods: MC-qPCR, qPCR on digest and the mouse bioassay.

The pilot study yielded a total of 15 seropositive pigs from where the actual presence of the parasite in hearts was confirmed in 13 pigs by the MC-qPCR while only in 9 pigs by the qPCR on digest and the mouse bioassay. In the light of the obtained results it is clear that the MC-qPCR is more sensitive than the two other methods. This higher sensitivity was also observed previously when testing tissues originated from experimentally infected pigs. Overall 16% of the organic pigs were found positive for *T. gondii* by MC-qPCR representing a potential risk for the consumers.

Here we present the successful application of the MC-qPCR to detect positive tissues at slaughterhouse level and the higher sensitivity of the method compared to other direct detection methods.

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Analysis of allelic diversity of two immunodominant antigen genes of *T. annulata* and *T. lestoquardi* in Oman

P 16

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Theileriosis is a widespread protozoan tick-borne disease of ruminants in Oman, causing high morbidity and mortality. Chemotherapy and acaracides are the main methods used to control theileriosis. There are a number of drawbacks associated with these methods and, as a consequence, there is great need for identification of putative vaccine candidate antigens.

Previous studies showed some success: with two candidate antigens, *Tams1* and *Ta9*, demonstrating potential for inclusion in a subunit vaccine against *T. annulata*. However, sequencing of the *Tams1* and *Ta9* genes from field isolates has demonstrated sequence divergence with evidence of positive diversifying selection of amino acid substitution. No information is currently available on diversity of antigens encoded by orthologous genes in *T. lestoquardi*, the major pathogenic *Theileria* species of small ruminants. The aim of the current study was to determine the extent of allelic diversity for these candidate antigens in *T. annulata* (*Tams1* and *Ta9*) and *T. lestoquardi* (*Tlms1* and *Tl9*) in Oman.

Analysis of allelic sequences derived from 11 *T. lestoquardi* and 9 *T. annulata* isolates from the same farm showed a total of 144 nucleotide positions (22.9 %) were polymorphic in *Tams1* while only 19 (3%) nucleotide positions were polymorphic in *Tlms1.* A small number of parsimony-informative sites (n=17) and singleton sites (n=2) were detected in *T. lestoquardi* sequences, while more of each were identified in *T. annulata* (Ps=98 and S=46). Haplotype diversity (expected Hd) was lower in *Tlms1* than *Tams1.* For the $CD8^+$ T cell antigen gene in *T. annulata* (*Ta9*), 250 polymorphic sites were detected consisting of 190 parsimony-informative and 60 singleton sites. In contrast, analysis of the *T. lestoquardi* orthologue (*Tl9*) revealed a lower level of diversity with 106 polymorphic sites (Ps=94 and S=12).

Global dN/dS values were computed as <1.0 for *Tams1*, *Ta9* and *Tl9*, implying these genes have evolved, in general, under purifying selection pressure. Tajima's D, Fu's Fs and Fu & Li's indices did not show significant departure from neutrality. Also, the dN/dS ratio was 1.899 for *Tlms1*, suggesting positive diversifying selection resulting in amino acid diversity may have occurred. In conclusion, all genes tested were found to be generally under a level of purifying selection, although evidence of positive diversifying selection in some areas of certain genes was present. The results imply that proteins encoded by these genes perform a biological function but that particular (antigenic) regions may display amino acid diversity. Characterization of any divergent Ab or T cell epitopes would be required for development of recombinant vaccines.

Genetics differentiation of *T. lestoquradi* in Africa and Asia

P 17

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T. lestoquardi, is associated with high mortality and morbidity among small ruminants in tropical and sub-tropical countries, and is a major constraint to the development of livestock industry. However, there is currently no information on the extent of genetic diversity in different geographical areas, and whether these parasites are isolated. The present study investigated the extent of genetic diversity of *T. lestoquardi* in Sudan (Africa), and compared to that in Oman (Asia). Such information will help to developing evidence based control measures and elimination strategy.

Blood samples (n= 110) were collected from apparently healthy sheep from River Nile state in Sudan. Nine genetic markers (micro- and mini-satellites) representing all chromosomes were examined. The resultant genetic data was analysed to provide the first insight into the genetic structure of the *T. lestoquardi* population in Africa. Out of 110 samples, 98 (89 %) were found to carry *T. lestoquardi*, indicating high of asymptomatic infection. A high rate of multiplicity of infection was observed with an average of 2.94 genotypes/ in infected animals. A moderate level of genetic diversity was observed, the mean heterozygosity (H_e) was 0.572, which is similar that seen among T. lestoquardi in Oman (H_e = 0.582). Significant linkage disequilibrium was observed among parasites in both Sudan and Oman. However, a significant genetic differentiation (F_{ST} = 0.332) was observed between the two populations, in Sudan and Oman

High rate of parasite multiplicity and genetic diversity were seen among T. lestoquardi in Sudan (Africa), comparable with that seen in Asia (Oman). There was significant genetic differentiation between parasites in Sudan and Oman, and a high level of genetic diversity was maintained within each sub-population. These findings are consistent with a high parasite transmission rate and limit movement of animals between two countries. The above findings are significant for control measures, based on vaccine or drug therapy.

Role of wildlife in the transmission of *Cryptosporidium parvum* to humans and livestock

P 18

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Cryptosporidium parvum is a zoonotic protozoan parasite of great public health concern. Upon infection hosts suffer profuse watery diarrhoea, dehydration and in severe cases death can ensue. Great economic losses occur each year from livestock contracting cryptosporidiosis some of which include treatments costs as well as loss of younger animals are more susceptible to developing the disease. It is therefore important to pinpoint sources of environmental contamination of oocysts and hence control their spread. *Cryptosporidium parvum* can infect multiple host species and it is thought that wildlife vectors may contribute to the spread of this parasite to both livestock and humans, however little documentation of species of *Cryptosporidium* found in Scottish wildlife, currently exists.

This study aims to investigate which species of *Cryptosporidium* are found within wildlife and to determine whether they contribute to the spread and prevalence of *C. parvum* in livestock.

Samples were collected (n=167) from various rodents killed by domestic cats all from within the same farmland. DNA was extracted from the gut content of voles (n=110), mice (n=37), rats (n=9), shrews (n=9) and 1 mole. DNA was amplified by PCR targeting the *Cryptosporidium* 18S rRNA and Actin genes. PCR positive samples were purified and the species present identified by sequence analysis.

Preliminary results have shown that of the 128 completed samples, 47 (36.72%) samples tested positive for *Cryptosporidium* spp. Two of these (from voles) were identified as *C. parvum.* Other genotypes identified in voles included: *Cryptosporidium* sp. UK E7 isolate (HQ822135.1)(n=11); *Cryptosporidium* sp. UK E4 isolate (GQ183525.1)(n=3) and *Cryptosporidium* sp. muskrat genotype II isolate (GQ183516.1)(n=1) In addition, three novel genotypes were identified in 3, 5 and 5 samples respectively. Samples from other host species are still to be sequenced and analysed.

Anti-cancer drugs affecting apicomplexan parasites: characterization of novel ruthenium-based compounds and their effects on *Toxoplasma gondii*

P 19

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The *in vitro* effects of a panel of dinuclear- thiolato bridged arene ruthenium complexes, (mono-, di- and tri-thiolato compounds), originally designed as anti-cancer agents, were studied in the apicomplexan parasite *Toxoplasma gondii* grown in human foreskin fibroblast host cells (HFF). Only few tri-thiolato compounds exhibited anti-parasitic efficacy at 250 nM and below. Among those, complex 1 and complex 2 inhibited *T. gondi* proliferation with IC_{50} values of 32 and 62 nM, respectively, and they did not affect HFF at dosages of 200 µM or above, resulting in selectivity indeces of > 16'000. The IC_{50} values of complex 3 were 1.2 nM for *T. gondii* and above 5 µM for HFF. TEM detected ultrastructural alterations in the matrix of the parasite mitochondria at the early stages of treatment, followed by more pronounced destruction of tachyzoites. However, all three compounds applied at 250 nM for 15 days did not act parasiticidal. Other complexes, such as complex 4 and 5, appeared to induce cyst formation as shown by immunofluorescence using bradyzoite markers such as mAbCC2 and anti-MAG1 antibodies after long-term treatments with these compounds. By affinity chromatography using complex 3 coupled to epoxy-activated sepharose followed by mass spectrometry, *T. gondii* translation elongation factor-1 alpha and two ribosomal proteins, RPS18, and RPL27 were identified as potential binding proteins. These results indicate that these compounds exhibit anti-parasitic properties due to interference in protein synthesis and mitochondrial function, and could thus induce bradyzoite formation.

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Semi-high throughput screening of the Pathogen Box for inhibitors with dual efficacy against *Giardia lamblia* and *Cryptosporidium parvum*

P 20

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Giardiasis and cryptosporidiosis, diseases characterized by diarrhea and malabsorption with increasing veterinary and public health implications, need more effective alternative therapies. Unfortunately, efficient cell-based assays amenable to high-throughput drug screening (HTS) are lacking for *G. lamblia*. Here, we report development of a screening method utilizing *G. lamblia* engineered to express red-shifted firefly luciferase, similar to the recently described luciferase expressing *C. parvum*. Parasite growth and replication were quantified using D-luciferin as substrate in a bioluminescent read-out platform. This semi-HTS assay was validated for reproducibility and reliability against the Medicines for Malaria (MMV) Pathogen Box compounds. For *G. lamblia,* 43 compounds (10.75 %) were identified with ≥75% inhibition of cell growth at 16 µM, of which 15 (3.75%) showed ≥95% inhibition and were selected for further analysis. For *C. parvum*, 85 compounds (21.25 %) had ≥75% cell growth inhibition at 10 µM, with 13 (3.25%) selected for further analysis. Seven compounds (1.75%) inhibited both parasites. Thirteen were further analyzed to determine the effective concentrations causing 50% growth inhibition (EC_{50}s) against both parasites and mammalian HepG2 cells. Compounds with EC_{50} values in the low micromolar range and no discernible cytotoxicity on mammalian HepG2 cells were deemed potent hits. Three of the seven dual hit molecules shared no obvious chemistry with any previously characterized anti-parasite drugs and offer new medicinal chemistry opportunities for therapeutic development. Our results suggest that bioluminescent assays are suitable for large-scale screening of chemical libraries against both *C. parvum* and *G. lamblia*. Moreover, this screen found molecules with dual-specificity, which may provide opportunities to treat giardiasis and cryptosporidiosis individually or in co-infections.

Evaluating the impact of pregnancy-associated immunomodulation on specific immune mechanisms against *Neospora caninum* infection in mice

P 21

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N. caninum is a causative agent of abortions in cattle worldwide leading to major economic losses. Parasite persistence in cattle populations depend largely in vertical transmission, which can derive either from reactivation of infection in persistently infected cows (endogenous vertical infection) or from primo-infections during pregnancy (exogenous vertical transmission). Mice models have been widely used to evaluate prophylactic strategies able to interfere with parasite vertical transmission, but the studies developed so far have not yet allowed the development of a safe and effective vaccine against congenitally acquired neosporosis in cattle. Here we confront immune parameters evaluated in non-pregnant versus pregnant mice aiming to understand the impact of pregnancy-associated immunomodulation on specific immune responses in infected and vaccinated mice. Antibodies and cytokines were evaluated in mice immunized with subunit antigens and challenged during pregnancy. A drop in antibodies directed against vaccine antigens was observed in some pregnant mice at 9 dpi but not in non-pregnant mice. Moreover, a shift in antibody isotypes towards IgG1 was observed in pregnant mice 30 dpi compared to non-pregnant mice. In an independent experiment, the role of CD8+ cells on the control of parasite recrudescence was evaluated by depletion of CD8+ T cells 4 weeks after the primary-infection. Recrudescence and higher morbidity was observed in depleted non-pregnant and pregnant mice, as well as higher morbidity and vertical transmission to pups, comparing with non-depleted mice. Together, these results suggest a role of CD8+ cells in controlling parasite recrudescence in chronically infected mice in both non-pregnant and pregnant mice and a potentially relevant role of pregnancy-associated immunomodulation in the impairment of vaccine-induced protective immune mechanisms in pregnant animals.

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Can multi-copy genes be an alternative for the diagnosis of TBDs in cattle? P 22

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Tick-borne diseaes (TBDs) affects domestic cattle and Asian buffalo, and impose serious constraints upon breed improvement programmes and livestock production and can cause major problems for the health and management of domestic cattle. Knowledge of risk factors associated with TBDs is an important pre-requisite in the design and implementation of control strategies in endemic and non-endemic regions. Within these, detection of parasites in carrier animals using specific, sensitive and reproducible laboratory tests is of great importance. The importance of selecting target gene sequences for development of molecular tests used to detect the very low levels of parasites that occur in carrier animals is evident. This study describes the presence of possible multi-copy gene family candidates within the genomes of *Theileria annulata*, *Babesia bovis* and *Anaplasma marginale*. *Sfi*, *Tar* and *SVSP* genes form the largest multi copy gene families encoded in the genome of *T.annulata*. However, none of these could provide a suitable target due to extensive nucleotide polymorphism across the paralogous gene sets. The genome of *A.marginale* comprises three major multicopy gene families (msp, ORFX and ORFY). Within these msp genes were found to be suitable targets for the detection of parasites in carrier animals. Within the multicopy genes encoded in the genome of *B.bovis*, *ves1* genes comprise the largest family with 119 identified *ves1* genes. Primers targeting conserved regions of *ves1* genes was found to be able to amplify a panel of different isolates with no evidence of length polymorphism. The use of multi-copy gene families as molecular diagnostic tools for the detection of carrier animals would be of beneficial.

Differential modulation of the Golgi and endosomal system in host cells infected with *Besnoitia besnoiti*, *Toxoplasma gondii* and *Neospora caninum*

P 23

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The Golgi apparatus is involved in transport and post-translational modification of proteins and glycosylation of lipids synthesized in the endoplasmic reticulum. The closely related apicomplexans *Toxoplasma gondii, Besnoitia besnoiti* and *Neospora caninum* recruit the host Golgi close to the parasitophorous vacuole (PV), which can be of great advantage when scavenging nutrients from the host cell. Using specific antibodies directed against *cis* (GM130) and *trans* (TGN46) Golgi proteins, we observed that there is a dispersion of the Golgi ribbon in *T. gondii* infected RPE-1 cells, compaction of the Golgi is seen in cells infected with *B. besnoiti*, and no remodeling of the Golgi can be detected upon *N. caninum* infection. The Rab 9A GTPase mediates endosome to *trans*- Golgi network (TGN) transport, Golgi targeting of glycosphingolipids, and lipid transport from late endosomes. At 24h post-infection, this protein was recruited to the vicinity of the PVs in all three parasites, indicating that these parasites manipulate the host cell endocytic pathway. Moreover, significantly increased Rab9A-mRNA expression was observed in *T. gondii* infected cells compared to non-infected cells, but not in cells infected with *B. besnoiti* and *N. caninum.* Overexpression of Rab9A was confirmed by Western blotting. Furthermore, overexpression of a Rab9A DN form in RPE-1 cells does not affect parasite invasion, and *B. besnoiti*, *T. gondii*, as well as *N. caninum*, do not show any relevant difference in terms of replication. Thus, despite exhibiting differential effects on Golgi morphology, these three parasites interact with Rab9A, showing a capacity for hijacking the host cell endocytic system, probably to efficiently acquire nutrients for intracellular proliferation and development.

A new indirect ELISA for the detection of Besnoitia besnoiti antibodies in individual and bulk milk samples

P 24

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Bovine besnoitiosis is of economic importance for cattle owners. Recent epidemiological data has confirmed an increase in the number of cases in cattle herds in both Europe and beyond. Serology may be used to identify infected animals and prevent the introduction of infected animals into disease-free herds. Antibody detection is currently performed by different techniques on plasma or serum samples. This poster presents validation data for a new commercially-available ELISA allowing for the detection of Besnoitia besnoiti antibodies in individual or bulk milks.

An infected herd (190 cattle, Ariège, France), where clinical cases had been reported as of 2012, was studied. Paired blood and milk samples collected from 97 cattle were tested with the ID Screen® Besnoitia besnoiti 2.0 Indirect ELISA and the ID Screen® Besnoitia besnoiti milk Indirect ELISA. For 98% of animals, the same status was obtained, regardless of the sample type tested (k=0.940), demonstrating that Besnoitia besnoiti antibodies may be detected in milk.

Specificity was evaluated through the analysis of 184 individual milk samples collected from 4 herds in France and Belgium with no history of bovine besnoitiosis. All samples were found negative, giving a measured specificity of 100% (IC95%: 99,6 – 100,0%).

In order to verify the absence of cross-reactions with other apicomplexan protozoa, 90 milk samples from an N. caninum-infected French herd. The measured prevalence of N. caninum in the herd was 50%. Abortions were observed. Samples were tested using the ID Screen® Besnoitia Besnoiti milk ELISA. All samples were found negative, indicating the excellent exclusivity of the test.

Sensitivity was assessed by testing samples confirmed by another serological method (ELISA or Western-Blot (WB)). Out of the 107 samples tested, 105 were found positive, giving a measured sensitivity of 98.1% (IC95%: [95,6 – 100%]).

To complete the initial study, individual, paired milk and blood samples from both infected and disease-free herds were tested using the serum and milk ELISAs. Out of the 333 samples tested, 316 samples gave the same positive / negative status for both milk and serum, for an overall agreement of 99,2%. The kappa value between the two tests was very high (k= 0.891, $CI_{95\%}$ [0.845 – 0.943]). Out of the 201 samples found positive by the serum test, 200 were also found positive on milk, for a relative sensitivity of the milk ELISA of 99,5% with respect to the serum ELISA.

This study demonstrates the first evidence of the possibility of detecting Besnoitia besnoiti antibodies by ELISA from milk. The kit shows excellent sensitivity and specificity, as well as excellent agreement with the serum ELISA. Bulk milk ELISA results from 12 herds exhibiting different seroprevalences were also tested. The kit is able to detect low to moderate prevalances. Field validation is ongoing to assess the possibility of using bulk milk samples for Besnoitia besnoiti herd monitoring/surveillance.

The IDScreen® *Besnoitia* indirect 2.0 serum ELISA perfectly correlates with confirmatory techniques

P 25

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Bovine besnoitiosis cause important economical consequences for cattle owners. Recent epidemiological data has confirmed an increase in the number of cases in cattle herds in both Europe and beyond. Serology may be used to identify infected animals and prevent the introduction of infected animals into disease-free herds. This poster presents validation data for the ID Screen® Besnoitia Indirect 2.0 ELISA. This kit detects antibodies against *B. besnoiti* in bovine serum or plasma samples.

Specificity was evaluated through the analysis of 814 cattle sera, collected in 2011 from French bovine herds from Ile et Vilaine, France, where besnoitiosis has never been reported (no clinical signs, no positive serology). All samples were found negative, giving a measured specificitiy of 100% (IC95%: 99,5 – 100,0%).

In order to verify the absence of cross-reactions with other apicomplexan protozoa, 15 sera from *T. gondii* and *N. caninum* seropositive animals were tested using the ID Screen® ELISA : all samples were found negative, indicating the excellent exclusivity of the ELISA.

Sensitivity was assessed by testing samples confirmed by another serological method (ELISA or Western-Blot (WB)). Out of the 107 samples tested, 105 were found positive, giving a measured sensitivity of 98.1% (IC95%: [95,6 – 100%]).

Finally, in a study conducted by two laboratories (ENVT Ecole Nationale Vétérinaire de Toulouse - Laboratoire Vétérinaire Départemental de l'Ariège, France), 520 cattle samples (195 positive, 325 negative), previously characterized by WB were tested. 516 / 520 samples gave the same result with both techniques, giving an overall agreement of 99,2%. The kappa value between the two tests was very high (k= 0.984, $CI_{95\%}$ [0.968 – 1]). With respect to the WB, the relative specificity (Sp), was 99,7% ($IC_{95\%}$: [99,1 – 100,0%]), and the relative sensitivity (Se), 98,5% ($IC_{95\%}$: [96,8 – 100,0%]).

The IDScreen® *Besnoitia besnoiti* ELISA shows excellent sensivitity and specificity, as well as an excellent agreement with the Western-Blot technique. Thanks to its high performance, the positive and negative predictive values remain high regardless of the prevalence of the tested herd, making the test a reliable method to prevent disease spread.

Experimental infection with *Besnoitia besnoiti* tachyzoites in calves and young bulls

P 26

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Bovine besnoitiosis is considered as a re-emergent disease in Europe. No treatments or vaccines are available for disease control. Therefore, the development of animal models of infection is urgently needed. The aim of the present study was to develop an experimental model of *B. besnoiti* infection in cattle.

Two experimental infections were realized. In experiment A, 12 Holstein Friesian 3-month old male calves were inoculated intravenously with either 3 different doses of tachyzoites (G1: 10^{8}; G2: 10^{7}; G3: 10^{6}) or with PBS (G4). In experiment B, 6 young bulls (14 months of age) were inoculated with the infection dose that showed the best results in experiment A (10^{6} tachyzoites). In both experiments, temperature and clinical signs compatible with acute and chronic besnoitiosis were monitored daily and blood samples were collected regularly for antibody and parasitemia detection. The experiment A was followed up to 70 days post-infection (pi), whilst the experiment B lasted 115 days pi. At the end of the trials, animals were euthanized and tissues from skin, eyes, respiratory and reproductive tracts among others, were collected for lesions and parasite detection. Clinical signs compatible with acute phase, such as lymphadenopathy and fever were observed in both calves (from 12 hours pi until 7 days pi) and young bulls (from 6 days pi until 9 days pi). Parasitemia was detected sporadically in calves from G1 on days 4 (n=2) and 7 dpi (n=3); and in G2 on 7 dpi (n=1). However, no clinical signs characteristic of the chronic stage of the disease, such as tissue cysts, were detected. All infected animals seroconverted around 16-19 days pi and antibody levels remained high until the end of the trials. In calves, parasite-DNA was detected in conjunctiva, ocular sclera, epididymis, as well as skin of scrotum and carpal zone (n=10, 6 of which belonged to calves inoculated with 10^{6} tachyzoites). However, in young bulls only 2 tissues (pampiniform plexus and testicular parenchyma) were positive by PCR.

The age seems not to be a key host-factor for inducing signs characteristic of the chronic stage. Despite the acute stage of the disease was successfully reproduced with a mild-moderate severity, the chronic stage was not developed. A further refinement of the present experimental model is needed and other parasite and host dependent factors should be considered.

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A new ELISA test to diagnose *Besnoitia* spp. infection in bovids and wild ruminants with improved specificity avoiding the use of a confirmatory test

P 27

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Recent studies have reported that routinely used whole or soluble *Besnoitia besnoiti* tachyzoite (TZ) extract based ELISAs may raise a high number of false positive results with subsequent limitations in control and epidemiological studies of bovine besnoitiosis. Thus, Western blot (WB) has been recommended as a confirmatory test. In the present study, a new ELISA test that employs lyophilized TZs (BbSALUVET ELISA 2.0) was developed and validated with cattle sera (n=606) under worst-case scenario. False positive and false negative soluble TZ extract based BbSALUVET ELISA 1.0 reactors were overrepresented and WB was considered as the reference test. One commercial test (PrioCHECK Besnoitia Ab 2.0 that employs whole TZ extract) and a recently developed membrane enriched ELISA (APure-BbELISA) were also tested. The three ELISAs showed high AUC values (> 0.9). However, the best diagnostic performance corresponded to BbSALUVET ELISA 2.0 and the APure-BbELISA [(92% sensitivity (Se); 98% specificity (Sp)] followed by PrioCHECK Besnoitia Ab 2.0 (88% Se; 98% Sp; 4.5% doubtful results). A different antigenic composition of lyophilized TZs compared with whole or soluble tachyzoite extracts may be responsible for the improved diagnostic performance. In addition, BbSALUVET ELISA 2.0 was validated with wild ruminant sera, and better performance (96% Se; 97% Sp; 4% doubtful results) was obtained when compared with a previously developed BbSALUVET ELISA1.0 for *Cervidae* (100% Se; 86% Sp; 41% doubtful results). This study offers the use of BbSALUVET ELISA 2.0, which largely avoids ambiguous test results and the use of confirmatory WBs in cattle prior to entry to herds free of the disease and samples prior to a selective culling.

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Development and characterization of monoclonal antibodies against *Besnoitia besnoiti* tachyzoites

P 28

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Only few reagents exist for experimental investigation of *Besnoitia* spp. biology. We here characterized eight monoclonal antibodies (MABs), raised against whole *Besnoitia* tachyzoites and a membrane enriched fraction. Genus- species- and stage-specificity were verified with the closely related protozoan *B. tarandi*, *Neospora caninum*, *Toxoplasma gondii* and *Sarcocystis* spp., together with the bradyzoite stage of *B. besnoiti,* respectively, by Western blot. IFAT and confocal laser scanning microscopy was employed to study the recognition pattern of these MABs during the lytic cycle of *B. besnoiti* tachyzoites at 30 min, and 1, 2, 6, 24, 48 and 72 hours post infection, and transmission electron microscopy (TEM) was employed to confirm the localization. Remarkably, most MABs developed were genus-specific. Indeed, none crossreacted with *T. gondii* and only MAB 2.F.3 reacted with *Sarcocystis* spp. Cross-reactions against *N. caninum* tachyzoites could only be evaluated for MABs 2.G.A, 2.A.12, 2.F.3 and 2.G.4 and all showed a negative result. Finally, all MABs developed were *B. besnoiti* tachyzoites stage-specific. MABs 3.10.8 and 5.5.11 labeled the surface of *B. besnoiti* tachyzoites, MABs 1.17.8, 8.9.2 and 2.G.A recognized the apical tip, and MABs 2.A.12, 2.F.3 and 2.G.4 reacted with granular content inside the tachyzoites compatible with a dense granule staining. TEM results corroborated dense granule localization for proteins recognized by MABs 2.F.3 and 2.A.12. Moreover, only MAB 2.F.3 seems to recognize a protein that is secreted during the lytic cycle of the parasite. In conclusion, we have generated MABs that will be useful to study key processes in the lytic cycle of the parasite or its differentiation into bradyzoites. However, the identity of the antigens recognized by these MABs remains to be elucidated.

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Serological and molecular epidemiology of *Toxoplasma gondii* infection in intensive pig farms in Northern Italy

P 29

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Toxoplasma gondii infection is a major public health issue, but though one of the most important ways of human infection is through the consumption of raw or under-cooked meat. Its control at slaughter is not mandatory. Further, information on *T. gondii* infection in domestic animals destined for human consumption is scarce as well as genotypes circulating among farms.
To acquire wider epidemiological data on *T. gondii* in pigs bred in Northern Italy, sera and hearth samples were collected from 219 fattening pigs and 151 sows from 23 intensive farms. Data on farm management and sanitary procedures were collected and for each farm a biosecurity score was calculatedfor statistical analysis.Sera samples were tested for antibodies anti-*T. gondii* using a commercial ELISA. On muscle samples, molecular analysis was carried out by 529bp-PCR and B1-Real Time-PCR coupled to sequencing for genotyping. To detect characteristics of farm management associated to the infection, a generalized linear model was carried out on intra-herd seroprevalence results.
Prevalence was 30.4% at farm level (63.3% in sows and in 6.7% in fattening pigs) and 3.8% at individual level (8.6% in sows and 0.5% in fattening pigs). *T. gondii* DNA was detected in nine animals by both 529bp-PCR and B1-Real Time-PCR. Sequencing revealed the presence of all three genotypes (Types I, II and III), with Type II the most frequently found, also in association with Type I or Type III.
Sows resulted to be at higher risk of infection than fattening pigs (OR=0.007, p=0.001). Furthermore, the risk of infection within a farm resulted to be enhanced by the decrease in the biosecurity score (OR=0.063, p=0.025).
Data obtained confirm the spread of *T. gondii* infection in pig farms in an intensive pig production area. Although low seroprevalence values were recorded, the application of stricter sanitary procedures may significantly reduce the infection in pigs, thus enhancing food safety of pig meat and derived products.

Sarcocystis neurona and *Neospora caninum* in Brazilian opossums (*Didelphis* spp.): Molecular investigation and *in vitro* isolation of *Sarcocystis* spp.

P 30

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Sarcocystis neurona and *Neospora* spp. are protozoa that induce neuropathy in horses and other animal species. Opossums (*Didelphis albiventris* and *Didelphis virginiana*) are definitive hosts of *S. neurona*. We aimed to determine the exposure of opossums to *S. neurona* and *N. caninum* by molecular methods, and to isolate *Sarcocystis* spp. from sporocysts shed by opossums. Carcasses of 39 opossums from Bahia state were available for molecular identification of *Sarcocystis* spp. and *N. caninum* in their tissues, and for sporocyst detection by intestinal scraping. In addition, *Sarcocystis*-like sporocysts from nine additional opossums, obtained in São Paulo state, were tested. *Sarcocystis* DNA was found in 16 (41%) of the 39 opossums' carcasses; *N. caninum* DNA was detected in tissues from three opossums. The sporocysts from the nine additional opossums were tested by bioassay and induced infection in nine budgerigars, but in none gamma-interferon knockout mice. *In vitro* isolation was successful using tissues from all nine budgerigars. Six of nine isolates could be continuously grown in cell culture (CV-1 and Vero cells). Genetic analysis of six isolates based on five loci showed that these parasites were different from each other and also distinct from *S. neurona*, *S. falcatula, S. lindsayi*, and *S. speeri*. In conclusion, opossums in the studied regions were infected with *Sarcocystis* spp. and *N. caninum*, and represent a potential source of infection to other animals. This is the first report of *N. caninum* DNA detection in tissues from black-eared opossum (*D. aurita* or *D. marsupialis*) and white-eared opossum (*D. albiventris*). Brazilian opossums are probably infected by *Sarcocystis* spp. distinct from previously described species, or present a high level of genetic recombination.

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Development of an alternative assay to study the infectivity of *T. gondii*

P 31

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The infection with *T. gondii* is one of the most widespread parasitic diseases worldwide. In humans clinical toxoplasmosis varies from slight visual weakness to lethal disease. The oral infection occurs by oocysts or by infectious meat. For the investigation of meat infectivity for humans, currently mouse bioassay is used (lethal experiment).

Movement and cell invasion of *T. gondii* is controlled by calcium-mediated secretion. The phytohormon abscisic acid (ABA) stimulates in plants the intracellular exposition from calcium. The effect of ABA on *T. gondii* activity was described by Nagamune et al. 2008.

In this study we aim at the development of an assay to replace the mouse- bioassay. ABA would be used as a catalyst.

As a pre-test the *T. gondii* tachyzoites (Me49) cultured in HFF cells was used. A high promotional effect of ABA on tachyzoite replication was shown and confirmed with Nagamune et al. 2008.

Bradyzoites were isolated from brain and muscles of infected mice with the trypsin digestion. ABA seems to exhibit significant effect on the stage conversion from bradyzoites which is essential for an *in-vitro* infectivity assay.

The results are promising regarding the development of an alternative infectivity test system.

Integrative transcriptome and proteome analyses define marked differences between *N. caninum* isolates throughout the tachyzoite lytic cycle

P 32

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Neospora caninum infection is one of the main causes of transmissible abortion in cattle worldwide. Intraspecific variation in virulence have been widely shown among *N. caninum* isolates. However, the molecular basis that govern such variability and the outcome of the infection have not been well-established yet. This study aims to improve the knowledge on the factors governing intra-specific variation in virulence in *N. caninum.* Quantitative label free LC-MS/MS was used to investigate proteome differences between the high virulent isolate Nc-Spain7 and the low virulent isolate Nc-Spain1H through tachyzoite lytic cycle phases: at the end of invasion, during exponential growth and at egress. The results showed higher differences in abundance of proteins at invasion and egress. Microneme protein repertoire, involved in parasite invasion, was more abundant in Nc-Spain1H isolate, which displays a lower invasion rate. Soluble effectors related to *Toxoplasma gondii* virulence (ROP and GRA), and proteins of carbohydrate and fatty acid metabolism and stress response also had different abundances between isolates. Interestingly, differential expression analyses by RNAseq during egress showed a higher expression of genes associated with the bradyzoite stage in the low virulent and low growing NcSpain1H isolate. The differences in expression profiles reveal interesting insights into virulence differences between isolates, which will be investigated further.

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Toxoplasma gondii infections in stranded marine mammals in France and Romania

P 33

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Marine mammals are a major sentinel species for the contamination of marine environment by terrestrial pathogens. Parasites such as *Toxoplasma gondii* were found worldwide in several species of marine mammals.

Since marine mammal meat consumption is frequent in some parts of the world, *T. gondii* zoonotic infections are a serious concern for public health. Extensive seroprevalence investigations have been made in USA, Japan, UK, Spain or Italy, showing a high presence of *T.gondii* infections in marine animals, yet little is known about it in France and Romania.

The objective of the present study was to investigate the prevalence of *T. gondii* in stranded marine mammals of the Atlantic coast of France and the Black Sea coast of Romania.

Forty-nine samples, originating from seven different species (grey seal, common seal, common dolphin, common porpoise, bottlenose dolphin, striped dolphin and sperm whale) were collected from both countries. *Toxoplasma gondii* seroprevalence was detected by MAT, while the presence of *T. gondii* DNA was assessed by quantitative PCR. The overall seroprevalence of *T. gondii* infection was 38% (with a 1:24 cut-off), with higher rates in common and bottlenose dolphins, species that are living close to the shores. Concerning the quantitative PCR detection of *T. gondii*, 32% of samples were found positive, most of them with a very small quantity of DNA only. A higher prevalence of *T. gondii* infection was noticed in Romania, partially explained by the close-in situation of the Black Sea. This study indicates the exposure of cetaceans and pinnipeds from France and Romania to *T. gondii.* This is the first identification of *T. gondii* in a sperm whale. A type II strain has been identified in a bottlenose dolphin.

Immune response induced by the Mic1-3 Knockout *Toxoplasma gondii* vaccine strain in the parasite definitive feline host

P 34

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Toxoplasmosis is a zoonotic disease caused by the protozoan *Toxoplasma gondii*. Up to a third of the global human population is estimated to carry a *T. gondii* infection, which can result in severe complications in immunocompromised individuals and pregnant women. The parasite is mainly transmitted to humans by consumption of contaminated meat, vegetables or water. *T. gondii* can infect any warm-blooded animal but its definitive host (DH) is the felid family (mostly cats) where it undergoes its sexual reproductive cycle with the release of infectious oocysts. Felidae are thus at the center of this public health problem enabling environmental and livestock contamination by oocysts.

Immune responses to the parasite have mainly been studied using intermediate host (IH) animal models and cat specific anti-*T. gondii* immune responses remain unexplored, mainly due to ethical issues and a lack of experimental tools. However, the study of these parameters in the DH of *T. gondii* will give a more relevant picture of host-pathogen interactions and may allow the identification of new targets for efficient vaccination strategies to interrupt the parasite life cycle. With this goal in mind, we studied the immune response of cats to an attenuated live strain of *T. gondii*, using sub-cutaneous injection or oral administration. Both vaccination routes induced a high specific antibody titer in animal sera, indicating that the live attenuated strain is highly immunogenic. However, the oral route required a higher vaccine concentration to induce antibody production, compared to the sub-cutaneous administration. We also noticed a delay in antibody production when the vaccine strain was given orally. Serum IL-2, IFN□, IL-4, IL-5 and IL-10 were also measured but no Th1 or Th2 signature could be observed with either oral or subcutaneous vaccination. As *T.gondii* antibodies are believed to be protective against a second infection in IH, we followed oocysts shedding by vaccinated cats showing high IgG titers, after oral challenge with a wild-type strain of *T. gondii* (76K). Surprisingly, a high antibody titer did not stop cats to shed oocysts from the challenge strain, regardless of the vaccination route. This striking result highlights the particular relationship between *T. gondii* and its unique DH which is now the focus of additional investigation in particular at the intestinal mucosal level.

Genus-specific antibodies for the diagnosis of *Neospora caninum* and *Toxoplasma gondii* using Immunohistochemistry in abortion cases of ruminants

P 35

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Abortion is a major problem for livestock operations and animal welfare worldwide, and the identification of a specific cause is particularly difficult and achievable in less than 50% of the cases, even in well-established diagnostic laboratories. Of the diagnosed causes of abortion, most losses are due to infectious diseases such as bovine neosporosis (a major cause of abortion in cattle worldwide) and toxoplasmosis (a major cause of abortion in sheep). In order to adopt adequate control measures, an accurate monitoring program is required to distinguish, confirm the presence or absence of protozoal parasites within a herd, and to estimate the levels of infection. As such, it is necessary to have access to specific diagnostic tools in order to confirm or rule out the presence of *Toxoplasma gondii* and *Neospora caninum* in a herd or flock. Current diagnosis using antibodies raised against the protozoal parasite *Toxoplasma gondii*, and *Neospora caninum* have shown cross-reactivity using diagnostic methods, which meant that infections could not accurately be distinguished. In this research, antibodies were raised against genus-specific recombinant proteins of *N. caninum* and *T. gondii,* and they were able to be used for the accurate and specific diagnosis of abortion cases in ruminants using Immunohistochemistry.

Characterization of *Theileria equi* antigen infecting donkeys in Egypt

P 36

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Equine theileriosis is a tick- borne disease caused by *Theileria equi* (TE) inducing varying degrees of hemolytic anemia and associated systemic illness. The mortality rate can vary from less than 10% to as high as 50% (OIE, 2008). Infected animals may remain carriers of these blood parasites for long periods and act as a source of infection for other ticks. The aim of this study was to characterize TE antigen for its further use in serological diagnosis of the parasite among donkeys in Giza governorate, Egypt. Blood samples were obtained from 133 donkeys and examined microscopally (ME) for TE infection. One of the naturally infected donkeys showing high parasitaemia was splenoctomized. Two lysate crude antigens (Ag); Ag with hemoglobin (L1), Ag without hemoglobin (L2) and the third sonicate Ag (S) of TE were prepared from this blood. These 3 Ag were tested through electrophoretic analysis (SDS- PAGE) using coomassie blue stain, silver stain and immunoblot analysis using serum from naturally infected donkey with TE. In this study, ME revealed that the incidence of TE was 24.8% in donkeys. Electrophoretic analysis of 3 antigens using SDS-PAGE showed 3 common bands for L1, L2 and S; at MWt 63, 54 , 31KDa by Coomassie blue stain and 5 bands at MWt 70, 54,31, 28, 23KDa by silver stain. Immunoblot analysis for L1, L2 & S antigens cleared that there were one immunodominant protein band at MWt 16 KDa. It could be concluded from these results that the sensitivity of silver stain toward protein characterization was higher than Coomassie blue stain as it can detect protein in the nanograme range. The common immunoreactive band in the three types of TE antigen was 16 KDa and the most immunogenic antigen was L2.

Coccidian in *Oryctolagus cunniculus* from Tenerife, Canary Islands, Spain

P 37

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Wild and farm rabbit populations are affected by viral and coccidian diseases, which lead to large economic losses worldwide. Coccidiosis is a common disease in *Oryctolagus cuniculus* that can cause important outbreaks, especially in farms. A total of 97 fecal samples from different rabbits were collected from 2015 to 2017 for coccidia examination. More than half of the rabbits were parasitized (55.7%) with *Eimeria* spp. and their oocysts were identified by morphology and morphometric characteristics. Since the degree of pathogenicity depends on the infectious species, so in some cases, a molecular characterization was carried out using a multiplex PCR diagnostic assay based on ITS1-5.8S rRNA-ITS2 fragments. Eight species were identified, three of them causing severe diseases in rabbits *Eimeria flavescens*, *Eimeria intestinalis* and *Eimeria magna*; and the others cause mild diseases *Eimeria perforans*, *Eimeria media*, *Eimeria coecicola*, and *Eimeria piriformis*. More than half (79.8%) of the infected animals presented coinfection mostly caused by *E. flavescens* and *E. coecicola*. The high prevalence detected in wild and farm rabbits, and the high pathogenicity of three of the species, suggest that coccidia may be causing more diseases than the estimated in Tenerife, and veterinarian should pay more attention on the diagnostic.

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Carotenoid-enriched corn protects poultry against coccidiosis

P 38

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Carotenoids are health-promoting organic molecules that act as antioxidants and essential nutrients. Chickens raised on a diet enriched with an engineered corn variety containing high levels of four key carotenoids (β-carotene, lycopene, zeaxanthin and lutein) accumulate higher levels of bioavailable carotenoids in peripheral tissues, muscle, skin and fat, and more retinol in the liver, than birds fed on standard corn diets.

In our study chickens were challenged with *Eimeria tenella*. Four groups of 15 one-day-old Ross 308 male chickens were housed in isolated cages. Two groups were fed on the control diet and two on the high-carotenoid diet. One group from each diet category was challenged orally with an inoculum of 30,000 *E. tenella* oocysts on day 13. The number of oocysts excreted (per g of feces x1000) were fewer ($P<0.05$) in chickens fed on the high-carotenoid diet than ones fed on control diet on days 6 (87±1.2 and 132.8±2.9) and 9 (14.2±0.9 and 56.9±4.3) post-challenge. Moreover, birds fed on the high-carotenoid diet suffered milder disease symptoms than birds fed on control diet on the challenged groups. Fewer levels ($P<0.01$) of foot pad dermatitis (1.30±0.33 and 2.43±0.25) and fewer number ($P<0.01$) of digital ulcers (2.54±0.57 and 6.50±0.49) were counted on slaughtered animals. We can conclude that carotenoid-rich corn improves poultry health.

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Toxoplasma inhibitor of STAT1 transcription effector protein IST and its relevance for virulence in closely related *Neospora* caninum and *Hammondia hammondi*

P 39

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The veterinary important and closely related apicomplexan parasites *Neospora caninum* and *Toxplasma gondii* show significant differences in virulence and host adaptation. The blockage of the STAT1-dependent type I and type II interferon (IFN) immune response by *T. gondii* serves as important virulence mechanism for the parasite. After infection of the host cell, *T. gondii* secretes the dense granule protein IST which translocates into the host cell nucleus where it recruits the Mi2/NuRD-complex to the chromatin and blocks STAT1-associated transcription of important host cell defense proteins such as iNOS, IRGs and GBPs. The IST protein is functional in all three classical *Toxoplasma* type strains (I – III) as measured by the blockage of the STAT1-inducible protein IRF1. Importantly, the close relative of *T. gondii*, *Neospora caninum* does not block IFN signaling pathways. Here we investigated whether *N. caninum* and its close relative, the avirulent parasite *Hammondia hammondi* possess IST protein orthologues. Using truncated protein versions, we show that a small domain of the rather large IST protein is sufficient to block STAT1 transcription. We further assess the functional differences in host parasite interference based on sequence comparison and forward and reverse genetic approaches.

Anticoccidial effect of naringenin and grape fruit peel extract in growing lambs naturally-infected with *Eimeria* spp.

P 40

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The current study aimed to determine the anti-*Eimeria* efficacy of an extract of grape fruit peels (GF) and commercial naringenin (NAR) in naturally-infected lambs, as well as the influence of these flavonoids on the oxidative status during ovine coccidiosis. Pharmacokinetic profiles were also determined. Extracts were administered *per os* to *Eimeria* naturally infected growing lambs during 90 consecutive days. The comercial anticoccidial drug toltrazuril (TTZ) was included in this trial as a standard. Twenty-four lambs were divided into four groups: NAR, lambs given a daily dose of 5 mg of a commercial extract of 98% higher purity per kg body weight; GF, lambs that received a daily dose of 5 mg of ethanolic extract of grapefruit peels per kg body weight; TTZ, lambs treated with 20 mg of toltrazuril/kg body weight on days 0 and 15 of the experiment; and CTRL, untreated lambs that received daily dose of 30 ml of water. Daily doses of GF and NAR were dissolved in 30 ml of water and orally given to animals; whereas toltrazuril was administered as a single dose of an undiluted suspension to lambs of the TTZ group. The CTRL group received 30 ml of water; as well as the TTZ group for the period after the single dose administration. Fecal and serum samples were collected from all lambs. Anticoccidial efficacy was estimated by coprological techniques. Generation of nitric oxide levels and antioxidant capacity of the experimental compounds were determined by the Griess and ABTS essay, respectively. The pharmacokinetic of NAR and the GF extract were obtained. On day 30 post-ingestion, anti-coccidial efficacy was 91.76% (NAR) and 89.65% (GF); whereas 99.63% of efficacy was achieved with TTZ 15 days after treatment. NAR, GF and TTZ significantly reduced oxidative stress in infected animals. The mean daily weight gain of each group was 122 g (NAR), 122 g (GF), 143 g (TTZ) and 98 g (CTRL). Following the oral administration of NAR and GF, values in plasma approached maximum concentrations within 2.1 and 2.5 h. In conclusion, the administration of NAR and the GF extract reduced *Eimeria* oocyst output, oxidative stress and promoted higher mean daily weight gains in infected lambs.

Microsatellite analysis reveals high diversity among geographically close isolates of *Cystoisospora suis*

P 41

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Microsatellites are short repetitive DNA sequences of two to six repeats interspersed in the genome which display a rapid mutation rate and consequently show high variation between individuals or populations. They have been used to characterise population diversity and structure and the level of variation between different isolates of a variety of organisms, including apicomplexan protozoa. Currently nothing is known about the genetic variability and population structure of *Cystoisospora suis*, the causative agent of piglet coccidiosis, and we made use of the recently available genome of *C. suis* (strain Wien-I) to generate sets of primers for the amplification of microsatellite regions (ca. 400 bp) in non-coding or intergenic regions to evaluate the applicability of fluorescence-labelled primers to analyse amplicon length variation at high resolution using capillary electrophoresis (CE) technique. Two phenotypically characterised isolates (Wien-I, toltrazuril susceptible; Holl-I, toltrazuril resistant) and three field isolates from Europe were compared in conventional PCR to evaluate the applicability of the method. Eight primer pairs amplified bands of the expected size for Wien-I and were labelled for CE analysis. High resolution CE of the amplicons revealed high diversity of the analysed strains, with differences even between two strains from neighbouring swine farms. In follow-up studies, adaptation of the PCR assay to multiplexing and amplification of small DNA quantities will provide a cost-effective tool to analyse field strains to reveal geographic diversity and possibly assignments to phenotypic traits. Stability of markers across stages and generations of the parasite must also be addressed.

Chicken line-dependent mortality after experimental infection with three type IIxIII recombinant *Toxoplasma gondii* clones

P 42

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Three genetically different clones of *Toxoplasma gondii*, also different in mouse virulence, were studied by experimental infection in chickens. For the experiments, four chicken lines were used, which differed in phylogenetic origin and performance level: two white egg layer lines, one with high laying performance (WLA), one with low (R11) and two brown layer lines, also displaying high (BLA) and low (L68) egg number. Chickens were intraperitoneally infected with three different *T. gondii* isolates representing type IIxIII recombinant clones, i.e. showing both, type II- and type III-specific alleles. These clones (K119/2 2C10; B136/1 B6H6; K119/2 A7) had exhibited virulence differences in a mouse model. In chickens, a significantly higher mortality was observed in white layer lines, but not in brown layer lines, suggesting that differences in the phylogenetic background may influence the susceptibility of chickens for toxoplasmosis. In addition, antibody (IgY) levels varied in surviving chickens at 31 days post infection. While low to intermediate antibody levels were observed in white layers, intermediate to high levels were measured in brown layers. Infection with a *T. gondii* clone showing low chicken virulence resulted in lower antibody levels in all chicken lines compared to infection with *T. gondii* clones of intermediate or high chicken virulence. This was in agreement with the parasite load as determined by real-time PCR. The progeny resulting from natural sexual recombination of *T. gondii* clonal lineages, may thus differ in virulence for chickens. Virulence patterns in mice are not identical to those in chicks.

Immune -endocrine patterns in dairy cattle experimentally infected with *N. caninum* in the second trimester of gestation

P 43

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Nowadays, immune-endocrine interactions during pregnancy in *Neospora*-infected cows remain largely unknown. This study examines plasma progesterone (P4) levels, humoral immune responses, *SERPINA14* and cytokine gene expression patterns in dairy heifers experimentally infected with *N. caninum* during the second trimester of pregnancy. Antibody levels and P4 were determined using ELISA. Gene expression of *SERPINA14, TNFA, IL8, IFNG, IL4* and *IL10* was performed by real-time PCR at the ovarian corpus luteum (CL), uterine lymph nodes (UTLN) and placental tissues. *N. caninum* infection up-regulated *IFNG* in placentome and *IL4* in UTLN but downregulated *SERPINA14* in intercaruncular tissues compared to uninfected dams. Negative correlation were observed between inter-caruncular *SERPINA14* and humoral response ($P< 0.05$) and Th1 response in cotyledon ($P< 0.01$) and Th2 response in UTLN ($P< 0.05$), but not in CL tissues. A dam with a mummified fetus upon euthanasia showed upregulation of *TNFA* and *IFNG* in CL and lower P4 values compared to control dams and non-aborting infected dams, which could be related to both functional (P4 decline) and structural (cell death) luteal regression. SERPINA14 has been linked to maternal immunosuppression during pregnancy. Reduced local immunosuppression by uterine serpin seems to be needed to improve maternal immune responses against the parasite to maintain gestation during *N. caninum* infection.

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Development of a recombinant protein based indirect ELISA for the detection of serum antibodies against *Cystoisospora suis* in swine

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Neonatal porcine coccidiosis, caused by an apicomplexan parasite *Cystoisospora suis,* is one of the predominant pathogens in suckling piglets with a global prevalence of over 70%. Nonetheless, the diagnosis is still cumbersome as individual oocyst excretion is highly variable and restricted to a very short period. Currently, for experimental studies, indirect fluorescent antibody test (IFAT) is considered as a gold standard for detecting antibodies despite several limitations, including relatively subjective interpretation and low throughput. In the present study, an indirect enzyme-linked immunosorbent assay (iELISA) was developed using a recombinant merozoite protein (rCSUI_005805) for the detection of specific serum antibodies against *C. suis* infection. rCSUI_005805 was expressed in *E. coli* as N-terminal HIS fusion protein, specificity of which was confirmed in an immunoblot probed with *C. suis* positive sera. Optimal dilutions of recombinant protein, sera and conjugate were determined by checkerboard titrations and the serum dilution that gave the greatest ratio between the positive and the negative sera was selected as a control for subsequent runs. The receiver operating characteristic (ROC) curve analysis based on 47 serum samples with known *C. suis* exposure tested in the reference IFAT was used to determine the cutoff value, sensitivity and specificity. The optimal cutoff based on ROC analysis was a 1:800 diluted serum sample OD value of 1.87 for which the sensitivity and specificity values were 100% (95% CI 76.84 - 100%) and 96.97% (95% CI 84.24 - 99.92%), respectively. This was comparable to the cutoff value of 2.1, calculated as the mean optical density of 33 *C. suis* negative sera plus three standard deviations. The diagnostic accuracy measured as the area under the ROC curve (AUC) index was 1.0, indicating excellent discriminatory capacity of the test and its possible application as a marker for infection or exposure in large-scale epidemiological studies.

Deciphering the host cues responsive circadian transcriptome of apicomplexan parasite *Plasmodium chabaudi*

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Organism's daily rhythm in behavior and physiology are regulated by circadian clock attuned to earth's rotational (circadian) period of 24 hours (h). Circadian rhythms have also been observed in organisms living within hosts that include parasites. *Plasmodium chabaudi*, an obligate intracellular parasite that causes malaria in mice, has an intra-erythrocytic developmental cycle of 24h, matching earth's rotational period. Whether intracellular parasites like *P. chabaudi* respond to rhythmic changes in host physiological and biochemical cues has not been elucidated at a molecular level. We have tested this by subjecting *P. chabaudi* parasites to a jet lag like effect where parasites were temporally mismatched with host circadian rhythms (12h difference). Initial microscopic observations showed that schizonts of mismatched parasites burst midday while matched parasites burst at midnight. We found that ~32% of genes in *P. chabaudi* expressed with circadian rhythm respond to changes in host environmental cues by loosing rhythmicity in mismatched host. Majority of the circadian genes expressed in a bimodal fashion, where they expressed maximum at two different phases of the day, a characteristic feature found in other eukaryotes. Gene ontology based enrichment analysis identified circadian genes to be enriched in cellular metabolic pathways including pathways involved in energy metabolism. This study for the first time reports the host cues responsive circadian transcriptome of a malaria parasite. Future studies will identify key molecules including core clock genes and receptors that can further be used for malaria intervention strategies.

Analysis of *Babesia bigemina* Apical Membrane Antigen-1 immunogenicity and its characterization in Apicomplexa

P 46

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Babesia bigemina and *Babesia bovis* are tick-borne haemoprotozoa causing cattle babesiosis worldwide with a negative impact in zootechnics. One of the molecules potentially involved in erythrocyte invasion is the Apical Membrane Antigen-1 (AMA-1), an apically located protein shared by many Apicomplexa and widely studied for vaccine and diagnostic purposes. This study aims to AMA-1 molecular characterization from several Apicomplexa and to the analysis of its ability to stimulate Interferon gamma (IFN-γ) production in *B.bigemina* infected bovine lymphocyte. Bioinformatics analysis showed conserved regions among AMA-1 proteins from Apicomplexa, as the Signal Peptide, the transmembrane domain or the cysteines involved in disulphuric bonds. Peripheral Blood Mononuclear Cells from *B.bigemina* infected cattle in vitro re-exposed to *B.bigemina* AMA-1 produced an INF-γ amount almost twice that the uninfected animals. The study provided new knowledge on AMA-1 characteristics in related organisms and on its immunogenicity in *B.bigemina*. IFN-γ has a key role in the immunity pathway of the host against *Babesia* infection and IFN-γ production by infected bovine lymphocyte following re-exposition to *B.bigemina* AMA-1 would suggest its ability to stimulate host immune reaction. These data could be useful for diagnostic and vaccine strategies in *Babesia* infected animals.

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Distribution of cattle babesiosis in Palermo province (Sicily)

P 47

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Cattle babesiosis is a tick-borne disease causing losses in zootechnics. This study aims to analyse babesiosis prevalence in cattle herds of Palermo province (Sicily) according to pedoclimatic characteristics of the area.

Palermo province, covering 4.992 km², was divided in 14 geographic quadrants where 1 to 2 herds were selected. During 2016, sera, whole blood and ticks were collected from a representative number of animals, for a total of 128 samples. Ticks were morphologically identified. Serological and molecular analyses were performed for *Babesia bovis* and *Babesia bigemina*. Confidence intervals of 95% (CI) were evaluated.

The analyses carried out showed serological prevalences of 71% (CI 63.2%-78.8%) and 38% (CI 29.6%-46.4%) for *B. bigemina* and *B. bovis,* respectively. *B. bigemina* prevalence was the highest in all the herds. Altitudes ranged from 239 to 823m and all the herds were positive at least to one *Babesia* species. In herds with high prevalence, competent vectors were present, i.e. *Rhipicephalus annulatus* in positive *B. bovis* herds.

B. bigemina prevalence was higher than *B. bovis* and uniformly distributed in the area, while *B. bovis* had a spotty distribution. Data underline also the tight relation between ecology and host-vector-pathogen interactions.

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Serological study of *Toxoplasma gondii* and *Neospora caninum* in a wildlife conservation area in southern Portugal

P 48

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A serological survey was performed to evaluate exposure to *Toxoplasma gondii* in cats and *Neospora caninum* in dogs and both parasites in wild animals in a wildlife conservation area in southern Portugal. The study involved 79 cats, 286 dogs, 36 European rabbits (*Oryctolagus cuniculus*), 34 Egyptian mongoose (*Herpestes ichneumon*), 26 wild boars (*Sus scrofa*), 25 foxes (*Vulpes vulpes*), 17 common genets (*Genetta genetta*), 14 red deer (*Cervus elaphus*), 6 wildcats (*Felis silvestris*), 17 mustelids and 6 rodents. Antibodies to *T. gondii* were screened with a commercial agglutination test in wild animals and cats. Antibodies to *N. caninum* were screened with an in-house agglutination test (N-MAT) in wild animals and by IFAT in dogs. Positive results in the N-MAT were confirmed by IFAT. The sample prevalence of *T. gondii* was 85.3% in Egyptian mongoose, 83.3% in wildcats, 47.1% in genets, 40% in foxes, 39.2% in cats, 29.4% in mustelids, 21.4% in red deer, 7.7% in wild boars and 2.8% in rabbits. Anti-*N. caninum* specific antibodies were found in dogs (32.5%), foxes (12%) and rabbits (25%). These results show that *T. gondii* is a ubiquitous and highly prevalent parasite in wild animals in the study area and gave the first evidence of natural exposure to *N. caninum* in foxes and rabbits in Portugal. The further monitoring of *T. gondii* in native animals is important for wildlife conservation strategies and human health protection and the sylvatic cycle of *N. caninum* in the area clearly claims for further studies.

Evaluation of *Eimeria* sp. infection and predictors of oocysts excretion in newly introduced beef cattle in northern Italy

P 49

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Eimeria spp. are protozoans causing economic losses in bovines. In 2016, prevalence and abundance of *Eimeria* infection in newly introduced beef cattle were evaluated in a farm sited in northern Italy. Individual fecal samples were collected from 130 animals purchased in 13 European Collection Centers, classified in 4 groups considering geographical position: Sardinia (SA, 16.9%), North-Continental (NC, 26.9%), South-Continental (SC, 52.3%), Ireland (IR, 3.9%). Age, sex and breed were recorded, together with date of sampling and origin. Samples were analyzed by FLOTAC® dual technique (NaCl s.g.=1200; $ZnSO_4$ s.g.=1350) to determine *Eimeria* sp. oocysts per gram of feces (OPG) values. The association between OPG and sex, age, breed, origin and season of sampling was analyzed through a GLM using SPSS (IBM).

Cattle were 73.1% male and 26.9% female and their mean age was 16.1 months old (s.d. 12.4). Breeds were Aubrac (9.2%), Sardo-Bruna (5.4%), Charolaise (29.2%), Gasconne (2.3%), Limousine (30.8%) and Salers (11.5%); 33.6% were crossbreeds. Samples were collected in spring (30.8%), autumn (49.2%) and winter (20%). *Eimeria* infection was detected in 65.4% of samples and mean abundance of excretion was 169 OPG (s.d. 858). In final GLM, age, breed, origin and season resulted significant predictors of OPG. OPG values were lower in older animals (age in months, continuous variable; coeff=0.970, $p<0.05$). All breeds and crossbreeds had higher OPG values ($p<0.001$) when compared with Salers (refer.) and the same was for animals from NC (coeff=41.864, $p<0.001$) and SC (coeff=27.645, $p<0.001$) when compared with those from SA (refer.). Lower OPG values were observed in spring (coeff=0.292, $p<0.05$) and autumn (coeff=0.085, $p<0.001$) than in winter (refer.). Individual features, origin and season of sampling affected OPG values; apparently a resistance to *Eimeria* infection in Salers breed could be hypothesized and should be verified in cattle raised in equal condition of breeding.

Mutual influences of the apicomplexan parasites *Toxoplasma gondii* and *Eimeria tenella* in poultry macrophages

P 50

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Toxoplasma (T.) gondii and *Eimeria (E.) tenella* are two common parasites in poultry. Mixed infections are likely to occur frequently in chickens due to the high prevalence of both pathogens. In this study, we investigate the co-occurrence of the two pathogens in the same immune-competent host cell population towards potential parasite-parasite as well as altered patterns of parasite-host interactions. Primary macrophages from chicken blood were co-infected *in vitro* with *T. gondii* tachyzoites (RH strain) and *E. tenella* sporozoites (Houghton) for 72h. Morphologic observations by light microscopy and assessments of parasite replication by quantitative real-time PCR (qPCR) were performed at 24, 48, 72h post infection. Immune factors such as TNF-α, IL6, IL10, IL12, IFN-γ, iNOS were evaluated. Higher catabolism of macrophages was distinct by mixed infection while immune activation of host cell was mainly seen at 24h p. i. mRNA expression of iNOS was significantly higher in mixed infection at 48h p. i. The total number of macrophages decreased distinctly at 72h p. i. Highest population of both parasites could be detected in mixed infection comparing with replicates in single infection group. *E. tenella* development was less suppressed during *T. gondii* co-infection while *T. gondii* replication was hampered in the presence of *E. tenella*. The obvious interaction between *T. gondii* and *E. tenella* demonstrated *in vitro* should be considered in further studies on naturally or experimentally co-infected chicken.

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Efficacy of VFO-IS-01, a live attenuated immunostimulant against *Salmonella Enteritidis*, *Eimeria Acervulina* and *Influenza* H7N1 infections in chicks

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VitamFero develops new prophylactic solutions for livestock aiming to prevent consequences of infectious diseases and decrease the use of antibiotics that causes growing AMR. Our prophylactic technology is based on live attenuated strains of Apicomplexa obtained by entire targeted deletion of genes of virulence and by targeted insertion of genes encoding functional proteins or antigens. The neonatal immunostimulant VFO-IS-01 was designed to stimulate the innate immune system in order to help newborns to protect themselves against infectious agents. The protective potential of VFO-IS-01 was tested firstly against *Influenza* H7N1 virus, secondly against *Salmonella enteritidis* bacteria carriage and finally against *Eimeria acervulina* parasite. In the first model, the Immunostimulation significantly reduces endotracheal excretion of *Influenza* viral particle, thus reflecting a lower viral replication in internal organs and a better control of the disease. In the second model, the gastro-intestinal carriage of *Salmonella Enteritidis* had been strongly reduced in immunostimulated groups for at least 4 weeks post challenge. In the third model, the body weight loss due to *E. acervulina* infection was significantly reduced in immunostimulated broilers which, vs. control group, showed an enhanced body weight gain in this experimental model of coccidiosis. The efficacity of VFO-IS-01 against, one virus, one bacteria and one parasite, confirmed that a stimulation of the innate immune system could be considered to prevent efficiently consequences of a broad spectrum of infectious diseases and thus could be integrated in a OneHealth program along with the replacement of chemical prophylaxis.

Effects of challenge dose and inoculation route of virulent *Neospora caninum* Nc-Spain7 isolate in pregnant cattle at mid gestation

P 52

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The standardization of bovine models remains as a challenging and high-priority issue to get a better understanding of immunopathological features occurring during *Neospora caninum* (NC) infection, but also to evaluate drugs and vaccines. Variables such as the stage of gestation, the parasite isolate, the challenge dose and the route of inoculation may lead to different patterns of foetal death presentation, as well as variable impact on parasite transmission rate. The aim of this study was to evaluate the effects of different challenge doses and routes of inoculation of the virulent Nc-Spain7 isolate on abortion and vertical transmission. For that, three doses of tachyzoites were inoculated intravenously (IV), and additionally subcutaneously (SC) for the mid dose, to 24 Asturiana pregnant heifers at day 110 of gestation (G1 (n=6): 10^7 IV, G2 (n=6): 10^5 IV, G3 (n=6): 10^5 SC, G4 (n=6): 10^3 IV and G5 (n=4): negative control). Foetal death (n=9) was observed in all infected groups; however, it occurred less frequently and later as dose decreased (G1: 66.7% (from 25 to 31 dpi); G2: 50.0% (from 29 to 39 dpi); G3: 16.7% (26 dpi); G4: 16.7% (38 dpi)). In terms of NC transmission, parasite DNA was found in the placental tissues of all abortion cases that could be examined. Lesions compatible with neosporosis were also confirmed in placental tissues from abortions of IV groups (7/7). All brain samples of fetuses from G1, G2 and G3 groups were positive for NC DNA (7/7) but only one brain (G1) resulted positive among calves (1/19). Indirect fluorescent antibody test was used for titration of thoracic fluids from aborted fetuses (n=6) and precolostral sera from calves (n=19). One aborted foetus from G2 at 39 dpi was positive at titre 1:16. Eighty percent of calves from G1 (2/2) and G2 (2/3) were seropositive, showing titres between 1:800 and 1:6400. Our results show that both dose and route of inoculation appear to influence clinical manifestations and NC transmission in experimental bovine neosporosis.

Significant reduction of *Neospora caninum* vertical transmission and postnatal mortality by a toll-like-receptor 2-targeting vaccine formulation in the pregnant mouse model of neosporosis

P 53

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Neospora caninum-associated abortions affect bovine herds worldwide, leading to high economic losses. Although attenuated live vaccines have shown good levels of protection in both mice and cattle they pose serious limitations in terms of marketing. Subunit vaccine development is of high interest to solve some of those drawbacks. Here, we formulated a subunit vaccine based on a cocktail of *N. caninum* antigens fused to OprI, a bacteria lipoprotein sensed by toll-like receptor 2 (G1) that is known to contribute to control acute neosporosis in mice. These antigens, in their non-fused version alone or in pairs, had previously been shown to confer a certain degree of protection in neosporosis mouse models. The vaccine was assessed in non-pregnant and pregnant mice, and was compared to the same OprI-fused antigens admixed with additional TLR3 and TLR7 ligands (G2), and to OprI-fused ovalbumin (G3). In two independent experiments, vaccination with OprI-fused antigens (G1) significantly reduced postnatal mortality by 25% and 27% and vertical transmission by 17% and 24%, respectively. No reduction was observed in G2 or G3. Whereas dams from G1 were not significantly protected against cerebral neosporosis, non-pregnant mice showed significant reduction of clinical signs and parasite burden. Immune response analysis revealed a more balanced IgG1/IgG2a response in G1 than in G2 and a significant IFNγ-based response against the vaccine in G1. More details on immune responses and correlation with protection will be discussed.

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Evidence that transfer of *Theileria annulata* parasites from infected to uninfected leukocytes occurs by spontaneous cell fusion

P 54

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The tick-borne protozoan parasites *Theileria parva* and *Theileria annulata* cause acute economically important lymphoproliferative diseases in cattle in Africa and Asia respectively. The parasites infect and transform leukocytes, in which they establish an intimate relationship with the host cell that enables them to divide at the same time as the host cell, ensuring that infection is retained in the daughter cells. This property permits establishment and maintenance of continuously growing parasitized cell lines *in vitro*. Successful vaccination against these parasites has only been achieved using live parasites. In the case of *T. annulata*, cultured parasitized cell lines are used successfully for vaccination, employing doses of 10^5-10^6 cells. Immunity against *T. parva* can also be achieved using infected cell lines, but doses of approximately 10^8 infected cells are required to obtain reproducible immunity. Hence, this method of vaccination is not economically viable for *T. parva*.

For both Theileria species, successful immunisation requires transfer of the parasites from the inoculated donor cells into cells of the recipient animals. This transfer occurs with much higher frequency with *T. annulata* compared to *T. parva*. Since the intra-leukocyte schizont stage of the parasite does not possess the organelles and proteins required for host cell invasion, the mechanism by which the parasites transfer between cells has been unclear. We have recently obtained evidence from *in vitro* experiments that this transfer occurs by cell fusion. Following co-culture of *T. annulata*-infected with MHC-disparate blood mononuclear cells for 2-3 days, a monoclonal antibody specific for the infected (donor) cells was used to deplete the parent infected cells from the cultures. Detection of Infected cells expressing the MHC of the (recipient) blood mononuclear cells among the surviving cells indicated that transfer of infection had occurred. However, a proportion of these cells expressed both donor and recipient MHC. Following cloning of the cells, analyses of a small set of clones, along with the parent donor and recipient cells, using a bovine SNP panel, demonstrated that the cells represented hybrids, indicating that transfer of infection had most likely occurred by cell fusion.

Placental lesions associated with abortions and stillbirths in goats naturally infected with *Neospora caninum* from Southern Minas Gerais, Brazil

P 55

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Neospora caninum is a protozoan reported as a cause of reproductive disorders in goats. Several aspects of the pathogenesis of neosporosis in naturally infected goats remains to be established. The aim of the present study was to characterize placental lesions in 14 goats naturally infected by *N. caninum* from southern Minas Gerais, Brazil, as well as to correlate these lesions with *N. caninum* IFAT titers, and with detection of *N. caninum* by immunohistochemistry (IHC) and PCR. The *N. caninum* IFAT titers from the goats whose placenta were analyzed ranged from 1:50 to 1:6400. Placental lesions were characterized mainly by necrosis involving the mesenchyme of the chorionic villi and trophoblast cells often alongside mononuclear inflammation composed mainly by lymphocytes, macrophages and few plasma cells. In some cases, there was a neutrophilic infiltration and calcification. Placental lesions were more severe in goats with titers higher than 1:800. The goat that had a 1:6400 titer aborted at 90th day of gestation. *N. caninum* DNA (NC5 gene) was detected in 10 of these 14 placentas, being negative in four goats with titers of 1:50, 1:200, 1:400, 1:800. Protozoan-like structures resembling cysts and tachyzoites were visualized in four of 14 placentas through conventional histological staining (hematoxylin and eosin, HE) and in the same cases by IHC (IFAT: 1:6400, 1:3200, 1:1600, and 1:1600). The present study demonstrates that severe lesions in the placenta of naturally infected goats are associated with abortions and stillbirths in caprine neosporosis and the placental alterations are likely involved in abortion pathogenesis. Moreover, the results highlight the importance of using more than one diagnostic technique for the detection of the protozoan in placentas because *N. caninum* cannot be reliably detected by histological and immunohistochemical tests. This is the first report of placental lesions associated to *N. caninum* in naturally infected goats in Brazil.

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Toxoplasma gondii tubulin-binding cofactor B a polarity factor required for host cell invasion and replication

P 56

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Tubulin -binding cofactors (TBCs) participate in the folding, dimerization and dissociation pathways of the tubulin dimer. Therefore, TBCs are implicated in microtubule dynamics *in vivo* and we hypothesise that they have a role in *Toxoplasma gondii* host cell invasion. TBCB is a CAP-Gly domain-containing protein that, together with TBCE, dissociate the tubulin dimer. *T. gondii* has one TBCB gene and the protein shows a polarized localization mainly at the anterior region of the cell, under the conoid, suggesting a close association with the polar ring and subpellicular microtubules. TgTBCB overexpression doesn't affect replication and egress rates but decreases host cell invasion.

Conditional TgTBCB knockout strains, using the CRISPR/Cas9 system, show a decrease in the glutamylated and acetylated tubulin of subpellicular microtubules and conoid and impaired host cell invasion and replication. In the absence of TBCB, parasite cells have an altered axis of division resulting in abnormal division which leads to polinucleated "big cell masses" with profound ultrastructural alterations.

Our data suggest that TBCB is a polarity marker, necessary to the correct establishment of division plan. TBCB does not present a clear co-localization with the apical complex secretory vesicles, but it is important for invasion. It is known that TBCB interacts with EB1 that is implicated in vesicular trafficking thus, considering TgTBCB localization, it is conceivable that TBCB can be involved in the toxoplasma secretory pathway, a hypothesis that is under analysis.

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Pharmacokinetics, safety and efficacy of Bumped Kinase Inhibitor (BKI) 1553 in a pregnant sheep model of neosporosis

P 57

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Neosporosis is considered a major infectious cause of bovine abortion worldwide and despite the economic importance, at present there is no approved treatment for cattle. It has been demonstrated that calcium dependent protein kinases (CDPKs) are promising drug targets by compounds from a focused bumped kinase inhibitor (BKI) library. BKI-1553 *in vitro* acted with IC_{50} of 0.18µM and in a pregnant mouse model of neosporosis reduced vertical transmission of *N. caninum* to pups and increased the rate of survival of offspring. The aim of this work was to investigate the pharmacokinetics, safety and efficacy of BKI-1553 compound in a pregnant sheep model of neosporosis. Thirty seven pregnant ewes were allocated to 6 groups. Group 1 (G1) (n=8), group 3 (G3) (n=8) and group 5 (G5) (n=8) were intravenously (iv) inoculated with 10^6 tachyzoites of the Nc-Spain7 isolate at day 90 of gestation. Group 2 (G2) (n=5), group 4 (G4) (n=5), and group 6 (G6) (n=3) were iv inoculated with PBS. Beginning 48 hours after infection, BKI-1553 was administered subcutaneously to G1 and G2: 1st dose 35 mg/kg and 2nd dose 10 mg/kg a week later, and G3 and G4: 10 mg/kg, 7 doses every 48 hours. Pharmacokinetics was evaluated in plasma by liquid chromatography-mass spectrometry. Safety was assessed by rectal temperature, local reaction in the inoculation points, hematological and biopathological parameters, fetal viability and weight of the lambs. Efficacy was assessed by fetal mortality, humoral and cellular immune responses, histopathology and parasite detection and load in target tissues. Fetal mortality was observed in G1 (5 out of 8 pregnant ewes), G3 (4 out of 8 pregnant ewes) and G5 in all inoculated animals. Parasite detection was found in 100% placentomes/cotyledons in all infected groups. Regarding fetal tissues, significant higher detection percentage in brain was observed in G5 compared to G1 ($P < 0.05$) and G3 ($P < 0.05$). Furthermore, brain parasite burden in G5 was significantly ($P < 0.01$) higher than in G3. In conclusion, BKI-1553 seems to allow partial protection against abortion and decrease detection and parasite load in target fetal tissues in a pregnant ruminant model of neosporosis.

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Cryptosporidium infections among animals and humans in Greece

P 58

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A cross-sectional study has been conducted in Greece in order to evaluate the prevalence of *Cryptosporidium* spp. in various animal species and humans. Faecal samples were collected from lambs (n=545) and goat kids (n=255) aged up to 120 days, calves (n=138) and foals (n=190) aged up to 6 months, dogs (household, shelter, shepherd) (n=879) and cats (household, shelter/catteries, feral) (n=1023) of all ages. Besides, 876 stool samples were collected from 822 adults and 54 children. A quantitative direct immunofluorescence assay was performed to evaluate the presence of *Cryptosporidium* oocysts. PCR followed by sequencing applied to genotype *Cryptosporidium* positive samples. The infection rates detected were: 16.7% for calves, 7.7% for lambs, 7.1% for goat kids, 5.9% for dogs, 5.2% for cats and 1.1% for foals. The prevalence of *Cryptosporidium* spp found in humans was 0.6%. Genotyping results revealed the presence of *C. parvum, C. andersoni, C. bovis in calves, C. parvum* (subtype IId), *C. ubiquitum* and *C. xiaoi* in lambs and goat kids, *C. canis* and *C. scrofarum* in dogs, *C. felis* in cats and *Cryptosporidium* horse genotype in foals. Regarding humans, sequencing was not successful for *Cryptosporidium* positive samples. In general, the genotyping results revealed a limiting but existing zoonotic risk from ruminants, whereas regarding the other mammals, sequencing indicated only the presence of host-specific genotypes.

Cryptosporidium parvum increases intestinal permeability through interaction with epithelial cells and IL-1β and TNF-α released by inflammatory monocytes

P 59

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Intestinal epithelial cells play a key role in maintaining homeostasis. We investigated the factors contributing to the alteration of the epithelial barrier function during *Cryptosporidium parvum* infection which represent the second most common cause of diarrheal diseases in infants in developing countries. Polarized epithelial cell monolayers infected by the parasite exhibit a drop in transepithelial resistance associated with a delocalization of E-cadherin and β-catenin from their intercellular area of contact, the adherens junction complex. In neonatal mice infected by *C. parvum*, the increased permeability is correlated with parasite development and with an important recruitment of $Ly6c^{+}$ inflammatory monocytes to the subepithelial space. $CCR2^{-/-}$ neonatal mice, which have few circulating inflammatory monocytes, were infected at similar level than conventional mice but with a lower increase in intestinal permeability suggesting a deleterious role of inflammatory monocytes during cryptosporidiosis. We next demonstrated that TNFα and IL-1β known to modulate tight-junction proteins are produced by inflammatory monocytes in the lamina propria of infected neonates and therefore can play a key role in the loss of barrier function. Our findings demonstrate for the first time that both the parasite and inflammatory monocytes contribute to the loss of intestinal barrier function during cryptosporidiosis.

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Investigation of tick-borne disease in Indian bovines: breed resistance and transmission blocking as strategies for improved productivity

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Apicomplexan parasites transmitted by ticks cause disease that is a severe constraint on productivity of cattle, globally. In India alone economic loss due to tick-borne disease has been estimated at US$800 million per annum, and TBD is likely to be of increased importance if the growing human population continues to rely on dairy products as a primary source of protein. Highly productive *Bos taurus* breeds are not reared in India due to high losses from TBD and milk is produced by less productive native cattle, buffalo, or by *Bos indicus*Bos taurus* crossbreed cattle reared in an attempt to improve production but retain resistance. To determine the effectiveness of this strategy we have surveyed bovines in several India states to model prevalence of infection with tick-borne pathogens, breed type and indicators of productivity. The results indicate that greater economic losses and prevalence of infection are manifest by crossbred cattle when in the carrier state, as well as those suffering overt disease.

A logical strategy to prevent the carrier state is to prevent transmission and studies on *Plasmodium* have shown efficacy of transmission blocking vaccines. We have conducted a transcriptomic and bioinformatic screen to identify putative transmission blocking vaccine candidates against, *Theileria annulata*. A number of candidates were identified with expression in the tick stages validated at the RNA level. Importantly, several candidates show good conservation across vector-borne Apicomplexa, indicating that they could be effective across a range of related parasites.

This study has highlighted the need for further research on identifying genetic loci that confer resistance and strategies to block pathogen transmission. Current work is aimed at providing increased knowledge in these areas in an effort to ameliorate future losses from TBD.

The relationship between presence of antibodies and direct detection of *Toxoplasma gondii* in slaughtered cattle from four European countries

P 61

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In cattle, the seroprevalence of *T. gondii* can be high but the presence of *T. gondii* tissue cysts is debated. To study the correlation between serological results and presence of tissue cysts, serum, liver and diaphragm samples of 167 veal calves and 235 adult cattle were obtained from slaughterhouses in the Netherlands, Italy, Romania and the United Kingdom. Liver samples were analysed by mouse bioassay and quantitative PCR on liver digest. In addition, a selection of diaphragms from negative cattle and all diaphragms of bioassay, digest PCR or serologically positive cattle were analysed by magnetic capture qPCR (MC-PCR). Serum samples were tested for anti-*T. gondii* IgG by modified agglutination test (MAT). For 17 cattle no bioassay results were obtained. Overall, 13 animals were considered direct detection-positive: 7 out of 151 in MC-PCR and 6 out of 385 cattle in bioassay (1.6%), indicating the presence of viable tissue cysts and thus a potential risk for consumers. These results demonstrate a lack of concordance between the identification of viable tissue cysts in liver and the detection of *T. gondii* DNA in the diaphragm. In addition, the probability to directly detect *T. gondii* in seropositive and seronegative cattle was similar, demonstrating that serological testing by MAT does not provide information about the presence of *T. gondii* in cattle and does not provide an indication of the risk for consumers.

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