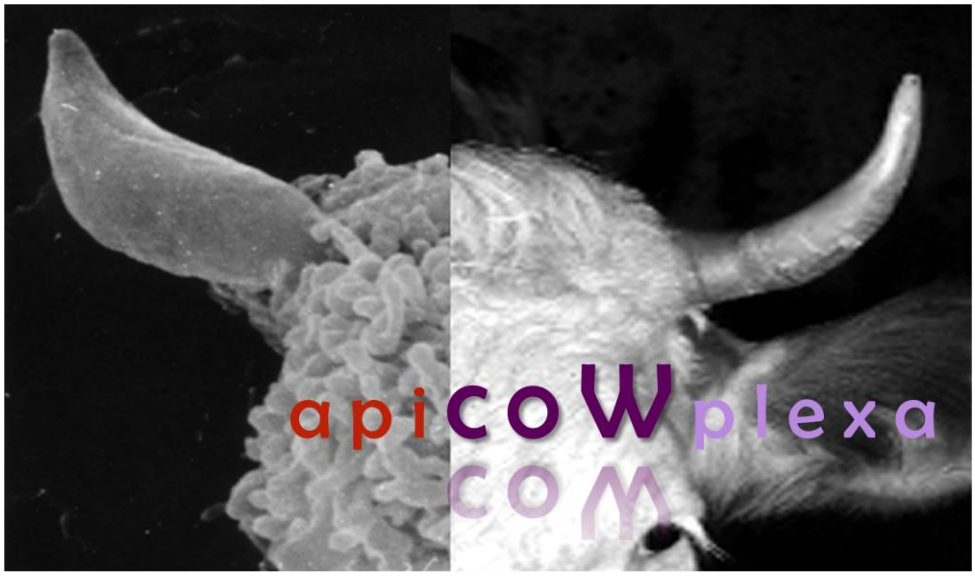

Proceedings

APICOMPLEXA IN FARM ANIMALS

3rd International meeting • Edinburgh, 30th June - 3rd July 2015

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apicowplexa

ApiCOWplexa 2015

3rd International Meeting on Apicomplexan Parasites in Farm Animals

PROCEEDINGS

30th June to 3rd July 2015

Moredun Research Institute

Edinburgh

Scientific committee

Alexandre Leitão (Technical University of Lisbon UTL and SPCV, Portugal)
Andrew Hemphill (University of Bern, Switzerland)
Anna Lunden (National Veterinary Institute, Sweden)
Brian Cooke (Monash University, Australia)
Damer Blake (Royal Veterinary College, UK)
Jens Mattsson (National Veterinary Institute, Sweden)
Gereon Schares (Friedrich-Loeffler Institut, Germany)
João Luís Garcia (Londrina State University, Brazil)
John Barta (University of Guelph, Canada)
Jonathan Wastling (University of Liverpool, UK)
Jitender P Dubey (United States Department of Agriculture, USA)
Julio Benavides (Livestock Health and Production Institute, ULE-CSIC, Spain)
Luís Ortega-Mora (Complutense University of Madrid, SALUVET, Spain)
Rachel Chalmers (Cryptosporidium Reference Unit, Public Health Wales, UK)
Tülin Karagenç (Adnan Menderes University, Turkey)
Joke van der Giessen (National Institute for Public Health and the Environment, The Netherlands)

Organising committee

Frank Katzer (Moredun Research Institute, Scotland)
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Stefano Guido (Moredun Research Institute, Scotland)
Alessandra Taroda (Londrina State University, Brazil)
Emily Hotchkiss (Moredun Research Institute, Scotland)

A warm welcome

ApiCOWplexa 2015

3rd International Meeting on Apicomplexan Parasites in Farm Animals

30th June – 3rd July

Edinburgh

It is our great pleasure to extend a warm welcome to all delegates to the third international scientific meeting dedicated to apicomplexan parasites in farm animals, which is being held in Edinburgh.

Moredun Research Institute and the John McIntyre Conference Centre offer ideal venues to meet your colleagues and to facilitate networking as well as scientific exchange.

The scientific sessions cover a range of interests within the field of apicomplexan parasites affecting farmed livestock, including evolution and genomics, epidemiology, diagnostics, vaccination, prevention and control, host-parasite interaction and food and waterborne zoonoses.

We thank you for attending the ApiCOWplexa 2015, and hope that you find it stimulating, enjoyable and fun.

The Organising Committee

Programme

Tuesday, 30th June 2015 – Pollock Halls (John McIntyre Conference Centre)	
16:00	**Registration**
	Opening Session
18:00	**Welcome reception**
19:00 - 19:45 **Keynote 01**	**The Lord Trees**, University of Liverpool and Moredun Research Institute, UK Opening address
19:45 - 20:30 **Keynote 02**	**John Barta** Molecular insights into the evolution of apicomplexan parasites across time and hosts: DNA barcoding and mitogenomics of coccidia

Wednesday, 1st July 2015 – Moredun Research Institute	
09:00 - 9:15	**Welcome to Moredun**
	Evolution and genomics, 9:15 – 10:45
	Chair: **Jonathan Wastling** co-chair: **Arnab Pain**
09:15 - 9:45 **Keynote 03**	**Mark Jenkins**, USDA, USA Approaches to combating avian coccidiosis through anticoccidial drugs and vaccination
09:45 - 10:00 S01	**Nadine Randle,** University of Liverpool, UK An integrated model of host-parasite interactions in Coccidian parasites
10:00 - 10:15 S02	**John Parkinson**, University of Toronto, Canada Systems based analysis of the *Sarcocystis neurona* genome identified pathways that contribute to a heteroxenous life cycle
10:15 - 10:30 S03	**Emanuel Heitlinger**, Humbold University, Germany *Eimeria-Mus* parasite-host coevolution in the house mouse hybrid zone
10:30 - 10:45 S04	**Kerry Woods**, University of Bern, Switzerland Using BioID to discover protein interaction networks at the *Theileria annulata* schizont surface
10:45 -11:15	**Coffee break**

	Epidemiology, 11:15 – 12:45
	Chair: **Gereon Schares** co-chair: **Fiona Tomley**
11:15 - 11:45 **Keynote 04**	**Damer Blake**, Royal Veterinary College, UK An epidemiological basis for the population, genetic and antigenic diversity of *Eimeria*?
11:45 - 12:00 S05	**Michael Grigg**, NIAID, USA Selective sweep of an inbred population of the protozoan pathogen *Neospora caninum*
12:00 - 12:15 S06	**Paula García-Lunar**, SALUVET, Spain *Neospora caninum* tachyzoite immunome study reveals differences among three biologically different isolates
12:15 - 12:30 S07	**Clare Hamilton**, Ross University, St Kitts Genetic diversity of *Toxoplasma gondii* in St Kitts, West Indies
12:30 - 12:45 S08	**Sonia Almería**, CReSA-IRTA, Spain *Toxoplasma gondii* in the exotic mustelid American mink (*Neovison vison*) in freshwater ecosystems in Spain.
12:45 -14:00	**Lunch**
12:45 -14:00	**EuPathDB workshop**
	Host Parasite Interactions 1, 14:00 – 15:30
	Chair: **John Barta** Co-chair: **Kerry Woods**
14:00 - 14:30 **Keynote 05**	**Andrew Hemphill**, University of Bern, Switzerland Interference in host cell invasion as an approach to develop vaccines and drugs against infection with *Neospora caninum* and related apicomplexan parasites
14:30 - 14:45 S09	**Marta González-Warleta**, INGACAL-Xunta de Galicia, Spain Endogenous transplacental transmission of *Neospora caninum* infection in naturally infected sheep
14:45 - 15:00 S10	**Caroline Frey**, University of Bern, Switzerland The lytic cycle of *Besnoitia besnoiti* in a standardized in vitro model: isolates display different invasion and intracellular proliferation rates
15:00 - 15:15 S11	**Daniela Chiebao**, University of Sao Paulo, Brazil Study of oral infection with *Toxoplasma gondii* in sheep: evaluation of congenital transmission in experimental infections by different strains in Brazil
15:15 - 15:30 S12	**Kyoko Hayashida**, Oita University, Japan Establishment of mouse/tick infection model for understanding *Theileria orientalis* biology
15:30 -16:00	**Coffee break**

	3 minutes poster presentations, 16:00 – 17:30
	Chair: **Rachel Chalmers** co-chair: **Frank Katzer**
OP01	**David Arranz-Solis**, Systemic and local immune responses in ewes after *Neospora caninum* experimental infection in the three periods of gestation
OP02	**Anamaria Balea**, Genetic characterization of *Toxoplasma gondii* strains isolated from stray cats in Romania
OP03	**Luciana Baroni**, Cloning, expression and characterization of an actin binding protein (ABP) from *Neospora caninum*: cyclase associated protein (NcCAP)
OP04	**Rita Isabel de Amorim Cardoso**, CCTα, a component of the tubulin folding pathway, in *Besnoitia besnoiti* and *Toxoplasma gondii* host cell invasion.
OP05	**Laila Darwich,** IFN-γ production in *Neospora caninum* experimentally infected dams at 110 days of gestation and in their fetuses
OP06	**Stefano Guido**, Identification of *Neospora caninum* bradyzoite-expressed antigens: developing improved diagnostics for the identification of chronically infected carrier cattle
OP07	**Turkan Gurbanova**, Correlation of Cryptosporidium infection of farm animals (cattle and sheep) and rodents in different ecosystems of Azerbaijan
OP08	**Chihiro Sugimoto**, Direct Blood Dry LAMP: A Quick, Stable, and Easy On-site Diagnostic Tool for tick borne-diseases in tropical countries
OP09	**Bożena Moskwa**, Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* infection in sheep, goats and fallow deer farming on the same area – preliminary results
OP10	**Stephen Larcombe**, Analysis of sequence diversity of transmission blocking candidate antigen genes reveals a potential association of SPAG1 indels with *Theileria* parasite speciation and host adaptation
OP11	**Emmanuel Liénard**, Experimental infections of rabbits by tachyzoites and bradyzoites of *Besnoitia besnoiti*
OP12	**Monica L. Mazuz**, Immunization of naturally infected pregnant cows against neosporosis-associated abortions with live tachyzoites
OP13	**Joana C. Silva**, Genomics of Whole-Organism Vaccine Composition and Design
OP14	**Justin A. Pachebat**, Technical advances in sequencing coccidian genomes directly from clinical samples – implications for genomics and veterinary diagnostics?

OP15	**Furio Spano**, Expanding the repertoire of TRAP-related proteins of Toxopl*asma gondii*: molecular and functional characterization of the micronemal adhesin TgMIC15
OP16	**Sofia Nolasco**, Mob1 protein: a critical factor in *Toxoplasma gondii* replication
OP17	**Kyle Tretina**, Re-annotation of the *Theileria parva* genome sheds new light into host-pathogen interactions
OP18	**Arnaib Pain**, The nuts and bolts of the making of an obligate intracellular parasite
	Poster session with drinks, 17:30 – 19:00

Thursday, 2st July 2015 – Moredun Research Institute	
	Diagnostics, 9:00 – 10:30
	Chair: **Julio Benavides** co-chair: **Sonia Almería**
09:00 - 9:30 **Keynote 06**	**Gereon Schares**, Friedrich-Loeffler Institute, Germany New tools for serological diagnosis and characterization of *Toxoplasma gondii* infections
09:30 - 9:45 S13	**Batol Al-Adhami**, Canadian FSA, Canada Performance of a new IgG ELISA-A/G compared to other serological tests for the detection of *Toxoplasma* infection in multiple animal species
09:45 - 10:00 S14	**Matthew Nolan**, RVC, UK Evaluation and application of a molecular tool to replace faecal oocyst count (FOC) testing of chickens
10:00 - 10:15 S15	**Brigitte Hentrich**, University of Bern, Switzerland Validation of a rapid test ("FASTest NEOSPORA caninum") for the detection of *Neospora caninum* antibodies in cattle, dogs and deer
10:15 - 10:30 S16	**Luis Fernando Pita Gondim**, Friedrich-Loeffler Institute, Germany In vitro isolation of *Hammondia heydorni* and evaluation of serologic cross-reaction with *Neospora caninum*
10:30 - 11:00	**Coffee break**

	Vaccination, 11:00 – 12:30
	Chair: **Luis Ortega-Mora** co-chair: **Mark Jenkins**
11:00 - 11:30 **Keynote 07**	**Ivan Morrison**, University of Edinburgh, UK Challenges faced in developing of a parasite vaccine based on induction of protective T-cell mediated immune responses
11:30 - 11:45 S17	**Adriana Aguado-Martinez**, University of Bern, Switzerland Evaluation of new bacterial lipoprotein-based vaccine formulations against neosporosis in mice
11:45 - 12:00 S18	**Virginia Marugan-Hernandez**, RVC, UK Study of the ability of microneme signal peptides of *Eimeria tenella* to deliver foreign antigens
12:00 - 12:15 S19	**Theo Schetters**, ProtActivity, The Netherlands Successful vaccination of calves against *Rhipicephalus microplus* and *R. annulatus* ticks using Bm86 and Subolesin antigens
12:15 - 12:30 S20	**Sarah Thomson**, Moredun Research Institute, UK Can infection with one genotype of *Cryptosporidium parvum* protect against infection with another?
12:30 -13:30	**Lunch**
12:30 - 13:30	**EuPathDB workshop**
	Prevention and control, 13:30 – 15:00
	Chair: **Damer Blake** co-chair: **Luis Fernando Pita Gondim**
13:30 - 14:00 **Keynote 08**	**Luis Ortega-Mora**, SALUVET, Spain Variability in *Neospora caninum* and its relevance to cattle infection
14:00 - 14:15 S21	**Isabel Hostettler**, University of Bern, Switzerland Development and application of a novel quantitative reverse transcriptase PCR (qRT-PCR) assay to identify drugs targeting intracellular *Theileria annulata* schizonts
14:15 - 14:30 S22	**Anja Joachim**, Institute of Parasitology, University of Vienna, Austria Neonatal porcine coccidiosis – do we understand it yet?
14:30 - 14:45 S23	**Kayode Ojo**, University of Washington, USA *Sarcocystis neurona* calcium-dependent protein kinase 1 is targeted in selective therapeutic development for Equine protozoal myeloencephalitis
14:45 - 15:00 S24	**Loredana Pop**, University of Cluj Napoca, Romania Effect of artemisinin and *Artemisia annua* on *E. tenella* infection in broiler chickens
15:00 -15:30	**Coffee break**

	Host parasite interactions 2, 15:30 – 17:00
	Chair: **Andrew Hemphill** co-chair: **Micheal Grigg**
15:30 - 16:00 **Keynote 09**	**Jens Mattson**, National Veterinary Institute, Sweden Disease management: more than a scientific challenge
16:00 - 16:15 S25	**Julio Benavides**, ULE-CSIC, Spain Foetal perivascular leucomalacia s the main lesion in abortions during the acute phase of ovine toxoplasmosis
16:15 - 16:30 S26	**Samuel Francisco**, University of Lisbon, Portugal Overexpression of TBCB protein affects *Toxoplasma gondii* host cell invasion
16:30 - 16:45 S27	**Ivan Pastor Fernandez**, SALUVET, Spain The expression of the non-secreted rhoptry protein ROP40 from *Neospora caninum* is up-regulated during tachyzoite egress and invasion
16:45 - 17:00 S28	**Jean-Michel Reperant**, ANSES, France Atypical forms observed among microgametes of two species of avian *Eimeria*
Thursday, 2st July 2015 – Pollock Halls (South Hall)	
19:00	**Conference dinner and ceilidh**

Friday, 3rd July 2015 – Moredun Research Institute	
	Food and Waterborne Zoonoses 1, 9:00 – 10:30
	Chair: **Karin Troell** co-chair: **Marieke Opsteegh**
09:00 – 9:30 **Keynote 10**	**Rachel Chalmers**, *Cryptosporidium* Reference Unit, UK *Cryptosporidium*: should we think more about food?
09:30 - 9:45 S29	**Beth Wells**, Moredun Research Institute, UK Detection of the protozoan parasites *Toxoplasma gondii* and *Cryptosporidium* in Scottish water
09:45 – 10:00 S30	**Stephane De Craeye**, University of Ghent, Belgium Is a terrestrial cat parasite really reaching marine mammals?
10:00 - 10:15 S31	**Joke van der Giessen**, RIVM, The Netherlands Anatomical distribution of *Toxoplasma gondii* tissue cysts and correlation between direct and indirect detection methods in the main livestock species
10:15 - 10:30 S32	**Berit Bangoura**, University of Leipzig *Toxoplasma gondii* in the meat of poultry following natural and experimental infections
10:30 -11:00	**Coffee break**
	Food and Waterborne Zoonoses 2, 11:00 – 12:30
	Chair: **Joke van der Giessen** co-chair: **Franz Conraths**
11:00 - 11:30 **Keynote 11**	**Marieke Opsteegh**, RIVM, The Netherlands *Toxoplasma gondii*: research priorities from a public health perspective
11:30 - 11:45 S33	**Alison Burrells**, Moredun Research Institute, UK Vaccination of pigs and lambs against *Toxoplasma gondii* reduces tissue cyst formation; safer meat for human consumption
11:45 - 12:00 S34	**Vitomir Djokic**, ANSES JRU BIPAR, France *Toxoplasma gondii* infection in pigs: correlation between direct and indirect detection methods and on farm risk factors
12:00 - 12:15 S35	**Sarah Macdonald**, RVC, UK A parasite, a bacterium and a chicken walk into a lab, what does the immune system say?
12:15 - 12:30 S36	**Heidi Enemark**, Norwegian Veterinary Institute, Norway *Cryptosporidium* and *Giardia* in Danish, organic pig farms: seasonal and age-related variation in prevalence, infection intensity and species/genotypes
12:30 - 12:45	**Closing address and student prizes**
12:45 - 14:15	**Lunch**

Opening session

Opening address — K01

Professor The Lord Trees

Professor the Lord Trees graduated in 1969 from Edinburgh. Following a year in mixed general practice, he completed a PhD on bovine babesiosis, and then worked in the pharmaceutical industry. In 1980 Lord Trees was appointed Lecturer in Veterinary Parasitology at the University of Liverpool, was appointed Professor of Veterinary Parasitology in 1994 and went on to become Dean of the Faculty of Veterinary Science from 2001-2008. His research has been funded by over £15m of external grants and produced over 170 scientific papers. He retired from the University of Liverpool in 2011. Lord Trees is currently Veterinary Editor in Chief of the Veterinary Record and In Practice, Chairman of the Board of the Moredun Research Institute, an elected RCVS Council member and Chair of the RCVS Science Advisory Panel. In 2012 Lord Trees was appointed to the Crossbenches through the Appointments Commission and is only the second veterinary surgeon to be appointed to the House of Lords.

Molecular insights into the evolution of apicomplexan parasites across time and hosts: DNA barcoding and mitogenomics of coccidia

K02

John R. Barta

Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, N1G 2W1, CANADA.
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The major 'groups' within the phylum Apicomplexa have been recognized for over a century: coccidia, piroplasms, plasmodia, haemogregarines, gregarines and cryptosporidia. However, considerable debate continues to surround discussions of the relationships among these parasites. Exploiting a variety of genetic targets, molecular phylogenetics have confirmed affiliations among parasites within these groups, but the relationships *among* these groups still remains unresolved. For the coccidia, use of the mitochondrial (mt) cytochrome c oxidase subunit I (COI) for use as a 'DNA barcoding' locus was shown to be far superior to many of the nuclear genetic loci used previously, especially nuclear ribosomal DNA and ITS regions. DNA barcoding at the mt COI locus is an excellent species-level molecular marker for most coccidia and, with some procedural modifications, can provide reliable species identifications from mixed samples using only the mt COI locus. In a manner analogous to 16S rDNA based estimates of bacterial biodiversity, incorporating next-generation sequencing technologies could provide simultaneous enumeration of known species and identification of previously unknown coccidia in a fecal sample (with concurrent partial molecular characterization) using only the mt COI locus. For many agricultural hosts of coccidia where the diversity of coccidia present may not be fully known, this may be a highly productive area of investigation.

Although complete mitochondrial sequences were obtained from *Plasmodium falciparum* and *Theileria parva* over 20 years ago, the first complete mitochondrial genome from a coccidium, *Eimeria tenella*, was sequenced in 2010; in the short time since this first coccidial mt genome was sequenced, more than 30 additional complete mt genomes have been generated from the eimeriid coccidia alone, with representatives from 6 eimeriid genera. Considerable conservation of mitochondrial genome content and gene order is typical within each major apicomplexan group but structure and content can vary dramatically between these major apicomplexan lineages. Combining mt genome sequences with complete nu 18S rDNA sequences was found to provide robust species delimitation as well as information on deeper relationships among apicomplexan parasites. Throughout various analyses, one overarching commonality was observed: Closely related definitive hosts were infected most commonly with closely related apicomplexan parasites suggesting that a general mechanism of coevolution of parasites with their definitive hosts underlies much of the evolutionary history of apicomplexan protists.

Evolution and Genomics

Keynote

Approaches to combating avian coccidiosis through anticoccidial drugs and vaccination. K03

Mark C. Jenkins and Raymond Fetterer

Animal Parasitic Diseases Laboratory, Agricultural Research Service, USDA, Beltsville, MD 20705

Avian coccidiosis remains an important disease problem for the poultry industry due to the ability of Eimeria to develop resistance to anticoccidial drugs, and to the lack of uniformity in vaccination of newly hatched chicks. Vaccination with low doses of live Eimeria oocysts remains the only alternative to controlling this parasitic disease because of discontinued use of anticoccidial drugs in many countries. In countries where medication of poultry feed with antibiotics is allowed, there is no a priori way for a poultry company to know the level of drug resistance in a particular operation. Our research is concerned with developing rapid, in vitro methods for assessing sensitivity of Eimeria in litter to ionophore drugs and synthetic chemicals, understanding the epidemiology of coccidiosis on poultry farms, and to improving the delivery of live Eimeria oocysts vaccines. Several in vitro methods coupled with PCR assay have been and are being developed for assessing salinomycin, monensin, diclazuril, and nicarbizin sensitivity in *E. acervulina*, *E. maxima*, and *E. tenella*. Our assay has shown excellent agreement with traditional in vivo drug sensitivity testing of ionophore-sensitive and –resistant strains of *E. tenella*. Measuring Eimeria oocysts levels in litter on commercial broiler farms during different phases of growout provided insight on Eimeria epidemiology. Vaccination against coccidiosis has been improved by incorporating a mixture of Eimeria oocysts in gelatin beads, and allowing chicks to ingest these gel-beads just after hatch. These studies are providing insight on Eimeria biology and prevention

An integrated model of host-parasite interactions in Coccidian parasites

S01

Nadine Randle, Dong Xia, Virginia Hernandez, Damer Blake, Fiona Tomley and Jonathan Wastling

University of Liverpool, Royal Veterinary College

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 a significant common biological characteristic: 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 quantitiative proteomics and transcriptomics we are simultaneously analysing gene expression, protein expression and protein phosphorylation from both the host and three coccidian parasites (*T. gondii*, *C. parvum* and *E. tenella*). Studies with *T. gondii* tachyzoites show that both host and parasites undergo wide-ranging transcriptional and protein expression changes within hours of invasion. These data are being 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. The functional role of specific parasite and host proteins implicated in the invasion process by our model, focussing on those shared across the species, will be validated *in vitro*.

Systems based analysis of the *Sarcocystis neurona* genome identified pathways that contribute to a heteroxenous life cycle

S02

Tomasz Blazejewski , Nirvana Nursimulu , Viviana Pszenny , Sriveny Dangoudoubiyam , Sivaranjani Namasivayam , Melissa A Chiasson , Kyle Chessman , Michelle Tonkin , Lakshmipouran Seshadri Swapna , Stacy S Hung , Joshua Bridgers , Stacy Ricklefs , Martin boulanger , Stephen F Porcella , Jessica C Kissinger , Daniel Howe , Dr. Michael E Grigg , **John Parkinson**

Hospital for Sick Children, , Toronto, Ontario, Canada;
University of Toronto, Toronto, Ontario, Canada;
NIAID, National Institutes of Health, Bethesda, Maryland, USA;
University of Kentucky, Lexington, Kentucky, USA;
University of Georgia, Athens, Georgia, USA;
University of Victoria, Victoria, BC, Canada;
Beltsville Agricultural research Center, Agricultural Research service, U.S. Department of agriculture, Beltsville, Maryland, USA;
Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, MT, USA

Sarcocystis neurona is a member of the Coccidia, a clade of single-celled parasites of medical and veterinary importance including Eimeria, Sarcocystis, Neospora and Toxoplasma. Unlike Eimeria, a single host enteric pathogen, Sarcocystis, Neospora and Toxoplasma are two-host parasites that infect and produce infectious tissue cysts in a wide range of intermediate hosts. As a genus, Sarcocystis is one of the most successful protozoan parasites; all vertebrates, including birds, reptiles, fish and mammals are hosts to at least one Sarcocystis species. Here we sequenced *Sarcocystis neurona*, the causal agent of fatal equine protozoal myeloencephalitis. The *S. neurona* genome is 127 Mbp, more than twice the size of other sequenced coccidian genomes. Comparative analyses identified conservation of the invasion machinery among the coccidia. However many dense granule and rhoptry kinase genes, responsible for altering host effector pathways in Toxoplasma and Neospora, are absent from *S. neurona*. Further, *S. neurona* has a divergent repertoire of SRS proteins, previously implicated in tissue cyst formation in Toxoplasma. Systems based analyses identified a series of metabolic innovations, including the ability to exploit alternative sources of energy. Finally we present a S. neurona model detailing conserved molecular innovations that promote the transition from a purely enteric lifestyle (Eimeria) to a heteroxenous parasite capable of infecting a wide range of intermediate hosts.

Eimeria-Mus parasite-host coevolution in the house mouse hybrid zone

S03

Emanuel Heitlinger

Humbold University and Leibniz Institute for Zoo and Wildlife research, Berlin

The house mouse is a "farm animal" associated with human agriculture as a commensal. When two different waves of human farmers met during the spread of agriculture to Europe a hybrid zone of the two subspecies *Mus musculus domesticus M. m. musculus* was established. This house mouse hybrid zone (HMHZ) often determines population structure of parasites and can thus be regarded a natural laboratory for adaptation and coevolution. Up to 16 species of Eimeria have been described from house mice, two of which are found at high prevalence throughout the HMHZ. We use a DNA capture approach based on our knowledge of the genome of *E. falciformis*. We describe the design of the capture baits and present an analysis of their effectivity allowing sequencing of target regions. Capturing up to 1 megabase of Eimeria spp. genomes we analyse population structure of the parasite and relate it to the HMHZ. Using this broad set of marker regions we can identify polymorphism correlating with host usage. This "specificity regions" put in their genomic context can identify candidate "specificity genes". We will test whether these genes drive differentiation analysing their evolution throughout the Apicomplexa. Some of the target genes analysed comprise (putative) pathogenicity factors described in Plasmodium, *Toxoplasma gondii* or *Eimeria tenella*. We test the hypothesis that especially variation in these factors determines the specificity of Eimeria species and strains. We give and outlook on the Eimeria-Mus model for host adaptation as a system approachable both in its original ecological settings and in laboratory experiments.

Using BioID to discover protein interaction networks at the *Theileria annulata* schizont surface

S04

Sandra Huber, **Kerry Woods**

Institute of Animal Pathology, Vetsuisse Faculty Bern, Switzerland

Theileria annulata, the causative agent of Tropical Theileriosis, manipulates its bovine host to an impressive extent. Theileria infection confers a cancer-like (transformed) phenotype upon the infected leukocyte, inducing anti-apoptotic signalling, uncontrolled proliferation and increased invasiveness. Transformation depends on the presence of the parasite within the host cytoplasm, and is linked to the modification of host cell signalling cascades. However the molecular mechanisms by which Theileria triggers these processes remain largely unknown. Several host molecules, including some kinases and microtubule-associated proteins, are reported to bind to the parasite surface. We are investigating the hypothesis that the schizont surface acts as a signal transduction platform, contributing to host transformation. We are using BioID technology to investigate protein interaction networks at the parasite surface. The principle of BioID involves the fusion of a promiscuous biotin ligase (BirA*) to a protein of interest, expression within cells, and the subsequent biotinylation and purification of interacting and proximal proteins. Because biotin is covalently bound to proteins, stringent conditions can be employed to solubilise protein complexes prior to purification – a huge advantage when dealing with membrane proteins. We used this powerful technique to identify the parasite binding partner of the microtubule stabilizing protein CLASP1. Excitingly, this approach also revealed the interaction of other host cell proteins, including several MAPs and signal transduction adaptor proteins, with the parasite.

Epidemiology

Keynote

An epidemiological basis for the population, genetic and antigenic diversity of *Eimeria*? K04

Damer P. Blake[1] and the *Eimeria* CIDLID Consortium

1 Pathology and Pathogen Biology, Royal Veterinary College, Hawkshead Lane, North Mymms, AL9 7TA, UK

Eimeria species parasites cause the disease coccidiosis, most notably in chickens where the global cost is thought to exceed US$3 billion every year. Every chicken is likely to be exposed to these parasites and control is an essential component of modern poultry production. Farmers most commonly rely on chemoprophylaxis, although drug resistance develops rapidly and is now widespread. Live and subunit parasite vaccines are available, but production capacity and relative cost limit uptake. In response, interest in the development of novel cost-effective recombinant or vectored anticoccidial vaccines has been rekindled in recent years. The successful translation of such vaccines to the field will depend in part on parasite population structure and the extent of pre-existing antigenic diversity, influencing opportunities for vaccine breakthrough and dissemination of resistant genotypes. For *Eimeria* these variables remain almost completely unknown. Using Sequenom massARRAY SNP-based genotyping we have defined population structure for *Eimeria tenella*, revealing an intriguing dichotomy between northern and southern populations characterised by distinctive spatial haplotype occurrence with evidence of clonality and panmixia. Targeted exon sequencing has revealed a conflicting lack of diversity for the vaccine candidates Apical Membrane Antigen 1 (AMA1) and Immune Mapped Protein 1 (IMP1), suggesting that for *E. tenella* protein functionality appears to outweigh immune evasion. This is in direct contrast to the situation in other apicomplexans such as *Plasmodium*, and is most likely underpinned by the biology of the direct and acute coccidian life cycle in the definitive host. Epidemiological analysis using multiple correspondence and hierarchical clustering methods has been applied to interpret the impact of environmental and production system parameters. Distinct, region- and system-specific clusters have been identified with a possible key role for regional humidity.

Selective sweep of an inbred population of the protozoan pathogen *Neospora caninum*

S05

Khan, A., A.W. Fujita, N. Randle, M. Quinones, K. Shen, A. Oler, N. Sundar, U. Ryan, J. Slapeta, G. Schares, L. Ortega-Mora, J.P. Dubey, J. Wastling, **M.E. Grigg**

Neospora is an obligate protozoan parasite of animals. *Neospora* propagates by both asexual replication, which includes horizontal and vertical transmission exclusively within intermediate hosts as well as sexual replication, which occurs only in canines. No evidence has been documented whether the sexual cycle, shown only experimentally, impacts the parasite's transmission in nature. Global population genetic diversity and whether parasite genotype contributes to phenotypic variations in neosporosis is understudied. To determine the population genetic structure, we characterized 49 *Neospora* isolates, collected from a variety of intermediate and definitive hosts from North and South America, Europe, Asia, and Australia using intron/antigen gene sequenced markers (SM) and microsatellite markers (MS). Although multilocus MS genotyping showed high genetic diversity, all 46 *N. caninum* isolates were monophyletic at the SM markers. This unprecedented finding was also supported by tight clustering using principle component analysis. Population structure analysis using a Bayesian statistical model demonstrated only two populations: *N. caninum* (n=46) and *N. hughesi* (n=3). Calculation of genetic distance indexes including F_{ST}, linkage disequilibrium, *ZnS* statistics, and association index demonstrated global expansion of a single clonal lineage of *N. caninum*. DNA sequencing of the mitochondrial and apicoplast genomes, however, identified incongruence between the nuclear and organellar genomes supporting evidence for intra-specific (within *N. caninum*) sexual recombination among strains. To produce a genetic ancestry model for the species and to determine the extent to which sexual reproduction is occurring, we sequenced the genomes of eight *Neospora* isolates. Genome wide pairwise comparison of single nucleotide polymorphisms identified nearly identical genomes that were in high linkage disequilibrium, but copy number variation plots identified significant structural variation. The identification of several haploblocks of introgressed sequence bearing different genetic ancestry established unequivocally that sexual recombination has impacted the population genetics of these highly similar *N. caninum* strains. Further, the presence of significant structural variation among largely identical, but independent, sister lines support a model whereby unisexual inbreeding has produced a global selective sweep of a single *Neospora caninum* lineage. Importantly, the existence of several strains that harbor limited SNP differences and no apparent structural variation support the co-occurrence of an asexual expansion model that is congruent with vertical transmission.

Neospora caninum tachyzoite immunome study reveals differences among three biologically different isolates

S06

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Pathogenesis of bovine neosporosis is determined by different host and parasite–dependent factors, including isolate virulence. A previous study identified several *Neospora caninum* tachyzoite proteins to be more abundant in virulent isolates, Nc-Liv and Nc-Spain7, when compared to the low-virulent isolate Nc-Spain1H. Herein, we explored differences in the immunomes of these three isolates. Thus, protein extracts from each isolate were revealed by 2-DE immunoblot using sera from experimentally and chronically infected mice with each isolate (in a 3x3 design). All protein extracts showed similar antigenic pattern when revealed by the same serum. Most of the reactive spots were located in the acidic region (pH 3-7) grouped in 3 antigenic areas (250-70, 45-37 and 35-15 KDa). Major differences depended on the sera, regardless the extract employed. Notably, 4 proteins related to metabolism (serine-threonine phosphatase 2C and superoxide dismutase), tachyzoite invasion (gliding associated GAP45) and dense granules (NcGRA1) identified by MS, failed to be consistently detected in all protein extracts by sera from Nc-Spain1H infected mice, apart from 4 non-identified spots and 2 spots chains located in 45-37 kDa area. Variations between Nc-Spain7 and Nc-Liv were limited to the absence of recognition of GAP45 and the spots chains located in 45-37 kDa area by sera from Nc-Spain7 infected mice. These results reflect intra-specific diversity on immunogenicity capacities of *N. caninum* in mice and the differentially recognized antigens should be investigated as putative virulence markers. This study was funded by AGL2013-44694-R.

Genetic diversity of *Toxoplasma gondii* in St Kitts, West Indies

S07

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Toxoplasma gondii is a ubiquitous protozoan parasite capable of infecting all warm-blooded animals. Humans become infected with *T. gondii* post-natally by ingesting tissue cysts from meat, or by ingesting oocysts from contaminated food or water, or directly from the environment. Disease outcome can vary depending on a number of factors, including genetic diversity of the infecting strain. We demonstrated a high prevalence of *T. gondii* in livestock on St. Kitts suggesting widespread environmental contamination with oocysts. The aim of this study was to determine the genetic diversity of *T. gondii* in free-roaming chickens on St. Kitts. Eighty one chickens were collected from 9 locations around the island and their hearts and brains were digested and inoculated into 243 CD1 mice in a bioassay. Aliquots of digested chicken material were examined for *T. gondii* DNA using quantitative PCR and 25% (20/81) were positive. Sera from chickens were examined for *T. gondii* antibodies using an in-house ELISA, and reactive antibodies were detected in 93% (75/81) of samples. Left lung was collected from all mice following bioassay for in vitro culture in Vero cells. Live *T. gondii* was isolated from 20% (16/81) of chickens, from 7 out of 9 locations on the island. Isolates were cryopreserved for genotyping and virulence studies. Right lung and brain were also collected from all mice following bioassay for genotyping using 11 genetic markers (5'SAG2, 3'SAG2, SAG3, GRA6, BTUB, SAG1, C22-8, C29-2, L358, PK1 and Apico). These results will be discussed.

Toxoplasma gondii in the exotic mustelid American mink (*Neovison vison*) in freshwater ecosystems in Spain.

S08

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Toxoplasma gondii is a zoonotic protozoan that causes serious illness in humans and infects animals worldwide. Felids are the definitive hosts, excreting oocysts in faeces to the environment. Several authors have suggested the important role of water-borne transmission of the parasite. The objective of the present study was to analyze the seroprevalence of *T. gondii* in American minks (*Neovison vison*), a widely distributed invasive species living in freshwater ecosystems in Spain. Serum samples were collected from 557 American minks (AM) from freshwater ecosystems from Castilla-León, La-Rioja and Catalonia regions (Northern Spain), from years 2011 to 2014. These animals come from the AM-eradication campaign (part of the National Strategy for the conservation of the endangered European mink (*Mustela lutreola*). Antibodies to *T. gondii* were assayed by the modified agglutination test (MAT titres ≥1:25). Antibodies were found in 440 (79.0%) of 557 American minks. Statistical significant differences were observed related to geographical area and to increased age, as indication of higher exposure to the parasite by age. No differences were observed related to sex. This study shows high and widespread natural exposure of American minks to *T. gondii* in freshwater habitats in Spain and indicates that water-borne transmission of oocysts may be an important mode of transmission. Although other food sources are also included in the diet of American minks, this species could be a sentinel species for *T. gondii* contamination in freshwater aquatic habitats.

Host and Parasite Interactions 1

Keynote

Interference in host cell invasion as an approach to develop vaccines and drugs against infection with *Neospora caninum* and related apicomplexan parasites

K05

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Neospora caninum is a leading cause of abortion in cattle, and is thus an important veterinary health problem of high economic significance. Vaccination has been considered a viable strategy to prevent bovine neosporosis. Different strategies have been investigated, and the one we have followed has been to employ subunit antigens, targeting components that are functionally involved in the physical interaction between the parasite and its host cell during invasion. The vast majority of experimental studies were performed in mice, but others have undertaken investigations in cattle and sheep. More recently, several studies have investigated drug treatment as an option to limit the effects of vertical transmission. In this context, bumped kinase inhibitors (BKIs) which are inhibitors of calcium dependent protein kinase 1 (NcCDPK 1) that is crucially involved in host cell invasion of *N. caninum* and other apicomplexans, have emerged as highly interesting drug candidates. In vitro studies showed that the BKI1294 inhibits host cell invasion, but also egress, but does not inhibit intracellular DNA replication, which leads to the formation of large multinucleated complexes that remain viable for extended periods of time in vitro. Nevertheless, BKI1294 has been shown to exhibit outstanding safety and efficacy in non-pregnant and pregnant mouse models for Neospora infection, rendering this candidate a prime candidate for future studies in large animal models. In addition, BKI1294 also exhibits promising in vitro activities against the related apicomplexans *Toxoplasma gondii* and *Besnoitia besnoiti*.

Endogenous transplacental transmission of *Neospora caninum* infection in naturally infected sheep

S09

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Neospora caninum has traditionally been considered an unimportant parasite in sheep. However, recent studies carried out in Spain have shown that this protozoan can cause severe reproductive failure in sheep. The importance of ovine neosporosis should therefore be reconsidered and the mechanisms of transmission studied in depth. This study aimed to evaluate the efficacy of endogenous transplacental transmission of *N. caninum* in sheep. A total of 28 seropositive sheep were housed in facilities in which horizontal infection was precluded. The sheep were mated, giving rise to 24 pregnant sheep in which ultrasound scans (for pregnancy follow up) and blood sampling (for antibody detection) were carried out monthly. Three of the sheep aborted 5 foetuses and 21 of the sheep gave birth to 11 stillborn and 39 live lambs. Ten lambs were born weak and died within a week of birth. Precolostral serum samples were collected from all live newborn lambs for antibody detection. Brain samples were obtained from all aborted, stillborn and dead lambs for parasite DNA detection and histological analysis. All sheep remained seropositive throughout pregnancy. Precolostral antibodies were also detected in 38 out of 39 newborn lambs. Parasite DNA was detected in all dead lambs (21) and in all foetuses except one (4/5). Scarce histological lesions consistent with protozoan infection (glial foci) were observed in 16 brains. In 13 out of these 16 cases, *N. caninum* tissue cysts were detected by immunohistochemistry. The results show conclusively that endogenous transplacental transmission of neosporosis is highly efficient in sheep.

The lytic cycle of *Besnoitia besnoiti* in a standardized *in vitro* model: isolates display different invasion and intracellular proliferation rates

S10

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Bovine besnoitiosis, caused by *Besnoitia besnoiti*, reduces productivity and fertility of affected herds. The disease continues its expansion in Europe and no effective control tools are at hand. Experimental models for drug evaluation *in vitro* are urgently needed. Herein kinetics and phases of the *B. besnoiti* lytic cycle were studied in MARC-145 cell culture. The invasion at 4, 6, 8 and 24 hpi and proliferation characteristics at 24, 48, 72, 96, 120, and 144 hpi of one *B. tarandi* and seven *B. besnoitia* isolates (Bb-Spain 1, Bb-Spain 2, Bb-Israel, Bb-Evora03, Bb-Ger 1, Bb-France, Bb-Italy) were studied. Key parameters of *Besnoitia* sp. lytic cycle differed from those of other Toxoplasmatinae. Remarkably, *Besnoitia* sp. displayed lower invasion rates and was slower in invading the host cells. Only 50% of invasion was reached after 3-6 hours, and parasites continued to invade for 24 hours, thus indicating a long extracellular survival. Overall, the predominant plaque forming tachyzoite categories were lysis plaques *versus* big and small parasitophorous vacuoles after 72 hpi, and a rather long doubling time of 20-40 h was observed. Significant intra-species differences were also detected. Invasion efficacy was significantly higher for Bb-France, Bb-Evora03 and Bb-Israel. Moreover, Bb-France and Bb-Evora03 showed a long half time of invasion. Finally, tachyzoite yield at 144 hpi was highest in Bb-Israel and *B. tarandi*. This study is the first comparing *in vitro* characteristics of different *B. besnoiti* isolates and provides the basis for a proof-of-concept standardized *in vitro* model to test drug candidates. This study was funded by AGL2013-46442R; CFF was funded by SNF PBBEP3 _141435.

Study of oral infection with *Toxoplasma gondii* in sheep: evaluation of congenital transmission in experimental infections by different strains in Brazil. **S11**

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Toxoplasma gondii is considered the primary cause of congenital disease, miscarriage and stillbirth. Chronically infected sheep may suffer recrudescence of the infection and transmit the parasite to the pregnant reproductive system. This study evaluated experimental infections by two atypical strains of *T. gondii* isolated from cats in Brazil. Four ewes (Group 1) were prime-infected orally with $2x10^3$ oocysts from genotype BrI whilst 9 ewes received genotype BrIII (Groups 2 and 3). After chronicity of infection, animals were mated. A second inoculation was held at 2 months pregnancy, group 1 inoculated with genotype BrIII, while Group 2 were infected with genotype BrI. Group 3 was reinfected with the same strain, genotype BrIII. Blood samples for serology using IFAT and abdominal ultrasound for fetus viability diagnosis were performed weekly. After lambling, all sheep were euthanized and tissue samples were collected for mice bioassay and PCR analysis. No abortion was observed, 19 lambs were born healthy. There was seroconvertion after prime-infection of infected ewes (cut-off ≥ 64). All lambs presented negative results from serology tests. During necropsy, a mummified fetus was found inside one ewe from group 2. Also from group 2, it was noted lymph nodes enlargement within ewes and lambs. PCR (ITS1) confirmed presence of *T. gondii* on group 2 lamb lymph nodes. From mice bioassays were analyzed serum, slide smears from brain and lungs for cysts and tachyzoites visualization and performed RFLP-PCR on positive samples. From ewes, only one mice bioassay of group 2 was negative. Considering mice bioassays from lambs, two animals from group 2 presented antibodies anti-*T. gondii*, one belonging to the mummy twin. The other was also brain cyst and PCR positive. PCR-RFLP reveled that apart from one ewe from group 1, all of them, including the positive lamb, were infected with the prime-infection strain. Considering the conditions applied on this study, it was observed a low rate of congenital transmission in seropositive ewes after reinfection. The prime-infection often provided cross protective immunity against a following reinfection.

Establishment of mouse/tick infection model for understanding *Theileria orientalis* biology

S12

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Theileria orientalis infection causes anemia and production losses in cattle, and thus the oriental theileriosis is considered to be one of the economically significant diseases of grazing cattle in East Asia, and more recently in Australia. However, the biology of this parasite is poorly understood. Inability for in vitro culturing and the lack of laboratory animal infectivity models are the major obstacles to the parasite research. Especially, research on tick stages of *T. orientalis* has rarely been undertaken, although such investigations are of paramount importance for the vaccine development targeting sporozoites. A previous investigation suggested that *T. orientalis* could proliferate in SCID mice transfused with bovine erythrocytes. In the present study, we employed this SCID mouse model to produce T. orientalis sporozoites in a tick vector. Injection of *T. orientalis* (Ikeda-type)-infected bovine blood to the splenectomized SCID mice was followed by biweekly intraperitoneal administration of bovine erythrocytes (To-SCID-Bo). After the detection of *T. orientalis* within the erythrocytes of To-SCID-Bo, *Haemaphysalis longicornis* larval ticks were allowed to feed on the infected mice. After the moulting, the resultant nymphal ticks were subjected to feeding on non-infected mice for 2-3 days to stimulate the sporozoite maturation. Finally, the mature sporozoites were demonstrated in the tick salivary gland using immune staining. These findings suggest that the mouse-tick laboratory infection model established in the present study is a promising research tool to understand the biology of *T. orientalis*.

Diagnostics

Keynote

Results of an extended literature review - The relationship between on farm risk factors and *Toxoplasma gondii* infection in farm animals — K06

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To reduce the risk of *Toxoplasma gondii* infection in humans, knowledge on potential risk factors associated with the infection of farm animals with the parasite is essential. An extended review of the relevant literature published since 1994 was performed in a systematic way. The review was restricted to the most important domestic food-producing animals in Europe and literature was excluded if the assessment was limited to risk or protective factors not applicable to European husbandry systems. A number of studies (mainly cross-sectional studies) provided information on various risk and protective factors for *T. gondii* infections in farm animals including definitive host related factors or factors characterizing the likelihood of fodder contamination. Most, but not all studies assessing the potential role of rodents identified a risk effect. Surprisingly, studies assessing the role of contaminated water as a risk factor for infection did not reveal a consistent association. The review shows that further studies are necessary to solve conflicting findings and to complete knowledge especially in cattle, equids and in poultry. *This research was conducted by a consortium within the framework of project n° GA/EFSA/BIOHAZ/2013/01 entitled “Relationship between seroprevalence in the main livestock species and presence of *Toxoplasma gondii* in meat”, grant agreement funded by the European Food Safety Authority (budget). This paper/publication is based on the results obtained in the framework of this mentioned project and it is published under the sole responsibility of the authors, and shall not be considered as an EFSA output.

Performance of a new IgG ELISA-A/G compared to other serological tests for the detection of Toxoplasma infection in multiple animal species

S13

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Toxoplasma gondii is a zoonotic protozoan parasite which can cause significant disease and losses in livestock, wild animals and humans. Effective control strategies require rapid, reliable and cost-effective detection methods for large scale surveys and diagnostic applications in a broad range of host species. An indirect ELISA using protein A/G was developed (ELISA-A/G) and evaluated on samples of serum and meat juice from a wide variety of experimentally or naturally infected livestock and wild animals, including pigs, sheep, cats, mice, and multiple wildlife species. Samples were also tested by indirect ELISA-IgG, the Modified Agglutination Test (MAT) and Western Blot analysis (WB). Comparative analysis of test results from samples obtained from experimentally infected pigs, cats, mice and seals showed excellent agreement between the ELISA-A/G, ELISA-IgG and MAT. High correlation was also observed when samples from naturally infected host species were tested by these same assays. Furthermore, a consistent band pattern was present on WB when protein A/G conjugate was used on samples from experimentally infected animals. Because the ELISA-A/G uses the same conjugate reagent for all mammalian species, it is an efficient and more rapid and convenient method for simultaneously testing multiple host species from domestic, wild and aquatic origins. A Modified WB using protein A/G conjugate was demonstrated as a potential confirmatory assay for the infection of *T. gondii*.

Evaluation and application of a molecular tool to replace faecal oocyst count (FOC) testing of chickens

S14

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Eimeria are recognised as highly pathogenic parasites of chickens. Presently, research aimed at reducing their impact is hindered by the lack of non-subjective medium/high throughput diagnostic tools. Here, we tested a pre-existing real-time PCR (qPCR) to quantify *Eimeria tenella* from chicken tissue and faecal samples. Chickens were inoculated with 500, 1500 or 4500 *E. tenella* oocysts; then, parasite burdens were quantified employing i) qPCR analysis of DNA extracted from caecal tissues collected at five and eight days post-infection (dpi) and ii) faecal oocyst counts (FOC) on samples taken six to eight dpi. Real-time PCR test results indicated a significant dose-dependent increase in parasite numbers among study groups ($p=0.0002$ and $p=0.0019$) for samples collected 5 dpi (i.e. prior to gametogony) but not in those from day eight (after most oocyst shedding). In contrast, no significant dose-dependent increase in FOC was observed in samples from the three groups collected 6-8 dpi. Further evaluation of this technique, via the quantification of *E. tenella* in naturally infected broilers from across the United Kingdom, indicated a concordant relationship ($r^2 = 0.8894$) between qPCR test results and FOC. This method overcomes the limitations of coproscopic quantification and allows reproducible medium to high-throughput examination of a variety of biological samples, thus representing a valuable diagnostic tool for determining the impact of Eimeria infections on chicken farms. Importantly, qPCR also has significant implications for animal welfare via improved statistical power and reduced group sizes in experimental studies.

Validation of a rapid test ("*FAST*est® NEOSPORA caninum") for the detection of *Neospora caninum* antibodies in cattle, dogs and deer

S15

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A rapid test **("*FAST*est® NEOSPORA** caninum**")** was developed for the detection of anti-*Neospora caninum* antibodies in whole blood, serum or plasma of dogs and cattle. The test is based on an immunochromatographic reaction, where *N. caninum* antibodies present in the sample are bound to membrane fixed recombinant *N. caninum* antigens. The interpretation is qualitative. At the Institute of Parasitology, 39 cattle sera positive for anti-*Neospora*-Ab, 19 negative cattle sera, 13 positive dog sera and 18 negative dog sera from the daily routine were examined by the rapid test. Additionally, sera from cattle experimentally infected with *N. caninum* (n=4) were tested. The relative sensitivity was defined using *N. caninum* positive sera with IFAT cut off ≥ 1:160. The relative specificity was defined using *N. caninum* negative sera with IFAT cut off < 1:160. From all 52 defined positive dog and cattle sera, 13 dog sera, 37 cattle sera from the routine diagnostic and 4 experimentally infected cattle reacted positive in the rapid test system. This leads to a relative sensitivity of 96.2%. All 37 IFAT negative defined dog and cattle samples also reacted negative in the rapid test system. This leads to a relative specificity of 100%. Potential cross-reactions were checked with *Babesia canis*, *Leishmania* sp., *Dirofilaria* sp. and *Toxoplasma* sp. positive dog sera (n=10) as well as with *Babesia divergens* and *Toxoplasma* sp. positive bovine serum samples (n=4). The rapid test showed no cross reaction with these samples. An additional validation of the ***FAST*est® NEOSPORA** caninum with 28 IFAT positive and 30 IFAT negative deer sera yielded similar results. The rapid test achieved a relative sensitivity of 96.4% and a relative specificity of 96.7% in this species No cross reactions occurred with *Besnoitia* sp. and *Toxoplasma* sp. positive deer sera (n=5).

In vitro isolation of *Hammondia heydorni* and evaluation of serologic cross-reaction with *Neospora caninum*

S16

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Hammondia heydorni was *in vitro* isolated from oocysts shed by a dog using a finite bovine heart cell line (KH-R). The oocysts were purified by sucrose flotation and suspended in 2% sulphuric acid for sporulation for five days at room temperature. The parasite was confirmed as *H. heydorni* by PCR using the parasite-specific primers JS4/JS5, and by negative reaction for *Neospora caninum* employing the primers Np6/Np21. 1 x 10^6 *H. heydorni* sporulated oocysts were treated with sodium hypochlorite and physically lysed by vortexing the oocyst suspension with glass beads. Zoites were clearly seen in the cells from three days after inoculation. Multiplying zoites, which were not recognized by a polyclonal rabbit serum against a bradyzoite-specific antigen (anti-BAG1), were classified as tachyzoites. Cysts with a length of up to 60 µm were observed in the cell monolayers at 15 dpi. They could be stained with the anti-BAG1 rabbit serum and with a rat monoclonal antibody (mAbCC2) against a cyst wall protein. The *H. heydorni* cysts increased in size during cultivation and reached a length of up to 135 µm. The parasite was maintained in the bovine heart cells up to 4.5 months. Sera from mice and sheep experimentally infected with *H. heydorni* oocysts reacted with the putative *H. heydorni* isolate by IFAT, but did not cross-react with *N. caninum* antigens using IFAT or immunoblot. These findings suggest that serologic cross-reactivity between *H. heydorni* and *N. caninum* seems to be of minor importance.

Vaccination

Keynote

Challenges faced in developing of a parasite vaccine based on induction of protective T-cell mediated immune responses	K07

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The tick-borne protozoan parasite *Theileria parva* causes an acute, often fatal disease in cattle known as East coast fever (ECF), which is present in 11 countries in eastern and southern Africa. The African buffalo (*Syncerus caffer*) is also a host for *T. parva*, but infected buffalo do not suffer disease.

Cattle that recover from infection with *T. parva* are solidly immune to challenge with the same parasite isolate but exhibit only partial protection against other isolates. Work carried out in the 1970s led to the development of a live vaccine incorporating 3 parasite isolates, which induced broad protection against both experimental and field challenge. Although this vaccine is deployed successfully in some regions to control the disease, logistical difficulties experienced in producing and distributing the live vaccine have highlighted the need to develop an alternative more sustainable method of vaccination.

A large body of evidence indicates that CD8 T cell responses specific for parasitized cells play a critical role in immunity to *T. parva.* Such responses frequently exhibit parasite strain restriction, which has been shown to correlate with cross protection between parasite isolates. Antigen screening has identified a number of parasite antigens recognised by CD8 T cells from immune cattle, some of which are conserved and others highly variable between parasite isolates. However initial attempts to immunise cattle by delivery of these antigens in viral vectors have been disappointing. These findings have focused current research on examining the role of other cellular responses, principally CD4 T cells, and determining the functional properties of the CD8 T cells that are responsible for protection. Further studies involving molecular typing of field populations of *T. parva* have provided insight into the nature and extent of antigenic diversity and helped to inform the choice of vaccine antigens to address the issue of strain restricted immunity.

Evaluation of new bacterial lipoprotein-based vaccine formulations against neosporosis in mice

S17

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Protective immunity against neosporosis by vaccination continues to be a difficult challenge. Some recombinant antigens were shown to be effective against cerebral neosporosis in mice but failed to induce protection during pregnancy. This was usually associated with a pro-inflammatory Th1 response incompatible with pregnancy or a predominantly Th2-biased response unable to control parasite proliferation. Unlike other Toll-like receptors, TLR2 ligands, such as bacterial lipoproteins, usually promote a Th1/Th2 response with a regulatory component which could be beneficial to maintain pregnancy and simultaneously prevent neosporosis. OprI (a lipoprotein of *Pseudomonas aeruginosa*) fused with the chimeric antigen NcMic3-1-R was constructed and assessed in a pregnant mouse model of neosporosis. The immune response and efficacy were compared with those induced by OprI-fused ovalbumin and the non-OprI NcMic3-1-R. The cytokine profile induced by NcMic3-1-R in the pre-challenge phase was different in OprI and non-OprI vaccinated mice: whereas non-OprI NcMic3-1-R induced a strong Th2 response with a lack of IFNg and IL17 but high levels of IL4, OprI-NcMic3-1-R induced a predominantly Th1-biased response with a strong regulatory component as high levels of IL10 were detected. However this immune response against the vaccine formulation was unable to protect against the infection as no protection against neither cerebral neosporosis nor vertical transmission to the pups were detected. How the immune response profile switches to a non-protective scenario after challenge will be analyzed and discussed.

Study of the ability of microneme signal peptides of *Eimeria tenella* to deliver foreign antigens

S18

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The ability to express foreign antigen in *Eimeria* spp has been reported before by several research groups. The development of these technologies has promoted the use of *Eimeria* spp as recombinant vaccines for the delivery of protective antigens against other pathogens. In order to improve the presentation of these antigens to the host immune system and therefore raise their potential protective attributes, different approaches have been carried out such as the use of stronger promoters to enhance protein expression, co-expression with adjuvants and cytokines or targeting to different compartments. In the present study, related to this last approach, endogenous signal peptides from three different microneme proteins of *E. tenella* (MIC2, MIC5 and MIC9) were cloned upstream of the mCherry fluorescent protein coding sequence in a plasmid also encoding the mCitrine fluorescent reporter. The plasmids were transfected into sporozoites of *E. tenella* and the ability of each signal peptide to deliver the mCherry was examined in vitro. After the shock, the parasites were incubated as free parasites as well as allowing them to invade MDBK cells for 48 hours. Only the signal peptide of MIC2 was able to localize the protein to the apical tip of the parasite.

Successful vaccination of calves against *Rhipicephalus microplus* and *R. annulatus* ticks using Bm86 and Subolesin antigens

S19

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Control of ticks and diseases transmitted by them is currently carried out using acaricides that inhibit the development of ticks. Their abundant use has led to the selection of tick strains that are resistant to these chemicals. Vaccination against ticks appears to be a promising alternative for the control of ticks and tick borne diseases. The Bm86 surface protein of midgut cells of *R. microplus* is the basis of a commercial vaccine that is still on the market. Results from the field showed that protection is partial, which impedes wide acceptance of the vaccine. More recently, Subolesin, a highly conserved protein, was described that showed protection in different tick-host models. Here we investigated whether vaccination of calves with both, Bm86 and Subolesin, could increase efficacy against tick infestation. Groups of five calves each were vaccinated with Pichia-derived Bm86 only, or with Bm86 and E. coli-derived subolesin on three occasions at three-week intervals. Montanide ISA50V2 (Seppic) was used as adjuvant. Three weeks after the final vaccination animals were infested with *R. microplus* and *R. annulatus* larvae contained in patches that were glued on either flank of the calves. Results showed that vaccination with Bm86 alone reduced the number of fully engorged ticks by 79%. When calves were also vaccinated with Subolesin protection was >95% against both tick species, thus transcending the strain and species level. Newly developed in vitro assays using *R. microplus* larvae that were fed serum of vaccinated calves confirmed the in vivo results.

Can infection with one genotype of *C. parvum* protect against infection with another?

S20

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Currently there is little knowledge about the development of immunity to Cryptosporidium; in humans it is possible to become infected with Cryptosporidium early in life and again in adulthood. In livestock however, diarrhoea caused by the parasite is generally only seen in neonatal livestock and not older animals. It is not known if infection with one genotype of *C. parvum* will provide protection against infection with another genotype. An experiment challenge was carried out to test the development of resistance to homologous and heterologous Cryptosporidium infections in lambs using two distinct *C. parvum* isolates. Thirty-six neonatal lambs were split into six groups: two to test age-related susceptibility, two homologous challenge groups and two heterologous challenge groups. The lambs were kept for 8 weeks until oocyst shedding stopped; total faecal output was collected from each lamb along with weekly blood samples. Clinical data (feed intake, demeanour and faecal consistency) was recorded daily. Oocyst counts are used to determine the shedding profile of each group along with PCR to confirm the genotype of the parasite being shed. Results show that lambs infected for the first time later in life exhibit few, if any, clinical signs of disease, although these lambs can still shed large numbers of oocysts. We also found evidence that some isolates can cause slightly more severe and prolonged diarrhoea than others.

Prevention and Control

Keynote

Variability in *Neospora caninum* and its relevance to cattle infection — K08

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Neospora caninum is an apicomplexan cyst-forming protozoan parasite phylogenetically closely related to *Toxoplasma gondii*. *N. caninum* infection in cattle may lead to abortion, stillborn or congenitally infected calves. It is unclear which host and parasite dependant factors play a major role in the outcome of the infection. *N. caninum* shares with *T. gondii* a world-wide distribution, ability to infect different intermediate hosts and capacity for genetic recombination in a definitive host that may give rise to genetic and biological diversity. So far, the molecular basis of the disease remains to be elucidated and studies on parasite variability and population structure are limited. Predominance of vertical transmission in cattle suggests the clonal propagation of this parasite and initial studies based on microsatellite sequences have shown evidence the geographic sub-structuring and predominant clonal propagation in some areas. In addition, studies on host-parasite interactions in in vitro culture systems revealed marked differences in lytic cycle kinetics, mechanisms to breach biological barriers for tachyzoite dissemination and stage conversion that can explain variability in vivo. Such variability has already been confirmed in mouse and bovine experimental models showing marked differences in pathogenicity, infection dynamics, tissue parasite distribution and induction of immune response. New approaches based on high-throughput sequencing methods and genetic manipulation tools together with well-defined ruminant models will be the key in unravelling the molecular basis of variability and its effect on cattle infection.

Development and application of a novel quantitative reverse transcriptase PCR (qRT-PCR) assay to identify drugs targeting intracellular *Theileria annulata* schizonts

S21

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Intracellular schizonts of the apicomplexan *Theileria annulata* immortalize bovine leukocytes, thereby causing fatal leukoproliferative disease in cattle. Buparvaquone (BPQ) is the only drug available against Theileria. BPQ is not licensed in in Europe, and emerging drug resistance underlies the need for identifying alternative compounds. Current Theileria drug assays monitor the proliferation of infected cells as host cell replication is tightly linked to the presence of a viable parasite, but they do not distinguish whether compounds act primarily against parasites or host cell targets. We here report on the development and validation of a novel Theileria-qRT-PCR assay for the rapid and reliable monitoring of parasite (non-) viability. In order to identify novel anti-theilerial compounds, we screened the open access MMV malaria box, which contains 400 compounds active against the blood-stage of *Plasmodium falciparum*. Primary screening was done on *T. annulata* infected bovine macrophages by alamar blue assay. The compounds with an IC50 comparable to BPQ were then further analyzed by Theileria-qRT-PCR assay in order to identify those that act specifically against Theileria. 5 compounds affected specifically the relative expression of TaSP (schizont surface protein) and these will be further analyzed in detail in terms of host cell cytotoxicity, minimum effective concentration and effects on parasite and host cell structural integrity.

Neonatal porcine coccidiosis – do we understand it yet? S22

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Cystoisospora suis is one of the most important causes of diarrhoea in suckling piglets worldwide. The parasite shares many features with other intestinal coccidia of livestock; however, there is a pronounced age resistance that is intertwined with the development of immunity on the one hand and the establishment of the microbiome in piglets on the other. Although *C. suis* is a primary pathogen, very little is known about the role of immunity in parasite control. Current research indicates that, unlike other coccidia, *C. suis* may be controlled primarily by maternal factors, possibly antibody-mediated protection through colostral antibodies. The clinical outcome of infection is highly variable, ranging from oocyst excretion without clinical signs to severe and sometimes fatal outbreaks of infection in the presence of other pathogens. This constitutes a challenge for both diagnosis and treatment. Current control schemes include hygiene and metaphylactic application of triazinones to suppress parasite development. However, consumer health and drug resistance concerns are increasing in livestock industries, and we are evaluating alternative control measures. This includes exploitation of genetic information about the genome and transcriptome of *C. suis* to aid the search for targets for chemical and non-chemical intervention, together with advanced in vitro cultivation and screening methods. Finally, we are investigating its interactions with the immune system and with the microbiome that might influence gut health and clinical outcome of infection in order to understand the mutual effects and their role in porcine coccidiosis.

Sarcocystis neurona calcium-dependent protein kinase 1 is targeted in selective therapeutic development for Equine protozoal myeloencephalitis

S23

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We have shown that inhibition of TgCDPK1 and subsequent interference with *T. gondii* mammalian host cell invasion by Bumped kinase inhibitors (BKIs) was due to the atypical glycine “gatekeeper” residue in the ATP-binding site of the kinase. We recently identified and sequenced an equivalent CDPK homologue (SnCDPK1) in S. neurona genome that has similar glycine “gatekeeper” residue. SnCDPK1 and TgCDPK1 have >85% amino acid identity. Here, we characterize BKIs for in-vitro efficacy against SnCDPK1 and *S. neurona* merozoites. Recombinant SnCDPK1 was expressed, purified and screened against a selected group of BKIs previously shown to have low IC50s against TgCDPK1 and *T. gondii* tachyzoites. Growth assays with a yellow fluorescent protein-expressing clone of S. neurona demonstrated that parasite growth was inhibited by BKIs at nanomolar concentrations. BKI-CDPK1 binding confirmation was performed using *S. neurona* lysates in thermal shift assays using CDPK1-specific antibody. SnCDPK1 was inhibited by low nM concentrations of BKIs. Analysis of Sarcocystis cell-inhibition data suggests that BKI interfered with an early step in S. neurona host cell invasion and egress processes. This study presents detailed molecular and phenotypic evidences that SnCDPK1 could be targeted for rational drug development as TgCDPK1 was previously validated for *T. gondii*. BKIs used in these assays have been chemically optimized with functional groups needed to improve potency, selectivity and pharmacokinetic properties. These compounds are good candidates for further investigation of their pharmacological properties and efficacy in a murine model of EPM.

Effect of artemisinin and *Artemisia annua* on *E. tenella* infection in broiler chickens

S24

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The anticoccidial effects of artemisinin and *A. annua* were assessed by experimental infection of 14 day-old chickens with $1x10^4$ *E. tenella* oocysts in a battery trial. Two independent experiments (A, B) were designed, with 3 control groups each: negative control, positive control and treated control (monensin). Experiment A contained 3 experimental groups (infected and treated with 5, 50 and 500 ppm artemisinin) and experiment B - 4 experimental groups (infected and treated with *A. annua* German [5 and 50 ppm artemisinin] and Romanian [5 and 21,3 ppm artemisinin] cultivars). The experimental feed was administered two days prior infection. The efficacy of artemisinin and *A. annua* against *E. tenella* infection was quantified by body weight gain, feed conversion ratio, oocysts shedded per gram of feces (OPG), lesion score and oocysts sporulation rate. Neither artemisinin nor *A. annua* had an effect on reducing the OPG, but artemisinin, in concentrations of 5 and 50 ppm, reduced the coecal lesions and the number of sporulated oocysts. Although *A. annua* had no anticoccidial effect, it improved the weight gain of the chickens, which was even greater than the control groups. Further studies need to be done in order to correlate the positive effect of the plant in broiler growth and the possible anticoccidial effect of artemisinin against *E. tenella*.

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Host Parasite Interactions 2

Keynote

Disease management: more than a scientific challenge — K09

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There is normally a complex interplay between several partners and disciplines in order to successfully handle diseases and disease outbreaks. Typically there is a tension between timely risk assessment and sound scientific advice on one side and the need to take immediate action on the other side. Add to that a paradox of uncertainties, deadlines and need to communicate with policymakers as well as the general public and you will find some very uncomfortable scientists along the way. So what can we learn? During my talk I will highlight some of the particular challenges that science face when the going gets tough. As an example I will discuss the problems to control coccidiosis that are currently facing the pan-European poultry industry.

Foetal periventricular leucomalacia as the main lesion in abortions during the acute phase of ovine toxoplasmosis

S25

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Toxoplasmosis is a major opportunistic disease of immunocompromised patients. It also represents a serious threat during pregnancy, causing severe foetal abnormalities or potentially leads to problems in childhood or later adult life. Undercooked or raw meat containing infective tissue cysts are a significant source of human infection. The production of *T. gondii* tissue cyst free meat could reduce the risk of human exposure to *T. gondii*. In two different animal studies a group of 23 pigs and 32 lambs were used to determine the efficacy of a commercially available vaccine, with an aim to reduce tissue cyst formation. Results from mouse bioassays, using a variety of porcine tissues, resulted in a 100% survival of mice that received tissues from vaccinated/challenged pigs. While bioassays of tissues from non-vaccinated pigs resulted in a survival rate of 51%. Parasite DNA was also identified in the homogenate used in bioassays from the non-vaccinated/challenged group but not in the vaccinated/challenged pigs. In a similar experiment, *T. gondii* DNA was tested for in the tissues of lambs. Following vaccination and challenge with 100,000 oocysts of the Moredun M4 strain, the parasite was detected at significantly lower levels in heart and skeletal muscle samples from the vaccinated/challenged group (0% and 5.9% respectively), when compared to the non-vaccinated/challenged animals (75% heart, 87.9% skeletal muscle). The results demonstrate that vaccination of pigs and lambs with the S48 attenuated *T. gondii* strain can reduce the formation of tissue cysts, resulting in potentially safer meat for human consumption.

Overexpression of TBCB protein affects *Toxoplasma gondii* host cell invasion

S26

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During the mammalian host cells invasion *Besnoitia besnoiti* and *Toxoplasma gondii* undergo dramatic modifications of the parasite microtubule cytoskeleton. Proteins involved in the regulation of microtubule dynamics are good candidates to control this microtubule remodeling. Tubulin cofactor B (TBCB) is a member of tubulin folding pathway but also controls microtubule dynamics through the recycling/degradation of the native tubulin heterodimers. *T. gondii* has a TBCB gene and to characterize its function we produced 3 overexpression transgenic lines: TgTBCB in fusion with c-myc tag; TgTBCB in fusion with GFP and TgTBCB in fusion with a destabilization domain, in which we are able to modulate the levels of expression in a Shdl1-dependent manner. The plaque assay showed that these clones have a decreased capacity in forming plaques, which is related to a decrease in the host invasion rate, as shown by invasion assays. Using a polyclonal antibody against TgTBCB produced in house, we observed that TgTBCB has a polarized localization being detected at the anterior region of the cell, immediately under the conoid. Striking, this staining pattern resembles that of the rhoptries suggesting a close association of TBCB with those organelles. Our data support that TBCB is involved in the invasion process of *T. gondii*. Experiments are in progress to characterize the TBCB loss of function by constructing a TBCB conditional knockout in *T. gondii*. How parasites gain entry into host cells is an important step and a potential target to design improved therapies.

The expression of the non-secreted rhoptry protein ROP40 from *Neospora caninum* is up-regulated during tachyzoite egress and invasion

S27

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Rhoptry proteins from ROP2-family have been extensively studied in *Toxoplasma gondii* due to their role in parasite virulence, but scarcely in *Neospora caninum*, where only NcROP2Fam-1 has been characterized. NcROP40 is a member of the ROP2-family and was found to be more abundant in virulent isolates. Both NcROP2Fam-1 and NcROP40 showed a synergistic effect against vertical transmission in vaccine trials performed in pregnant mice, which suggests that they may be relevant for parasite pathogenicity. Preliminary studies carried out by TEM and confocal microscopy demonstrated the presence of NcROP40 in the rhoptry bulbs of tachyzoites, its apparent lack of secretion during or following host cell invasion and the absence of association with the parasitophorous vacuole membrane, contrary to what has been shown for *T. gondii* rhoptry proteins and NcROP2Fam-1. Herein, in silico analyses showed that NcROP40 is a pseudokinase that exhibits degenerated RAH domains. Similarly to NcROP2Fam-1, the expression of NcROP40 transcripts is highly up-regulated during tachyzoite egress and invasion, despite it does not appear to be necessary to trigger DTT-induced egress. In addition, contrary to NcROP2Fam-1, NcROP40 phosphorylation was not associated with egress. The fact that NcROP40 and NcROP2Fam-1 exhibit different characteristics indicates that they carry out different functions. These findings highlight the need to elucidate the role of NcROP40 within the lytic cycle and to explain its relative abundance in tachyzoites. Financed by the AGL2010-22191/GAN project. IPF was supported by the AP2009-0354 fellowship.

Atypical forms observed among microgametes of two species of avian *Eimeria*

S28

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The life cycle of the protozoa belonging to the genus *Eimeria* can be divided in three major stages: schizogony, gamogony and sporogony. During gamogony, which is the reproduction stage, microgamonts mature to produce microgametes with two or three flagella. After escaping from the microgamont, these microgametes move actively to fertilize the female stage. We performed controlled infections of chickens with E. acervulina and turkeys with *E. meleagrimitis* in order to study in details the maturation of the microgamonts, aiming to harvest gamogonic stages for further studies. During microscopic examination, we observed what we called eel-like organisms, with a size similar to that of microgametes, but these organisms were moving undulating. During observation of a disrupting mature microgamont, we saw the biflagellated microgametes leaving rapidly, then the « eels » remained attached to the central core of the gamont, and finally some of them detached and moved away. We also observed some « eels » in the mucosal scrapings at several occasions. These forms are definitely produced in the microgamont, and they might be alternative forms of microgametes that can be favored in liquid contents compared to the microgametes with two flagella that could possibly move more easily in semi-solid media. Further studies are needed to elucidate whether eel-like forms are fully functional or if they participate in another way of fertilization. These forms observed with *E. acervulina* and *E. melagrimitis* may occur for other Apicomplexa protozoa. These observations suggest that much is unknown about the reproductive stage among *Eimeria* species.

Food and Waterborne Zoonoses 1

Keynote

Cryptosporidium: should we think more about food? K10

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Cryptosporidium is traditionally considered a waterborne parasite, yet the attributes that enable waterborne transmission can also facilitate transmission via food, so why are foodborne outbreaks of cryptosporidiosis rarely detected and reported? Two consecutive reviews found that globally, up to the year 2010, there were a total of 285 reports of waterborne outbreaks of cryptosporidiosis (Karanis et al., 2007; Baldursson and Karanis, 2011), which contrasts with just 19 foodborne outbreaks found by other researchers (Robertson and Chalmers, 2013). There are multiple possible explanations for this difference: maybe foodborne outbreaks occur but are not detected or reported; perhaps outbreaks are detected but links to food are not made, or it could be that foodborne transmission genuinely occurs only very rarely.
It is possible that failures in drinking water sources, treatment and supply increase the likelihood of drinking water transmission compared with food. However, it seems unlikely that these present a greater risk than those in the food supply chain, since contamination may occur during food production, processing, or preparation and *Cryptosporidium* can survive may control measures.

In England and Wales a total of 66 drinking waterborne and three foodborne outbreaks of cryptosporidiosis have been reported since 1992. Most of the waterborne outbreaks were prior to the year 2000; regulatory changes and water company investments since then have reduced the number of mains water-related outbreaks to just five, one in 2002, two in 2005, one in 2008 and one in 2013. With the exception of the outbreak in 2008 which was caused by *C. cuniculus*, these more recent outbreaks were caused by *C. hominis* gp60 subtype IbA10G2 which is anthroponotic. Two of the foodborne outbreaks were linked to pasteurisation failures at on-farm dairies and the third outbreak was strongly associated with the consumption of pre-cut mixed salad leaves sold by a single retailer. *Cryptosporidium* genotyping was undertaken in two of the outbreaks, and identified zoonotic *C. parvum* gp60 subtypes IIaA15G1R1 (milk outbreak) and IIaA15G2R1 (salad outbreak). The contrasting risks in these outbreaks, and controls within the food and water industries, as well as the limitations in outbreak identification and investigation, will be discussed at the conference.

Detection of the protozoan parasites *Toxoplasma gondii* and *Cryptosporidium* in Scottish water

S29

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Waterborne transmission of the zoonotic protozoan parasites *Toxoplasma gondii* and *Cryptosporidium* is a water quality and public health threat worldwide. In a first study Using 1427 samples collected from 147 public water supplies throughout Scotland, *T. gondii* DNA was detected using 529bp real time PCR and was found to be present in 8.79% of samples from one third of the water plant locations. Interestingly, over 30% of the samples positive for *T. gondii* DNA came from one water supply with a history of *Cryptosporidium* contamination. A further study was then carried out to establish *Cryptosporidium* prevalence, species and genotypes present in livestock, deer and water samples from this catchment using a novel method of processing adult ruminant faecal samples followed by nested species specific multiplex PCR, targeting the 18S rRNA gene to detect and speciate *Cryptosporidium.* Results indicated a very high prevalence of *Cryptosporidium* with a predominance of *C. parvum* in livestock, deer and water samples*.* A multilocus fragment typing (MLFT) tool and GP60 sequencing was used to genotype *C. parvum* positive samples. Four GP60 subtypes were detected within *C. parvum* with the majority IIaA15G2R1 which was detected in all host species and on all farms. MLFT further differentiated these into 6 highly related multilocus genotypes. It is anticipated that project outputs, including knowledge exchange activities with farmers and land agents, alongside fencing off the water courses above the public water supply and provision of water troughs for livestock, will reduce the cycles of *Cryptosporidium* contamination in this catchment.

Is a terrestrial cat parasite really reaching marine mammals? S30

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Toxoplasma gondii is a zoonosis of worldwide distribution: Felids act as the final-host in the lifecycle of *T. gondii* and it is assumed that all warm-blooded animals, including humans, can act as intermediate hosts. Because of the absence of felids in the marine environment, the presence of *T. gondii* in marine mammals is surprising. Still, several studies have shown the presence of this parasite in marine animals. Although disease or pathology associated with toxoplasmosis is rarely observed in marine mammals, they can develop clinical symptoms, mostly due to immunosuppression. Different transmission routes (such as coastal run-off, mechanical vectors) and predisposing factors (such as *Polychlorbiphenyls, Morbillivirus*) have been hypothesized, but these are still insufficiently investigated. Since *T. gondii* is able to infect humans, research on the spread of *T. gondii* in the marine habitat is relevant for public health in areas where marine mammals are consumed. Furthermore, marine mammals, being at or near the top of the food chain, act as sentinels for the marine environment. This investigation can thus act as an indicator for the marine pollution originating from land-based sources.

Anatomical distribution of *Toxoplasma gondii* tissue cysts and correlation between direct and indirect detection methods in the main livestock species.*

S31

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Toxoplasma gondii is an important pathogen that causes a high disease burden in humans. To assess the role of meat, the European Food Safety Authority (EFSA) has requested information on the presence and infectivity of *T. gondii* cysts in edible tissues of the main meat-producing animals and its relationship with T. gondii seroprevalence. An extensive literature review was conducted to identify knowledge gaps and to guide sampling in the experimental phase. After searching MEDLINE, BIOSIS and EMBASE using search terms to cover the subjects ‘Toxoplasma’, ‘animals’, ‘detection’ and ‘tissue’, and initial screening on relevance and eligibility, data was extracted from 270 records that reported direct detection of *T. gondii* in pigs, bovines, small ruminants, poultry or horses. The data from these publications was used to prepare an overview of the anatomical distribution of *T. gondii* tissue cysts, the performance of direct detection methods and the correlation between indirect and direct detection methods. Results and implications for further research will be discussed. *This research was conducted by a consortium within the framework of project n° GA/EFSA/BIOHAZ/2013/01 entitled “Relationship between seroprevalence in the main livestock species and presence of *Toxoplasma gondii* in meat”, grant agreement funded by the European Food Safety Authority (budget). This paper/publication is based on the results obtained in the framework of this mentioned project and it is published under the sole responsibility of the authors, and shall not be considered as an EFSA output.

Toxoplasma gondii in the meat of poultry following natural and experimental infections* S32

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Toxoplasma gondii infections are widespread in domestic poultry. The prevalence is highly variable and depends mainly on husbandry factors. Our study investigates the relationship of seropositivity and the presence of *T. gondii* in poultry tissues as a potential infection risk for consumers. Experimental *T. gondii* infections were performed in broiler chickens and turkeys and the serological status of the birds at slaughter in MAT, ELISA and IFAT was linked with the detection of *T. gondii* DNA in heart and muscle by magnetic capture (MC-) PCR. In addition, laying hens from organic farms and backyard husbandries were sampled in order to correlate seropositivity in MAT, ELISA and IFAT with a direct parasite detection by MC-PCR or mouse bioassay in the hearts and muscles of the animals. In experimentally infected animals, *T. gondii* DNA was detected regularly and in laying hens seropositivity was related to the isolation of *T. gondii* tissue cysts after positive mouse bioassay. *This research was conducted by a consortium within the framework of project n° GA/EFSA/BIOHAZ/2013/01 entitled “Relationship between seroprevalence in the main livestock species and presence of *Toxoplasma gondii* in meat”, grant agreement funded by the European Food Safety Authority (budget). This paper/publication is based on the results obtained in the framework of this mentioned project and it is published under the sole responsibility of the authors, and shall not be considered as an EFSA output. A part of this work was also funded by the German Ministry of Education and Research (BMBF, grant n° 01KI1002C).

Food and Waterborne Zoonoses 2

Keynote

Toxoplasma gondii in European slaughtered calves and cattle – serology, mouse bioassay and magnetic capture qPCR

K11

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The role of beef in human infections with *T. gondii* is unclear as high seroprevalences have been reported for cattle, but direct detection of *T. gondii* by bioassay and PCR is limited. In addition, there is an indication that detection by PCR is more common in seronegative than seropositive cattle. To study the relationship between mouse bioassay, real time PCR (qPCR), magnetic capture quantitative PCR (MC-PCR) and serology (modified agglutination test); serum, a tissue sample from a predilection tissue, and a sample representative for edible tissue was collected from 100 cattle slaughtered in Italy, The Netherlands, Romania and the United Kingdom. Based on the results from an extensive literature review, liver was considered the predilection site and diaphragm was collected as a representative of edible tissue. Liver (200g) of all 400 cattle is tested by mouse bioassay, regardless of the serological result. The diaphragms (100g) of cattle positive in mouse bioassay or PCR on the digest, and 100 cattle negative in mouse bioassay are tested by MC-PCR. The results of this multi-national slaughterhouse study will be presented. *This research was conducted by a consortium within the framework of project n° GA/EFSA/BIOHAZ/2013/01 entitled “Relationship between seroprevalence in the main livestock species and presence of Toxoplasma gondii in meat”, grant agreement funded by the European Food Safety Authority (budget). This paper/publication is based on the results obtained in the framework of this mentioned project and it is published under the sole responsibility of the authors, and shall not be considered as an EFSA output.

Vaccination of pigs and lambs against *Toxoplasma gondii* reduces tissue cyst formation; safer meat for human consumption

S33

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Toxoplasmosis is a major opportunistic disease of immunocompromised patients. It also represents a serious threat during pregnancy, causing severe foetal abnormalities or potentially leads to problems in childhood or later adult life. Undercooked or raw meat containing infective tissue cysts are a significant source of human infection. The production of *T. gondii* tissue cyst free meat could reduce the risk of human exposure to *T. gondii*. In two different animal studies a group of 23 pigs and 32 lambs were used to determine the efficacy of a commercially available vaccine, with an aim to reduce tissue cyst formation. Results from mouse bioassays, using a variety of porcine tissues, resulted in a 100% survival of mice that received tissues from vaccinated/challenged pigs. While bioassays of tissues from non-vaccinated pigs resulted in a survival rate of 51%. Parasite DNA was also identified in the homogenate used in bioassays from the non-vaccinated/challenged group but not in the vaccinated/challenged pigs. In a similar experiment, *T. gondii* DNA was tested for in the tissues of lambs. Following vaccination and challenge with 100,000 oocysts of the Moredun M4 strain, the parasite was detected at significantly lower levels in heart and skeletal muscle samples from the vaccinated/challenged group (0% and 5.9% respectively), when compared to the non-vaccinated/challenged animals (75% heart, 87.9% skeletal muscle). The results demonstrate that vaccination of pigs and lambs with the S48 attenuated *T. gondii* strain can reduce the formation of tissue cysts, resulting in potentially safer meat for human consumption.

Toxoplasma gondii infection in pigs: correlation between direct and indirect detection methods and on farm risk factors

S34

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Pork has historically been considered an important source of *T.gondii* infection in humans. Although some putative risk factors for pigs to get infected have been identified, the need of more accurate data in order to propose potential control strategies to mitigate the risk of foodborne exposure to *T.gondii* has been highlighted by EFSA. A nationwide study was conducted to evaluate the prevalence of *T.gondii* in pork meat, in France during 2013, investigating pigs from two different housing systems: indoor and outdoor, and three different ages: piglets, finishing pigs and sows. Tissue fluid from hearts and diaphragms, and sera were tested serologically (Modified Agglutination Test). Direct detection of parasites was performed by mouse bio-assay following the artificial digestion of 200g of heart from the serologically positive samples and 50 negative ones. Based on the correlation between sero-prevalence and presence of tissue cysts in the predilection site (heart) and election site (diaphragm) obtained, a cross-sectional study was performed in the UK. Serological samples and a standardised questionnaire were used in order to identify farm characteristics that may increase risk of infection with *T.gondii*. The results from both studies will be presented.
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A parasite, a bacterium and a chicken walk into a lab, what does the immune system say? S35

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Eimeria causes the intestinal disease coccidiosis, most notably in poultry. While the direct impact of coccidiosis on animal health and welfare is clear, its influence on the enteric microbiota and knock-on effects on chicken health and production remains largely unknown. *Campylobacter* is the most common cause of bacterial foodborne illness in humans in the developed world; with raw or undercooked poultry meat identified as one of the major sources of infection. Concerns relating to zoonotic potential, as well as impact on economic food production and animal welfare, are now elevating interest in *Campylobacter* in the chicken. Nonetheless, the influence of the enteric microbiota on *Campylobacter* colonisation within the avian intestine and deeper tissue remains a neglected area of research. Quantification of early Campylobacter jejuni colonisation of the chicken caeca, liver and spleen has revealed significant variation in the presence or absence of concurrent *Eimeria tenella* infection. Intriguingly, parasite co-infection associates with an elevated *C. jejuni* load within the caecal lumen three and ten days post bacterial challenge, but reduced colonisation of the liver and spleen. Thus, while faecal shedding of C. jejuni is at least temporarily increased by concomitant *E. tenella* infection, deep tissue bacterial contamination is decreased. Underlying mechanisms including the role of the innate immune system are being investigated along with analysis of the enteric microbiome in commercial poultry in order to further understand the effect that *Eimeria* has on bacterial colonisation of the chicken gut and poultry health.

Cryptosporidium and *Giardia* in Danish, organic pig farms: seasonal and age-related variation in prevalence, infection intensity and species/genotypes

S36

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During quarterly visits in 3 organic, Danish farms, seasonal and age-related variations in *Cryptosporidium* and *Giardia* prevalence and infection intensity were assessed from 152 piglets, 234 starter pigs, 230 fatteners and 240 sows. Zoonotic potential and correlation between species/genotypes and infection intensity were quantified from representative subsamples by amplification and partial sequencing of SSU 18S rRNA, *hsp70*, and *gdh* genes. *Cryptosporidium* or *Giardia* was found in 40.9% and 14.0% of the pigs, respectively, of which 8.2% were dual-infected. Prevalence and infection intensity were stable through the year except for dual-infections which were more common in September and December. Significantly higher prevalences of both parasites were found in starter pigs compared to other age-groups. Intensity of *Cryptosporidium* infection was highest in piglets, while *Giardia* intensity was highest in starter pigs. Infection intensity tended to be lower in dual-compared to mono-infected pigs. All infections were subclinical without correlation between faecal consistency and (oo-)cyst excretion. Of the genotyped isolates, 69.0% were *C. scrofarum* and 29.3% were *C. suis* while *G. duodenalis* Assemblage E was detected in 84.6% and the potentially zoonotic Assemblage A was identified in 15.4%. Piglets predominantly hosted *C. suis,* while starter pigs and fatteners predominantly hosted *C. scrofarum.* As organic pigs are reared outdoors, environmental contamination with *Cryptosporidium* and *Giardia* is inevitable. Yet, the results indicate that potential public health risk associated with these parasites in Danish organic pigs seems negligible.

Posters

Population genetic analysis of *Theileria annulata* in Oman

P01

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Theileriosis is a widespread protozoan tick-borne disease of ruminants in Oman, causing high morbidity and mortality. However, little is known about the population genetic structure of the main bovine-infecting species present in the country, namely *Theileria annulata*. In the present study, we examined cattle in four regions of Oman in order to investigate some basic features of the *T. annulata* population and to determine whether parasites in the different regions comprise a single inter-breeding population or if they are geographically sub-structured. We examined 310 *T. annulata* isolates collected from cattle across four regions, where theileriosis is evident, with farm location and host breed type recorded in each case. PCR-RFLP of the 18S rRNA gene was used for the identification of *Theileria* species. Ten genetic markers (micro- and mini-satellites) were developed and used to generate a multi-locus genotype for each isolate in order to provide a dataset for population genetic analysis of *T. annulata* in Oman. Allelic diversity for each marker was computed and the level of inter-subpopulation differentiation was determined. The multi-locus genotyping data was then compared to a dataset representing *T. annulata* genotypes isolated from Tunisian and Turkish cattle. The predominant multi-locus genotype for each isolate was determined and allelic diversity, as measured by estimated heterozygosity (He), was found to be high all regions, i.e. Al Sharqia (He = 0.83), Al Batinah (He = 0.85), Al Dhira (He = 0.82) and Dofar (He = 0.83). An array of alleles was identified for every marker in each population and 'private' alleles, i

Experimental infection of pregnant cattle with *Neospora caninum* Nc-Spain 7 strain at 110 days of gestation

P02

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Neospora caninum infection is considered one of the main causes of abortion in cattle worldwide. Experimental infection of cows early in gestation, i.e. 70–90 days of gestation, usually results in foetal death whereas experimental infections in naïve cattle in the third trimester are related to the birth of full-term congenitally-infected calves. On the other hand, foetopathy can occur in experimental infections at 110 days of gestation if the experimental period is at least 6 weeks post-infection (wpi). In the present study we confirm that infection in dams on gestation day 110 with 107 tachyzoites of the high pathogenic strain Nc-Spain7 causes abortion in some animals but not in others. Three of 6 infected dams suffered foetopathy. Two dams had aborted fetuses at 2-3 weeks after infection and one dam had a foetus with signs of mummification at euthanasia. All the experimental animals were febrile at 3 and 4 dpi. *N. caninum* total specific antibodies reached seropositive threshold in all the infected animals by 3-4 wpi. Aborted animals showed higher mean antibody levels compared to non-aborted animals but high individual variations were observed. Some of the infected foetuses showed specific antibodies at euthanasia and *N. caninum* DNA was detected in all the infected foetuses and in the placenta. Although dams could abort at later times after infection, these results indicate that this model of experimental neosporosis allows the comparison of parasitological and immunological data in aborted and non-aborted dams in the same experiment, which could help in clarifying the exact causes of *N. caninum*-associated abortions.

Dose titration of the *Neospora caninum* virulent Nc-Spain7 isolate in a pregnant mouse model

P03

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Despite the evident physiological and immunological differences between ruminants and mice, pregnant mice represent a suitable model for investigation on the *Neospora caninum* infection biology and drug/vaccine proof-of-concept studies. Inbred mice are commonly challenged with 2-5x10^6 tachyzoites (tchz) of a virulent isolate in order to detect substantial clinical signs of disease. However, different groups have been conducting experiments using different mouse strains, various Neospora isolates and dissimilar inoculation routes, rendering a comparison of results a difficult task. In addition, for experimental infections in cows and sheep, similar doses (10^7 and 10^6 tchz, respectively) are commonly used. Thus, the currently employed inoculation in mice could be inappropriate, and model refinement and standardization is advisable. Herein, a dose titration experiment in the pregnant BALB/c model was performed, employing the virulent and well-characterised Nc-Spain7 isolate in an attempt to set up a standardized model. Mice were subcutaneously inoculated with 2x10^6, 10^5, 10^4, 10^3 and 10^2 tchz, and clinical outcome, vertical transmission, parasite burden and antibody levels were compared. Dams from all infected groups displayed nervous signs and the percentage of surviving pups at day 30 *post-partum* was low (24%), even with the lowest dose. Importantly, infection with105 tchz resulted in similar antibody levels, parasite burden and mortality in pups (100%) as infection with 2x106 tchz. Hence, for the assessment of vaccines and drugs against *N. caninum*, we propose a standardized BALB/c model infected with 10^5 Nc-Spain7 tchz.

Systemic and local immune responses in ewes after *Neospora caninum* experimental infection in the three periods of gestation

OP01

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Recent studies have shown that *N. caninum* poses a high risk as abortifacient for small ruminants. In a previous work, pregnant ewes were infected with 106 Nc-Spain7 tachyzoites in the 3 terms of gestation. Results showed that, as in cattle, the outcome of infection relies heavily on the time of gestation when infection occurs. In this study, local and systemic immune responses from the same sheep were assessed. Serology revealed that early (G1) and mid (G2) gestation ewes elicited an earlier and stronger IgG and IFN-γ response compared to ewes infected at late gestation (G3), whereas all groups showed no variations regarding IL-4 serum levels throughout the study. While parasite antigen, as free tachyzoites in the foetal mesenchyme, was found almost only in G1, placental lesions were more severe and diffuse in G2 and G3. These lesions appeared as multifocal necrotic foci surrounded by inflammatory cells, mainly T-lymphocytes, but also macrophages. Cytokines and TLR mRNA expression levels in placentomes exhibited a similar pattern in all groups: IFN-γ and IL-4 showed the highest increases, whereas modest increases were observed for TNF-α and IL-10. No differences to the uninfected group were detected regarding IL-12, TLR-2 or TLR-4. Moreover, IFN-γ, IL-4 and IL-10 expression appeared to be higher in G1, likely associated with the high parasite presence. These results confirmed that immune response to *N. caninum* varies along the gestation and is related to the outcome of infection. This study was supported by the SNF (310030_146162) and CYTED (113rt0469).

Genetic characterization of *Toxoplasma gondii* strains isolated from stray cats in Romania

OP02

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T. gondii oocysts were detected coproscopically and by PCR only in 2 fecal samples. These samples were confirmed by bioassay in mice. Antibodies against *T. gondii* were detected by modified agglutination test (MAT titer ≥ 1:6) in 13 (52%) cats. Heart samples of 24 cats were bioassayed individually in mice and 4 *T. gondii* isolates were obtained. In addition, 2 heart digests were positive for *T. gondii* DNA by PCR. *T. gondii* isolates (n=8) were subsequently genotyped by PCR-RFLP using 8 markers (SAG1, 5'- and 3'-SAG2, altSAG2, SAG3, BTUB, L358, c22-8). Seven *T. gondii* isolates were genotype II and one isolate was genotype III. The study is first report of *T. gondii* genotypes in cats from Romania.

Cloning, expression and characterization of an actin binding protein (ABP) from *Neospora caninum*: cyclase associated protein (NcCAP)

OP03

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The filamentous form of actin has a central role in the generation of the invasion and gliding motility at the motor complex of Apicomplexan parasites. However, most of the total actin in the cell is maintained in monomeric form, suggesting that actin turnover is tightly regulated. This regulation is made by actin binding proteins (ABP) that have domains capable of binding in the actin molecule. Thus, in this study we aimed at cloning, expressing and characterizing an ABP – Cyclase associated protein or NcCAP – from the Apicomplexan protozoan *Neospora caninum*, a parasite associated with abortion in cows. Multiple alignment of NcCAP was made through Clustal W method with orthologues; conserved domains were analyzed in Pfam. Subsequently, the ORF was cloned in pET32a( ) and expressed in E. coli BL21. The recombinant protein was loaded on a Ni-resin column and the purification was analyzed on SDS-PAGE. After that, mice were immunized with recombinant form and the pooled sera were tested by western blot and immunofluorescence. CAP-C domain was present in NcCAP and this protein had 81% identity with *Toxoplasma gondii* CAP. Besides, NcCAP was expressed at 18°C for 18 hours with ~50 kDa in SDS-PAGE. The 1:15000 serum dilution of anti-NcCAP revealed two endogen bands of ~30 and ~35 kDa in western blot (24,6 kDa predicted). In immunofluorescence, NcCAP was localized in two forms: with a diffuse pattern or in the periphery of the cytoplasm in 1:5000 serum dilution. This study initiated the characterization of NcCAP towards the elucidation of its functional role in *Neospora caninum*.

Identifying pig herds at risk for *Toxoplasma gondii*: prevalence and test characteristics

P04

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Toxoplasma gondii has repeatedly been named as one of the most important zoonotic infections in Europe, in terms of its impact on human health. To control *T. gondii* in the pork supply chain, Vion, a pork slaughter company in the Netherlands and Germany, initiated an active serological monitoring on pigs entering their slaughterhouses, using the PrioCHECK Toxoplasma Ab porcine ELISA. To estimate the prevalence of *T. gondii* infections in pigs and to identify herds at risk, the collected data were evaluated. To define herds at risk, estimating the prevalence of *T. gondii* infections in pigs was the first objective of the monitoring. To evaluate the data, an analysis of appropriate cut offs of the ELISA test for active field monitoring was also required. We found that for the studied population a very high test sensitivity can be obtained with a cut-off value around 10 percent positivity (PP). A high specificity can be obtained with a cut-off value around 20 PP. Using a cut-off value of 20 PP, we fond that 2% of the pigs at slaughter are infected with *T. gondii*. The seroprevalence on organic farms (with outdoors areas) was twice as high. Furthermore we fond a clear seasonality in the data, with a higher *T. gondii* risk for pigs that go to slaughter in the first quarter of the year, as compared to the third quarter. The results of this serological monitoring will be used to develop and implement intervention measures in the pork chain.

Occurrence of *Eimeria* sp. in faeces of calves from dairy farms in northern Paraná, Brazil

P05

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Coccidiosis is a disease of great importance in cattle herds around the world. This disorder is caused by protozoa of the genus Eimeria and primarily affects calves and steers, and *E. bovis* and *E. zuernii* are the species of greatest clinical importance. The aim of this study was to evaluate the occurrence of Eimeria in faeces of calves from dairy farms in Northern Paraná, as well as to identify the species found. 400 fecal samples from calves, aged between 20 and 210 days, of both sexes, from 44 different dairy farms were used. The Modified Gordon & Whitlock technique was used to evaluate the presence of Eimeria oocysts. Positive samples were subjected to morphometric analysis by light microscopy for identification of species of Eimeria. Of the 400 samples evaluated, 205 (51.25%) were positive, and 10 different species of Eimeria were identified. The frequencies of the species were: *E. bovis* (30.13%), *E. alabamensis* (26.84%), *E. zuernii* (22.03%), *E. ellipsoidalis* (18.48%), *E. aubernensis* (13.67%), *E. canadensis* (8.10 %), *E. cylindrica* (7.34%), *E. subspherica* (5.06%), *E. bukidnonensis* (3.04%) and *E. brasiliensis* (0.76%). All farms studied had at least one positive animal. Therefore, this study shows the high occurrence of Eimeria in the northern region of Paraná State, demonstrating the need for further studies to assess the health importance of this disease in those herds, since a high incidence of pathogenic species was detected, which is probably causing losses due to treatment costs and reduced production by the animals.

CCTα, a component of the tubulin folding pathway, in *Besnoitia besnoiti* and *Toxoplasma gondii* host cell invasion.

OP04

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B. besnoiti and *T. gondii*, the etiological agents of besnoitiosis and toxoplasmosis, respectively, are two apicomplexan parasites unable to replicate outside the host cell. The host cell cytoskeleton, but also the cytoskeleton of the parasite, participate in the process of host cell invasion. In fact, upon interaction with the host cell, B. besnoiti seems to re-organize the microtubule cytoskeleton: tachyzoites lose their crescent cell shape and acquire an irregular surface. This way, components of the tubulin folding pathway are good candidates to regulate cytoskeleton dynamics and reorganization during host cell invasion. Thus, we addressed the immunolocalization of the CCTα-subunit of the chaperonin containing TCP-1 in invading and non-invading *B. besnoiti* and *T. gondii* tachyzoites. Preliminary results show that in free tachyzoites CCTα is detected as globular like structures at the anterior pole; and in invading tachyzoites localizes at the region that seems to correspond to the moving junction, possibly playing a role during cell entrance. We also studied the gene expression of CCTα in *B. besnoiti* and *T. gondii* at different times post cell infection. The transcript levels of this gene decrease in the first minutes of host cell invasion and increase during parasite replication, a step in the cell cycle that requires microtubule reorganization. This way, a dynamic microtubule cytoskeleton in the parasite could be important in later events of the tachyzoite cell cycle, namely during replication inside the parasitophorous vacuole.

Toxoplasma gondii experimental infection in pregnant sheep at early, mid and late gestation. Pathological response and parasite distribution.

P06

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The relation between gestational age and foetal death risk in ovine toxoplasmosis is already known, but the mechanisms involved are yet not clear, especially in sheep. In order to study these mechanisms, pregnant sheep of the same age and genetic background were orally dosed with 50 oocysts of *T. gondii* (M4 isolate) at days 40 (G1), 90 (G2) and 120 (G3) of gestation. In each group, four animals were culled on the second, third and fourth week post infection (wpi) in order to evaluate parasite distribution and loads and lesions in target organs. There were no significant differences between groups in either rectal temperatures, with a peak temperature at days 6 and 7 pi, or serological maternal antibody kinetics. Parasite DNA and lesions were found in the placentomes and foetal viscera of sheep from G1 only at the fourth wpi. In G2, parasite was found from the third wpi, also in placenta and foetal viscera, increasing both at the fourth week, but lesions were only present at the fourth wpi. These lesions were the most severe found in whole experiment. In G3, parasite DNA but also placental and foetal lesions were found in the third wpi. Three out of four sheep from G3 aborted at this time. At the fourth wpi, parasite burden in G3 foetal viscera was lower than those on G1 and G2. These results suggest that the period of gestation influence the parasite multiplication and development of lesions in the placenta and foetus, and as a consequence the clinical course in ovine toxoplasmosis.

EuPathDB.org - An integrated database resource for protist pathogen Omics data sets

P07

Kathryn Crouch on behalf of Jessica C. Kissinger and the EuPathDB Team

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The Eukaryotic Pathogen database (EuPathDB: http://eupathdb.org) is a portal to a family of free online resources that serve scientists working on a variety of eukaryotic pathogens and related organisms from a number of different perspectives. Supported organisms include the Apicomplexa (*i.e. Plasmodium*, *Toxoplasma*, *Cryptosporidium*, *Babesia* and *Theileria*), kinetoplastida, Amoeba, fungi and others (i.e. *Giardia*, *Spironucleus* and *Trichomonas*). EuPathDB offers a graphical interface that enables users to combine search results from a wide variety of diverse data. Data types include genomic sequences and their annotations (close to 100 genomes represented), transcript level data (i.e. microarray and RNA sequence data), protein expression data (including quantitative), epigenomic data (ChIP-chip and Chip-seq), population-level (SNP) data and clinical & environmental isolate data, as well as host-response data (antibody array, host transcriptomic and proteomic data). In addition, genomic analyses provide users the ability to search for gene features, subcellular localization, motifs (InterPro and user defined), function (Enzyme commission annotation and GO terms) and evolutionary relationships based on gene orthology.

New additions to EuPathDB include:
-HostDB (http://hostdb.org), a database that integrates host functional genomic data generated in response to parasite infection.
-Metabolic pathways for all EuPathDB databases.
-Gene Ontology (GO) and pathway enrichment tools.

Stop by the EuPathDB poster or attend the workshop if you would like to learn more about this community resource.

Humoral Immune Responses in pigs immunized with recombinant proteins (rROP2) of the *Toxoplasma gondii* plus Iscomatrix

P08

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This study aimed to evaluate the humoral immune response in pigs immunized intranasally and intramusculary with rROP2 of *T. gondii* in combination with ISCOMATRIX. 12 mixed breed pigs were divided into three groups, G1 (vaccinated with rROP2, 200mg/dose + Iscomatrix, n=4), G2 (Iscomatrix, n=4), and G3 (unvaccinated, control group, n=3). The treatments were performed at days 0, 14, 28, 42, 56 e 72 intranasally and days 86, 93 e 100 intramusculary. On day 110 all animals were challenged with $4x10^4$ infective oocysts of the VEG strain. The animals were examined daily for clinical signs. The humoral immune response (IgG, IgM and IgA) was evaluated weekly by ELISA. The presence of *T. gondii* in blood and tissues (tongue, masseter muscle, heart and diaphragm) was assessed by bioassay in mice. The clinical signs observed in all animals were fever >40.0°C, anorexia and prostration. Animals from G1 produced antibodies above the cut-off point on the day of challenge (OD_{Mean} IgG = 0.893 ± 0.132, p <0.001; OD_{Mean} IgM = 0.778 ± 0.544, p <0.05; OD_{Mean} IgA = 0.895 ±, 0368, p <0.05). G2 and G3 remained with titles below the cut-off point before the challenge. High titers of antibodies were produced in the immunized animals and seroconversion was observed 9 DPI on G3. Partial protection against parasitaemia and tissue cysts formation was observed in G1 compared to G3. The protection factors were 40.0% and 6.1% in G1 and G2, respectively, compared to G3. In conclusion, there was total stimulation of humoral immune response with high production of antibodies IgG and IgA and it was possible to detect the parasite in muscle tissues.

IFN-γ production in *Neospora caninum* experimentally infected dams at 110 days of gestation and in their fetuses

OP05

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The production of IFN-γ has been related to protection against abortion in *Neospora caninum*-infected cows. However, excessive production of IFN-γ may be involved in the pathogenesis of fetal rejection during gestation. In the present study, IFN-γ concentrations were analyzed in control uninfected (n=4), naturally infected (n=3) and experimentally infected (n=6) pregnant dams and in their fetuses. Experimental animals were infected at 110 days of gestation and euthanized at 6 weeks post-infection. Peripheral blood mononuclear cells (PBMC) and lymphocytes from spleen and uterine lymph nodes of dams and spleen and thymus of the fetuses were isolated and cultured for 24 hours with medium (mock), ConA (10µg/mL) and *N. caninum* antigen (NC-1, 5µg/mL). After ConA stimulation, PBMC of infected dams produced significantly higher IFN-γ levels (11850±4260pg/mL) compared to naturally infected (3950±1183pg/mL) and to control uninfected (2471±1235pg/mL) dams, as well as after NC-1 stimulation (18885±7564) compared to naturally infected (2990±2860). In the fetuses, IFN-γ production was detected in the spleen of infected fetuses after ConA (2285±1300) or NC-1 (1255±772) stimulation but not in the uninfected ones. However, no IFN-γ release was seen in plasma and amniotic or allantoid fluids of infected fetuses that remained alive (n=3) but was very high in samples of the fetus found dead at euthanasia (1466pg/mL in plasma, 22886pg/mL in fetal fluids). These results suggest that exaggerated IFN-γ response, possible as part of the immune response trying to control the high parasitation, might backfire and be the cause of fetal death.

Optimization of an in vitro assay to certificate disinfectants against oocysts of coccidia

P09

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Coccidian parasites are a common threat in animal husbandry and human health. Preventive and accompanying control measures like hygiene and environmental disinfection play an important role in the combat of coccidia- associated diarrheal diseases in livestock. Disinfectants are currently tested mainly by *in vivo* tests assessing the infectivity of disinfected oocysts, e. g. of *Eimeria spp.* to chickens (like nationally recommended in Germany by the DVG, German Veterinary Medical Society).
Cryptosporidium parvum oocysts are suited to be used as model organisms to test disinfectants *in vitro*, because of their exemplary behaviour with regard to their resistance against many disinfection measures and environmental conditions. Furthermore they are able to infect cells *in vitro* and replicate within them. We aim to establish and standardize an efficacy assay based on *in vitro* disinfection of *C. parvum* oocysts and combining germ carrier use, cell culture infection and quantitative PCR in order to replace the *in vivo* tests. Parameters which have been optimized are e.g. the excystation medium and excystation time. The optimal concentration of sodium taurocholate hydrate was found to be 0,20 vol.% based on experiments combining the aspects of cytotoxicity and maximal infectivity. By using fluorescence-activated cell sorting we found out that excystation is completed after approximately 120 minutes. Furthermore results regarding the earliest expected time point for harvesting infected cells as well as the establishment of an internal positive control are presented.

Toxoplasma gondii infection in horses: correlation between serological status and parasitological findings in meat*

P10

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Horse meat and products are an important source of quality protein. An extensive literature survey showed that data on the presence and infectivity of *T. gondii* tissue cysts in horse tissues is very limited. Therefore, a study to assess the relationship between the serological status of Toxoplasma infection in horses and the presence of the parasite in horse meat has been initiated in France and Serbia. By the end of the study, a total of 100 horses per country will have been examined for Toxoplasma antibodies (by modified agglutination test), as well as for the presence of live parasites (by mouse bioassay) within the heart as the predilection site, and for parasite DNA (by MC-PCR) in the diaphragm as representative of edible tissue. The preliminary results show a seropositivity of 21.4% in Serbia. A number of discrepant results between serological status and detection of the parasite in the heart obtained so far seem to point to a low correlation between serological status and presence of tissue cysts in horse meat. An overall analysis of the findings obtained with direct and indirect methods in both countries will be presented. *The research was conducted by a consortium within the framework of project n° GA/EFSA/BIOHAZ/2013/01 entitled "Relationship between seroprevalence in the main livestock species and presence of *Toxoplasma gondii* in meat", grant agreement funded by the European Food Safety Authority (budget). This publication is based on the results obtained in the framework of this mentioned project and it is published under the sole responsibility of the authors, and shall not be considered as an EFSA output.

The semen of *Besnoitia besnoiti* infected bulls: poor quality and source of infection? P11

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Bovine besnoitiosis is an emergent disease in Europe. The clinical apparent form of *Besnoitia besnoiti* infection in bulls exhibits fever and nasal discharge in the acute phase, scleroderma, orchitis and infertility. But it was not clear if the semen quality remains to normal levels in sub-clinically infected bulls or returns to normal levels when infected animals had recovered clinically. In parallel, the role of semen in a putative sexual transmission of bovine besnoitiosis has not yet been investigated. Forty bulls coming from an area of emergence in southern French Alps, eleven seronegative, seventeen sub-clinically infected and twelve clinically infected (chronic phase) were included in the study. Pre-sperm and sperm fractions were collected by electroejaculation. No *B. besnoiti* DNA was detected in both fractions by quantitative real-time PCR whatever the category of bulls. The sperm concentration and motility were not significantly different between non infected and sub-clinically infected bulls. By contrast, in clinically infected animals, more genital tract alterations were observed (in particular testis calcifications and hyperkeratosis), and the sperm quality was deteriorated. In conclusion, the transmission of *B. besnoiti* by the semen seems to be unlikely in natural conditions and the impact of sub-clinically infection on reproductive male function appears to be limited.

Ocular toxoplasmosis: a combination of assays to improve diagnosis and the use of pigs as a biological model

P12

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Toxoplasma gondii is the most important agent of posterior uveitis in humans worldwide. In the present study we experimentally infected pigs with *T. gondii* tachyzoites, bradyzoites and oocysts in order to evaluate this species as a biological model for detection of human ocular toxoplasmosis. Herein, we used 18 pigs divided into four groups, G1 (infected with tissue cysts of M4 strain (type II) at day 28, n=5), G2 (infected with 1,000 oocysts of M4 strain at day 28, n=5), G3 (infected with tachyzoites of S48 strain at day 28, n=5), and finally G4 (uninfected unchallenged, control group n=3). At day 70 of the experiment all animals were culled, and serum and eye samples were collected to perform indirect ELISA, immunoblotting, and PCR (nPCR, and qPCR). All control pigs (G4) were serum negative throughout the experiment as well as ELISA and PCR from humors. The average of optical density (OD) from sera, in the cull day were OD_{G1}=1.21±0.38; OD_{G2}=1.44±0.27; and OD_{G3}=0.73±0.20. Nine pigs (60%) out of 15 were positive in vitreous humors (VH), and 7/15 (46%) were positive in aqueous humors (AH). Imunoblotting from humors showed a reaction against a major protein of approximately 29-30 kDa. Animals from G1 had more humors positive in both ELISA and PCR than the other groups. As a conclusion, we observed that pigs may be successfully used as an animal model for human ocular toxoplasmosis.

Characterization of an IgG monoclonal antibody targeted to sporocyst walls of *Toxoplasma gondii*

P13

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We report here the generation of monoclonal antibodies (mAb) using mice immunized with total extracts of hypochlorite-treated *T. gondii* sporulated oocysts. One particular IgG mAb, named K8/15-15, recognized antigens in sporocyst walls of the parasite. This mAb also reacted against sporocyst walls of the closely related parasites *Hammondia hammondi*, *H. heydorni*, and *Isospora felis*. Sporocyst walls of *Sarcocystis* sp. from dogs, and *Eimeria bovis*, were not stained by the mAb K8/15-15. The Toxoplasma antigens recognized by the mAb presented molecular weights of approximately 80 to 350 kDa using protein extracts from purified sporocysts. SDS PAGEs of different acrylamide/bis-acrylamid concentrations were employed (12.5%, 10%, 7.5%, and 5%) to better separate the high molecular weight bands recognized by the mAb. 5% SDS PAGE presented the best protein separation and resolution. mAb K8/15-15 seems to be a practical tool for the identification of sporocysts of *T. gondii* and related parasites. We are currently employing the mAb K8/15-15 to separate and identify protein components of *T. gondii* sporocysts by mass spectrometry analysis.

In vitro effects of different phytohormones on *Toxoplasma gondii* replication

P14

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Introduction: Movement and cell invasion of *Toxoplasma (T.) gondii* are related to calcium availability enhanced by the second messenger cyclic ADPR (cADPR) which activates the signal transduction and signalling pathways. Phytohormones are incriminated as modulators of *T. gondii* metabolism because of the plant-like character of the apicoplast and linked functional similarities.
Aim: To determine the effect of different phytohormones on the *in vitro* replication of *T. gondii*.
Material and methods: Tachyzoite cultures were grown in HFF (human foreskin fibroblast) cells. The influence of three different phytohormones (abscisic acid, ABA; kinetin, KIN; gibberellic acid, GIBA) was tested for different concentrations (500 pg/µl to 1.25 µg/µl, range based on the host cell tolerance as confirmed by MTT assay). $1x10^5$ tachyzoites were seeded per well and the respective treatment was performed for 2 hours. After 48 hours of monitoring, cells were detached and the number of *T. gondii* replicates was measured by quantitative PCR (based on the specific 529 bp fragment).
Results: Compared to untreated controls, ABA induced an increase of up to 65 % of the *T. gondii* replicate number. KIN treatment did not lead to statistically significant changes in the *T. gondii* replicate number. GIBA treatment reduced parasite replication by 98% in comparison to the control wells. The observed effects of ABA and GIBA on tachyzoites were concentration dependent.
Conclusion: Different phytohormones are able to modulate the replication rate of *T. gondii*. Further studies are pending.

Identification of *Neospora caninum* bradyzoite-expressed antigens: developing improved diagnostics for the identification of chronically infected carrier cattle

OP06

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Neospora caninum represents a major cause of reproductive failure due to abortion and neonatal mortality in cattle worldwide. At present, control of bovine neosporosis relies mainly on biosecurity and diagnostics as effective vaccines and licensed pharmacological treatments are unavailable. Although widely used for the *in vivo* diagnosis, serological tests show limitations deriving from the drop of antibody levels against the actively growing tachyzoite stage of the parasite in chronically infected cows. In these animals, *N. caninum* resides in form of the slowly replicating bradyzoite stage developing within tissue cysts and expressing a different antigenic repertoire. In order to identify *N. caninum* antigens expressed by the bradyzoite stage, a set of genes putatively encoding antigenic proteins was selected. As *N. caninum* and *Toxoplasma gondii* genomes display a high degree of synteny with many protein-coding genes showing a one-to-one correspondence, *T. gondii* was used as a model. Selection of *N. caninum* candidate genes was performed either by orthology or predicted protein homology with already characterised *T. gondii* antigens that are known to be expressed by the bradyzoite stage. Recombinant proteins were produced using bacterial expression systems and their immunoreactivity was evaluated through Western-Blot using a panel of reference sera. Besides reacting with sera from both naturally and experimentally infected animals, some of the candidate antigens reacted with sera from animals that tested negative with commercial serological tests but in which tissue cysts were detected. The aim is to employ these recombinant antigens for the development of a serum antibody ELISA able to identify cows chronically infected with *N. caninum*.

Correlation of Cryptosporidium infection of farm animals (cattle and sheep) and rodents in different ecosystems of Azerbaijan

OP07

Turkan Gurbanova

It is known that in Azerbaijan Cryptosporidium are common in farm animals and some wild animals. Cryptosporidium oocysts found in the isolates of feces of wild and farm animals have matching morphometric characteristics. They were diagnosed as *C. parvum* and *C. muris*. We compared the published data on the dynamics of infection of farm animals with the results of our own research of infection in rodents inhabiting the pastures near areas where previously investigated farm animals live. Cryptosporidium oocysts were previously reported in fecal isolates of cattle and sheep in the highlands of Gobustan (51.9% and 33.6% respectively). In our investigation of 123 red-tailed gerbils–a wild rodent widely spread in this region–we found that 30.9% of them were also infected with Cryptosporidium oocysts. In the farms of Absheron Peninsula, mainly in the vicinity of the city of Baku, oocysts were found in 21.0% of cattle, 22.7% of sheep, 31.7% of gray rats and 24.5% house mice. In the farms located in the northwest part of the Great Caucasus (Balakan, Gakh, Shaki, Zagatala) Cryptosporidium oocysts were detected in 59.3% of cattle, 48.1% of sheep and 72.7% of gray rats. The investigated forest mice in the natural ecosystems in Khachmaz region revealed a much smaller number of individuals infected with Cryptosporidium-11.1%. Therefore it can be concluded that the infection of farm animals (cattle and sheep) and rodents with Cryptosporidium in different ecosystems is interconnected. At this stage of our research the species composition of Cryptosporidium has not been defined, but our research is continued in this direction.

Wild ruminants as sentinels of endemic bovine besnoitiosis and first report of *Besnoitia besnoiti* infection in roe deer

P15

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Besnoitia besnoiti and *B. tarandi* infect bovids and Cervidae species, respectively. *B. besnoiti* infection is endemically present in beef cattle in the Pyrenees. Thus, we studied the spread of *Besnoitia* spp. infection in red deer (n=309), roe deer (n=418) and Spanish Ibex (n=288) located in areas where bovine besnoitiosis is endemically present, and whether these species may act as intermediate hosts of *B. besnoiti*. The serosurvey conducted only confirmed two seropositive roe deers and one seropositive red deer by western blot. Macroscopic tissue cysts were observed in sclera of one seropositive roe deer and later on visualized by histopathology in nasal turbinates. Genotyping of *Besnoitia* spp. roe deer isolate was carried out by means of multilocus microsatellite analysis (MS). In addition, Protein Disulfide-Isomerase (PDI) gene sequencing was carried out. Eight Besnoitia spp. in vitro grown isolates (7 *B. besnoiti* and 1 *B. tarandi* isolates) and biopsies from a roe deer, infected cattle and a donkey (infected with *B. bennetti*) were analyzed. MS analysis revealed differences between *B. besnoiti*, *B. tarandi* and *B. bennetti* species but a high genetic uniformity among *B. besnoiti* isolates. Surprisingly, *Besnoitia* spp. detected in the roe deer showed a *B. besnoiti* MS pattern supported by 100% similarity of PDIBb sequences from *B. besnoiti* isolates and *Besnoitia* spp. roe deer isolate. This work describes for the first time that roe deer may act as an intermediate host of *B. besnoiti* although low prevalence rates indicate that wild ruminants do not pose an epidemiological risk for cattle. This study was funded by AGL2013-46442R.

Direct Blood Dry LAMP: A Quick, Stable, and Easy On-site Diagnostic Tool for tick borne-diseases in tropical countries

OP08

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Loop-mediated isothermal amplification (LAMP) is a rapid and sensitive tool used for the diagnosis of a variety of infectious diseases. One of the advantages of this method over the polymerase chain reaction is that DNA amplification occurs at a constant temperature, usually between 60-65°C; therefore, expensive devices are unnecessary for this step. We have developed several LAMP tests for tick-borne pathogens including *Theileria parva*, *Theileria equi* and *Ehrlichia ruminantium*. However, LAMP still requires complicated sample preparation steps and a well-equipped laboratory to produce reliable and reproducible results, which limits its use in resource-poor laboratories in most developing countries. In this study, we made several substantial modifications to the technique to carry out on-site diagnosis of Human African Trypanosomiasis (HAT) in remote areas using LAMP. The first essential improvement was that LAMP reagents were dried and stabilized in a single tube by incorporating trehalose as a cryoprotectant to prolong shelf life at ambient temperature. The second technical improvement was achieved by simplifying the sample preparation step so that DNA or RNA could be amplified directly from detergent-lysed blood samples. We also developed battery-driven portable devises for DNA amplification and detection. With these modifications, on-site diagnosis of HAT in local clinics or villages in endemic areas becomes a reality, which could greatly impact the application of on-site diagnosis not only for HAT but also for other tick-borne diseases.

Theileria annulata interacts with the bovine adaptor proteins CD2AP and CIN85

P16

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The tick-transmitted apicomplexan parasite *T. annulata* causes fatal leukoproliferative disease in cattle. By dissolving the surrounding leukocyte membrane after invasion, the parasite gains access to an ideal position in the host cell cytoplasm to interact with host cell proteins. The schizont induces uncontrolled proliferation and confers resistance to apoptosis in the infected cell by modulating various host cell signalling pathways, and ensures its propagation by stably interacting with the host cell microtubule (MT) network. Our main interest is the study of host-parasite interactions that occur between the Theileria schizont and its bovine host. We recently found that two host cell adaptor proteins, CD2AP and CIN85, bind to the parasite surface. Adaptor proteins are molecules possessing two or more protein-binding domains that function to allow the formation of large signalling complexes. In this way, they can play important roles in signal transduction while usually possessing no catalytic activity themselves. CD2AP and CIN85 are similar in terms of sequence and structure, and contain several SH3 domains and a proline-rich domain (1). Many binding partners have been reported for CD2AP/CIN85, and they have been implicated in both signal transmission and cytokinesis, cellular processes that are used by the parasite to transform its host cell and to ensure its distribution to both daughter cells. By studying the way in which CD2AP and CIN85 interact with Theileria, we hope to learn more about the strategies this fascinating parasite employs to survive within its host.

An investigation on the effectiveness of *Babesia ovis* apical membrane antigen (BoAMA1) and *Babesia ovis*-infected cell culture in protecting against ovine babesiosis.

P17

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Babesia ovis, the main causative agent of ovine babesiosis, is a tick-borne hemaprotozoan parasite of small ruminants in tropical and subtropical regions. Although the economic impact of the disease is very high, babesiosis remains to be one of the neglected diseases of small ruminants. In the present study, the effectiveness of *B. ovis* apical membrane antigen (BoAMA1) was compared with *B. ovis*-infected erythrocyte cell culture (*B. ovis* Aydın/Akcaova) in protecting against ovine babesiosis. Forty sheep were divided into five groups (eight sheep per group) to establish four experimental and one control group (Group 5). Animals were immunized either with BoAMA1 (Group 1) or *B. ovis* cell culture (Groups 2, 3) or both with BoAMA1 and *B. ovis* cell culture (Group 4). Animals in all groups were challenged one month after the primary infection with homolog parasite stock of *B. ovis* Aydın/Akcaova cell culture. All sheep infected with *B. ovis* cell culture developed a similar clinical reaction to primary infection with 3-7 days of pyrexia along with an obvious reduction in RBC and PCV values and a slight decrease in WBC numbers. No clinical reactions were observed in Group 1 following the immunization. Animals in Groups 1 and 5 showed more severe clinical reactions compared to animals in Groups 2 and 3 following the challenge. Increased body temperature, notable decrease in RBC and PCV were among the clinical symptoms observed in Groups 1 during the first week of challenge. One sheep died due to severe disease in the control group. Animals in Groups 2 and 3 did not develop pyrexia and there was only a slight decrease in RBC and PCV following the challenge. The least and the highest percentage reduction in PCV and RBC following the challenge were observed in Group 4 and Group 5, respectively. Western blot analyses indicated that while animals in Groups 1 and 4 generated a good antibody response against BoAMA-1, sera obtained from animals immunised solely by *B. ovis* did not show any reactivity. BoAMA1- ELISA demonstrated that all animals immunized with BoAMA-1 became positive fifteen days after the primary immunization and remained positive until the end of trial. In contrast, only a few animals in Groups 2 and 3 were positive by BoAMA-1 ELISA. It appears on the basis of these observations that BoAMA-1 does not provide any protection against ovine babesiosis. However, biological basis of findings demonstrating that the percentage reduction in PCV and RBC following the challenge was the least in Group 4 warrant further investigations.

Occurrence of anti-*Neospora caninum* antibodies in cattle from a rural settlement in Arapongas, Paraná State, Brazil

P18

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Neosporosis is one of the main causes of reproductive failures in cattle, causing losses such as abortion, and is also responsible for significant economic losses in dairy farming. Seroepidemiology studies confirm the presence of the parasite worldwide including in Brazil. There are no pathognomonic signs during this disease and therefore is dificult to diagnose. Identifying the occurrence of the parasite is very important to determine preventive and control measures. The goal of this study was to evaluate the occurrence of anti-*Neospora caninum* antibodies in cattle from a rural settlement in Arapongas City, Paraná State, and also to assess the risk association between positivity and disease. Epidemiology questionnaires were answered by all farmers from the settlement and 406 bovine sera samples from 30 farms were analysed by IFAT. Anti-*N. caninum* antibodies were detected in 23.1% (94/406) of bovines, with titers ranging from 1/100 to 1/25600. A smaller proportion (17.8%) of seropositive cattle were found in crossbreed Gyr x Holsteins Black-and-White (HBW) compaired to pure HBW (28%), Jersey (20.1%), cross-breeding (31.1%) and other breedings (0). There was no statistic significant association between age and positivity, although the occurrence of seropositive in animals aged between 13 and 36 months indicated that keeping infected animals as replacements from the herd itself may complicate disease control strategies.

Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* infection in sheep, goats and fallow deer farming on the same area – preliminary results

OP09

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Toxoplasma gondii and *Neospora caninum* closely related tissue-dwelling Coccidian parasites are important causes of reproductive failure and production losses in farm livestock worldwide. The aim of the study was to monitor the seroprewalence of *N. caninum* and *T. gondii* infection in sheep, goats and fallow deer farming on the same area. The studies were conducted from 2012 to 2014 in the breeding station in Kosewo Górne, in the Mazurian Lake District, north-east Poland (latitude 53o41' North, longitude 21o25' East). The presence of antibodies against *T. gondii* was detected using ID Screen Toxoplasmosis Indirect Multi-speciec kit (ID.vet Innovative Diagnostics). The presence of antibodies against *N. caninum* in sheep and goats were detected by c-ELISA (VMRD, USA) but in fallow deer by an enzyme-linked immunoassay (IDEXX, USA) with some modifications. In fallow deer antibodies against *T. gondii* were detected in 5.4% of examined samples and against *N. caninum* in 10.8%. Antibodies against both parasites were detected in 0.6% of examined samples. The results of ELISA test were confirmed by Western blot. Antibodies against *T. gondii* were confirmed in 50% of examined sheep and 18% of goats serum samples. The study on the presence of *N. caninum* antibodies are in progress. The results of the studies may suggest different sensitivity of examined animals farming on the same area on *T. gondii* and *N. caninum* infections. The study was partially carried out in the Polish-Slovak Joint Research Project “Observation on serious protozoonoses (neosporosis and toksoplasmosis) in domestic and sylvatic cycle”.

Analysis of sequence diversity of transmission blocking candidate antigen genes reveals a potential association of SPAG1 indels with *Theileria* parasite speciation and host adaptation.

OP10

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Theileriosis in cattle and small ruminants caused by *Theileria annulata* and *T. lestoquardi* impose significant economic burden in countries endemic for these parasites. In addition to clinical disease much of the economic burden is imposed by the carrier state, and modelling has indicated that targeting questing adult ticks would have a major impact on disease control . Such findings highlight the benefit of control aimed at blocking transmission of infection. One way this could be achieved is to target proteins that are required for establishing infection in the tick or ruminant host. However, divergent antigens that evade host immunity compromise this strategy. In this study, genes with bioinformatic and transcriptomic profiles that indicate they encode potential transmission blocking antigens were identified. These included the major merozoite piroplasm surface antigen gene (Tams1), the major sporozoite surface antigen (SPAG-1) and a third gene encoding a Serine Repeat Antigen (Ta-SERA1) that is highly expressed by stages within the tick vector. Alleles of these three genes representing parasite from a number of geographical regions were sequenced. The results indicated that for *T. annulata*; Tams1 showed a high level of diversity with evidence for selection of amino acid substitution, while the gene encoding Ta-SERA1 was considerably less variable. Investigation of SPAG-1 alleles showed that in all genomes of *T. lestoquardi* analysed, a a large indel in the region encoding PGVGV repeats was apparent, resulting in a small version of the gene. In contrast, in *T. annulata,* potentially all alleles amplified from infections in both cattle and sheep lacked this indel, resulting in large versions of the gene. We speculate that this indel is associated with speciation of *T. lestoquardi* from *T. annulata* and is either a signature of genotype selection or has functional significance for adaptation to the ovine host.

Experimental infections of rabbits by tachyzoites and bradyzoites of *Besnoitia besnoiti*

OP11

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Cattle besnoitiosis due to *Besnoitia besnoiti* is in expansion in Europe and is responsible for severe economic losses in newly infected herd. Experimentally, rabbit has been found to be susceptible to this parasite. The adaptation of *B. besnoiti* to the rabbit could offer a new, easiest to handle and cheapest model of investigation to this disease. This experimental study has compared the virulence between tachyzoites and bradyzoites of *B. besnoiti* on rabbits. 18 New Zealand rabbits were allocated into three groups of six animals. The control group (group C) received subcutaneous injection of 30 µl of ovalbumin at the right flank. 2.106 tachyzoites, at the passage 125 on Vero cells, were subcutaneously injected at the right flank of the group “Tachyzoites” (group T). 2.106 bradyzoites of *B. besnoiti* taken from cutaneous cysts of chronically infected cow were injected subcutaneously at the right flank of the group “Bradyzoites” (group B). Clinical follow-up and serological survey were performed during ten weeks until the euthanasia. Only the group B has exhibited a febrile syndrome above 40°C from day 8 to day 11 after the injection with positive qPCR in blood. 25 samples of skin and organs were investigated for detection of parasite DNA by qPCR and parasites by histology. Cysts of *B. besnoiti* were found only on the group B in skin samples and organs corresponding to positive qPCR results. These results suggest a high virulence of bradyzoites in rabbits. The proposed model could be used for the assessment of efficacy of vaccine or treatment.

PINning down the parasite : understanding how *Theileria* transforms its host cell

P19

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Infectious agents develop intricate mechanisms to hijack the genetic and epigenetic machinery of their host cells to change phenotypic states. Studying host-parasite interactions can provide insights into the signaling pathways controlling cellular phenotypes. *Theileria* is the only eukaryotic parasite which can induce transformation of bovine host leukocytes. We have been studying how the parasite hijacks host signaling pathways to maintain cell transformation. We have identified epigenetic events in the host cell nucleus that are induced by the intracellular parasite. One example is the regulation of a positive feedback loop involving microRNAs (Marsolier *et al*., 2013); the parasite induces oncogene addiction which maintains host cell phenotypes. We also found examples of host methylation events induced by the parasite which lead to stable changes in host nuclear chromatin and gene expression (Cock-Rada *et al*., 2012). In addition to investigating changes in the host genome and cellular states, we explore unique features of the parasite genome. We are mining the *Theileria* genome in search of parasite-encoded onco-proteins. We recently identified a parasite-encoded Pin1 protein that is secreted into the host cell and rewires host cell metabolism and oncogenic signaling (Marsolier *et al*., 2015). We study *Theileria*-infected bovine leukocytes as a model to explore the plasticity of cellular phenotypes, the determinants of cell identities and the evolutionary strategies of interacting cellular systems.

Immunization of naturally infected pregnant cows against neosporosis-associated abortions with live tachyzoites

OP12

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Neosporosis in cattle, caused by the intracellular protozoan *Neospora caninum*, is a major cause of abortion and reproductive failure worldwide. The principal transmission route of neosporosis is via in utero infection of the offspring. There is no effective prophylactic treatment or vaccine available against bovine neosporosis; however, vaccination is considered as a preferred option for cost-effective control of the disease. A *N. caninum* NcIs491 isolate was examined for its ability to reduce abortion rate in naturally-infected herd under field conditions. *N. caninum*-seropositive pregnant dams were inoculated during mid-term pregnancy with freshly prepared or frozen live tachyzoites in two separate trials performed in endemic dairy herds. A total of 520 N. caninum seropositive pregnant cows were included in the study; of these, 146 were immunized with 108 freshly prepared live tachyzoites and 374 served as non-immunized control group. A significantly lower abortion rate was observed in immunized compared to the control group, 16 and 26% respectively (P = 0.0156, immunization efficacy of 39%). In the trial immunization with 2x108 frozen live tachyzoites, a total of 122 *N. caninum* seropositive dams were divided into immunized (n=78) and control (n=44) groups. As in the first trial, significantly lower abortion rate was observed in immunized compared to non-immunized cows, 29.5% and 10.3 % respectively (P = 0.011,immunization efficacy of 65%). In both trials the number of seropositive offspring in immunized cows remained similar to non-immunized dams. The results obtained showed that immunization of naturally infected cows with live N. caninum tachyzoites was effective in reducing abortions caused by neosporosis in naturally infected herds.

Genomics of Whole-Organism Vaccine Composition and Design

OP13

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Vaccination remains the most effective means of inducing long-term protection against infectious diseases, but efficacious vaccines against eukaryotic parasites have been notoriously difficult to develop. For subunit vaccines, efficacy appears to be tightly linked to the epitope composition in the vaccine preparation, with vaccine evasion observed for more divergent genotypes. However, it is unclear the extent to which vaccine evasion is also an issue for whole organism vaccines. East Coast fever, caused by the apicomplexan *Theileria parva*, kills one million cattle each year in sub-Saharan Africa. A highly effective, live, multi-strain vaccine against *T. parva* exists but it is not known how representative the vaccine strains are of the genetic variation in the field. We have sequenced and compared the genome of the three strains that compose this vaccine, and show that two of the strains are nearly identical, with most differences located in members of rapidly-evolving multigene families, while the third isolate differs from these two in close to 40,000 nucleotides located throughout the genome, including >50% of the genes. Despite the large number of SNPs identified, the variation among the three isolates of this vaccine cocktail represent only a small proportion of the overall genetic variation in the species, demonstrating that successful vaccines against parasites can be developed that do not require extensive representation of population variation.

Anti-*Neospora caninum* antibody detection and vertical transmission rate in pregnant zebu beef cows (*Bos indicus)*

P20

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The aim of the present study was to evaluate anti-*Neospora caninum* antibodies and the vertical transmission rate in naturally infected pregnant zebu beef cows (*Bos indicus*) reared on pasture. The present study began with 200 cows from four farms (50 cows from each farm), and these animals were submitted to timed artificial insemination (TAI). After ultrasonography, 76 pregnant cows were selected, 22, 15, 22, and 17 respectively from farms 1, 2, 3, and 4. Blood samples were taken from cows thrice during the first, second, and third trimester of gestation, and a blood sample was collected from 31 calves before colostrum milking. From 76 cows 23 (30.3%) had anti-*N. caninum* antibodies detected by indirect ELISA (Idexx), and 53 (69.7%) did not. Sixty-four cows that initiated the experiment were negative to *N. caninum* and 11 became positive either during the second or third trimester of gestation, this mean an infection incidence of 17.2% (11/64). OD for ELISA was higher (OD=2.08) during the second and third (OD=2.10) trimesters of pregnancy when compared with the first (OD=1.81), however, there were no statistical differences (P=0.45). The vertical transmission was calculated to be 29.0% (9/31), and the risk of vertical transmission of *N. caninum* in seropositive dams was 26.25 times higher than seronegative animals (OR=26.25, 2.38<OR<289, P=0.007). In conclusion, the rate of vertical transmission of *N. caninum* in pregnant zebu beef cows was 29%, and the risk was 26.25 higher in seropositive dams relative to than seronegative animals.

Risk factors related to *Toxoplasma gondii* infection in indoor-housed Dutch dairy goats

P21

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Toxoplasma gondii causes economic loss for goat farmers, but also has impact on human health through food-borne transmission. Our aim was to identify the risk factors for *T. gondii* infection in Dutch dairy goats. Fifty-two out of ninety approached farmers with indoor kept goats (58%) participated by answering a standardized questionnaire and contributing 32 blood samples. Serum samples were tested for *T. gondii* SAG1 antibodies by ELISA (ID Screen®). At least one seropositive animal was found on 61.5% of farms, and the overall animal seroprevalence was 13.3%. To evaluate potential risk factors on farm-level, three modeling strategies (Poisson, negative binomial and zero-inflated) were compared. The negative binomial fitted the data best, and the number of cats present at the farm (more than five cats: IR: 5.4, 95% CI: 1.8-16.5; no cat: IR: 0.4, 95% CI: 0.2-0.9) and mean animal age (IR: 1.5, 95% CI: 1.1-2.1) remained in the final multivariable model. In conclusion, the prevalence of *T. gondii* infection in indoor-kept Dutch dairy goats is relatively low and exposure to cats seems an important risk factor for *T. gondii* infection. *This research was conducted by a consortium within the framework of project n° GA/EFSA/BIOHAZ/2013/01 entitled “Relationship between seroprevalence in the main livestock species and presence of *Toxoplasma gondii* in meat”, grant agreement funded by the European Food Safety Authority (budget). This paper/publication is based on the results obtained in the framework of this mentioned project and it is published under the sole responsibility of the authors, and shall not be considered as an EFSA output.

Technical advances in sequencing coccidian genomes directly from clinical samples – implications for genomics and veterinary diagnostics?

OP14

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Cryptosporidium, Eimeria and Toxoplasma species are single-celled, intracellular, spore forming parasites. They are understudied and whilst some species can be cultured in vitro, there is a lack of suitable culture systems for others such as *Cryptosporidium* spp. There is an urgent need for high-quality genomic sequences to aid in bio-marker discovery, in-silico drug-target/-vaccine screening, and geno-epidemiology. Next generation sequencing (NGS) has the potential for increasing our knowledge of the biology, transmission, virulence and epidemiology of these species and so improve diagnosis, control and treatment of coccidial infections in animals and humans. However, significant challenges exist for generation of sequence data directly from animal and human samples. Parasite cells may be present in very low numbers, whilst samples are often contaminated with host/bacterial cells. Here we discuss the development of protocols to enrich for, and purify, Cryptosporidium oocysts from stool samples, optimisation of DNA extraction methods, the pro's and con's of whole genome amplification (WGA), and use of transposon based techniques to sequence sub-nano-gram quantities of genomic DNA. We have sequenced and created PAGIT-improved assemblies of several Cryptosporidium genomes directly from clinical samples, and characterised new human species i.e. *C. viatorum* - first isolated by Elwin et al, (2012), from patients with gastro-intestinal symptoms returning from the Indian sub-continent. We also discuss the application of these techniques to characterise *Eimeria tenella* and *Toxoplasma gondii* isolates.

Expanding the repertoire of TRAP-related proteins of Toxoplasma *gondii*: molecular and functional characterization of the micronemal adhesin TgMIC15

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In apicomplexan parasites, zoite motility and host-cell invasion rely on transmembrane (TM), apically secreted micronemal proteins (MICs) retrogradely transported by the parasite actomyosin motor. The ectodomains of these MICs contain adhesive motifs involved in binding to poorly characterized host-cell receptors, whereas their cytoplasmic tails are essential for microneme targeting and interaction with the parasite actomyosin motor. The thrombospondin type I (TSP1) repeat is present in various MICs, e.g., the thrombospondin-related anonymous protein (TRAP) of Plasmodium, MIC2 of *Toxoplasma gondii* and TRAP-C1 of Cryptosporidium. We characterized two novel TRAP-related proteins of *T. gondii*, MIC14 and MIC15, sharing 26% amino acid identity and the same overall architecture, suggesting a paralogous relationship between the two genes, which lay far apart on chromosome XII. MIC14 (2344 aa) and MIC15 (2924 aa) are type I TM proteins, characterized by i) an ectodomain harbouring three TSP1 repeats, ii) a C-terminal TM region devoid of a bona fide rhomboid cleavage site and iii) a cytoplasmic tail containing putative microneme targeting signals and aldolase-binding residues. Based on our western blot and immunolocalization analyses and available proteomic data, MIC14 does not appear to be expressed in *T. gondii* tachyzoites, bradyzoites and sporozoites, while MIC15 was localized in micronemes and detected in all three invasive stages. A tetracycline-regulatable, MIC15 knockdown mutant has been recently obtained and preliminary data point to an important role of MIC15 in the invasion process.

New tools for serological diagnosis and characterization of *Toxoplasma gondii* infections

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Toxoplasmosis is a common parasitic infection of humans and other warm-blooded animals. Serological tools play an important role in diagnosis, but also in epidemiological studies. One important aspect of human toxoplasmosis is congenital infection. Both symptomatic and asymptomatic primary infection during pregnancy can lead to transmission of the infection to the fetus, with associated disease (ocular and neurologic defects) appearing in the child months to years after birth. By contrast, a latent *T. gondii* infection, acquired by the mother prior to pregnancy, very rarely leads to congenital infection. Thus, the challenge to physicians caring for pregnant women with serologic evidence of an infection with *T. gondii* is to distinguish between recent and past infection. Since *T. gondii* specific IgM and IgA may persist for years after a primary infection, Toxoplasma IgG avidity testing is currently the most reliable tool to differentiate between acute and chronic infection. To develop additional methods to differentiate between acute and latent *T. gondii* infection, we screened microarrays carrying >1000 synthetic peptides mimicking in-silico predicted epitopes of *T. gondii* antigens (GRA, MIC, ROP, SAG) with sera collected from acutely and latently infected and non-infected human patients. 72% (771/1077) of the peptides were significantly stronger recognized by *T. gondii*-positive sera as compared to *T. gondii*-negative sera ($p<0.05$; LIMMA). Analysis of group-specific peptide reactions revealed that 21% (160/771) of these peptides were significantly stronger recognized by acutely infected individuals, while 3% (24/771) were recognized significantly stronger by latently infected individuals ($p<0.05$; LIMMA). The results provide a promising base for the development of a peptide-microarray that may be able to confirm acute infection. Another important aspect of human toxoplasmosis is the predominant route of infection, which may vary depending on various factors, including climate or consumer habits. European risk factor analyses suggest that the ingestion of infectious tissue cysts containing bradyzoites in raw and undercooked meat is the predominant route of human infection. However, a recently published study form North America, which made use of a sporozoite-specific antigen called TgERP, suggests that infection via *T. gondii* oocysts excreted by felids may represent an as yet underestimated source of human infection. However, available information on test specificity suggests that results gathered with TgERP based serological assays should be interpreted with care.

Mob1 protein: a critical factor in *Toxoplasma gondii* replication

OP16

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The control of protozoan parasite replication is a matter of great relevance in the infection process and, being so, members of pathways controlling cell proliferation/division, like Hippo and MEN (mitotic exit network) pathways, are excellent targets to study this process. *Toxoplasma gondii* presents one mob1 gene, encoding a component of the core kinase module of these both pathways. A phylogenetic analysis of Mob1 showed it to be similar to other Apicomplexa but distant from protozoan parasites like the Trypanosomatida. We confirmed that this gene is expressed and our data show that its expression dramatically decreases (94%) during the parasite replication inside the host cell. We have constructed a transgenic parasite strain that overexpresses Mob1 and these parasites show a significant delay in the replication process. Using an in house polyclonal antibody against this protein we observed a very clear localization of the protein in the parasite posterior pole, where the basal complex, a structure involved in cytokinesis in *T. gondii*, is localized. Additionally, we observed a dot localized in the middle of the cell. However, differently from other organisms, this Mob1 signal did not co-localize with the centrosome. Experiments are in progress to characterize the Mob1 loss of function. Altogether, the data presented above support that Mob1 is involved in the control of *T. gondii* replication. The identification of proteins involved in the regulation of parasite replication and the establishment of their interactions network can be a platform to investigate the control of parasite replication.

Re-annotation of the *Theileria parva* genome sheds new light into host-pathogen interactions

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Theileria parva, an apicomplexan parasite of cattle in eastern, central, and southern Africa, kills ~1 million cattle per year at a cost of >US$150 million. It causes pathogenesis by inducing cancer-like phenotypes, such as hyper-proliferation, in the leukocytes it infects, a trait commonly known as host cell transformation. An understanding of the genetic mechanisms underlying the induction of host hyper-proliferation may lead to the identification of proteins involved and generate new targets for anti-Theileria chemotherapies. Much like *T. parva*, *Theileria annulata* also transforms host cells, while other Theileria species, such as *T. orientalis* and *T. equi*, do not. Here, we use comparative genomics to identify genes that are specific to the host-transforming Theileria species. Using new RNA-seq data for *T. parva*, we generated a much improved genome annotation, including the discovery of 121 new genes and correction of the structure of 48% of the existing genes, identification of the genome coordinates of transcripts, CDSs and introns and quantification of gene expression levels. Using the updated *T. parva* genes and the available genome annotation for *T. annulata*, *T. orientalis* and *T. equi*, we then created Jaccard-filtered clusters of orthologous genes, and identified 83 *T. parva* proteins that are shared only with *T. annulata*, and were predicted to interact with the host, including proteins in known multi-gene families or with known functional domains. Using this list, we have generated several hypotheses regarding parasite gene function as a basis for future experimentation into Theileria-induced host proliferation.

The nuts and bolts of the making of an obligate intracellular parasite

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Apicomplexans are a diverse group of obligate parasites of humans and animals, with tremendous impact on human health and global food security. To understand how parasitism originated and diversified in this group of protists, we sequenced and analyzed the nuclear genome of two photosynthetic algae closely related to the Apicomplexa, *Chromera velia* and *Vitrella brassicaformis*. The algal genomes contained many genes implicated in parasitic processes in apicomplexans, including broad repertoires of extracellular proteins, a motility apparatus, and families of DNA- and putative RNA-binding proteins. Analyses of ancestral gene contents during apicomplexan evolution revealed that radiation to present-day apicomplexans was accompanied by lineage-specific, progressive gene losses such as losses in metabolic pathways and endomembrane trafficking systems. This was accompanied by re-tooling and re-wiring of existing cellular infrastructures and gene control mechanisms. I will provide an account of our current understanding of how apicomplexans, arguably the most successful group of parasitic protists known to man, have arisen and adapted to diverse terrestrial host niches including farm animals.

A systems biology approach to characterizing host-parasite interactions during Toxoplasma cell invasion

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Understanding host cell-pathogen interactions is crucial in determining the key factors controlling virulence and disease pathogenesis in *Toxoplasma gondii*. In this study we adopted a systems based approach to look simultaneously at dynamic modulations in both host and parasite transcriptomes and proteomes during Toxoplasma cell invasion. Our study was focussed on the early stages of cell invasion and used quantitative label-free proteomics and RNASeq to characterise protein and gene expression simultaneously from both host cells and parasites across various strains of T. gondii. This study permitted a parallel interrogation of host and pathogen responses, as well as a direct comparison of transcript and protein expression data. Results from clustering and network analysis identified dynamic changes in host and parasite responses during infection, as well as clusters of co-regulated proteins, enabling us to build a preliminary systems-model of host-parasite interactions. Statistical and functional bioinformatics analyses showed that transcriptional profiles did not always match quantitative proteomics data, which may indicate complex mechanisms of gene expression and regulation. The established workflow and data analysis pipeline are readily adaptable to study host-parasite interactions under various treatments or physiological conditions. The expression data acquired from both host and parasite have been integrated into EuPathDB and are now freely available to the user community.

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