IT01

If you cant get *Toxoplasma gondii* to reveal its secrets, its family members are MORE than willing to talk.

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Toxoplasma gondii and *Hammondia hammondi* are closely-related coccidian intracellular parasites with distinct life cycle characteristics and ability to cause disease. *T. gondii* is capable of infecting all warm-blooded animals and humans where it can be highly pathogenic. In contrast, *H. hammondi* has a narrower host range and is not known to naturally cause severe disease in animals or humans. The overall objective of our work is to use molecular, biochemical and functional genomic comparisons between these species to ultimately identify the precise molecular evolutionary events that determine these critical differences in pathogenicity. To address this goal we have compared the *in vitro* development of *T. gondii* and *H. hammondi* and found that in contrast to *T. gondii*, *H. hammondi* is refractory to extracellular pH stress for the majority of life as a tachyzoite. We have also compared the transcriptomic response of a monocyte cell line (THP-1) to infection by these two parasite species. We found that *T. gondii* and *H. hammondi*-infected cells had unique transcriptome profiles, with *H. hammondi* inducing significantly higher levels of proinflammatory cytokine production and *T. gondii* being capable of suppressing this effect. We are now investigating the precise nature of the cell state differences between *T. gondii* and *H. hammondi* infected THP-1 cells with respect to cell cycle and identifying the parasite effectors responsible for suppressing resposes to *H. hammondi* using reverse genetics. Overall our work has led to the identification of at least two phenotypes as being unique to *T. gondii* compared to *H. hammondi*, and this comparative system should be useful for elucidating developmental regulatory processes and immune suppression in *T. gondii*.

IT02

"Hidden" Stages of the Toxoplasma life cycle: Cats, Mice, and Petri Dishes

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The natural cycle of Toxoplasma gondii in cats and rodents/birds comprises all asexual and sexual developmental stages. While the biology of tachyzoites has been elucidated in considerable detail we are still struggling to get traction with transmissible and/or persistent developmental stages. Here, the challenges of developing in vitro conditions which promote stage differentiation and recapitulate the development in vivo remain. Experimental infection of animals, in particular companion animals such as cats, cannot serve as a sustainable platform for molecular studies of T. gondii and is increasingly disapproved by our society. Hence, novel developments in tissue engineering as well as imaging technology will drive creation of new and complex infection models which will serve as experimental platforms for the next decades of research into the cell biology of this model Apicomplexan.

IT03

The oocyst of coccidian parasites: a Trojan horse for special invaders

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The presence of "walls" is key to the survival, transmission and pathogenesis of a wide array of infectious microbes, including bacteria, fungi and protozoan parasites. Among the latters, the Coccidia are characterized by the production of sporozoite-containing, environmentally resistant oocysts, which are the result of sexual reproduction within suitable hosts. The presentation aims to offer an overview on scarcely investigated aspects of the biology of the oocyst-sporozoite binomial within different genera of Coccidia, with a special attention to *Toxoplasma gondii*. In addition to a survey of the literature concerning the molecular structure of the oocyst/sporocyst wall, recent data on the phenomenon of host cell traversal by *T. gondii* sporozoites will be discussed.

IT04

Comparative genome analysis of tissue-cyst forming coccidian parasites of diverse host range.

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Tissue-cyst forming coccidia of the subfamily Toxoplasmatinae encompass a group of closely related obligate intracellular parasites of the genera Besnoitia, Hammondia, Neospora and Toxoplasma. They cause highly prevalent infectious diseases worldwide, infecting many species of wild and domestic animals and causing zoonotic infections in humans. Notably, despite of being structurally and genetically closely related, this group of parasites presents significant differences in their life cycles, way of transmission, and host range, but the molecular bases for these differences are poorly understood. To gain insights into the genetic factors that may contribute to differences in host range and virulence among Toxoplasmatinae parasites, we sequenced the genomes of three Besnoitia species that infect cattle in Europe (Besnoitia besnoiti), North American donkeys (Besnoitia bennetti) and caribous (Besnoitia tarandi) and performed comparative genome analyses together with already available genomes of Toxoplasma gondii, Hammondia hammondi and Neospora caninum. Here, we will present preliminary results from these analyses and discuss their potential implications on parasite evolution and pathogenesis.

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IT05

Apicomplexan exploitation of host cell signaling: between rewiring and malignant transformation

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As a way of enhancing intracellular survival, apicomplexan pathogens alter host cell signaling pathways. The extent to which these protists are capable of modulating host pathways ranges from short-term rewiring, as in acute toxoplasmosis, to permanent transformation, as in malignant theileriosis. There is even evidence that epigenetic changes do not fully reverse after clearing of the parasite in the case of *Theileria* infection. For *Toxoplasma*, a wealth of studies over the past years has identified and characterized rhoptry or dense granule proteins such as TgIST that are secreted into the host cell and are responsible for monitored gene expression changes. Even though *Toxoplasma* is not *per se* considered a transforming pathogen, it is surprising how many parallels can be drawn between the cellular manipulations of these different apicomplexan taxa and how they must have independently found ways to manipulate central host pathways such as the NFκB signaling. Despite the huge quantity of details reported on altered host signaling that is proposed to mediate the phenotypic changes in *Theileria*-infected cells, the parasite effectors involved in this transformation are not well understood. One reason that so few *Theileria* effector proteins have been fully characterized is the lack of effective tools to genetically modify the parasite. Comparison of *Theileria* proteins with databases of proteins linked to cell transformation and tumor progression has not identified obvious kinases, phosphatases or proto-oncogenes thus far. However, the parasite as a multinucleated, syncytial body in the cytoplasm of the host cell during schizogony is perfectly positioned to interfere with cell signaling pathways by means of secreted proteins and parasite proteins located directly on the schizont membrane. To fully understand the biology of host cell transformation, it is therefore crucial to investigate the composition and roles of putative *Theileria* effector proteins. In this talk, the mechanisms by which different apicomplexan parasites manipulate their hosts will be explored, with a particular emphasis on the unique host-parasite relationship that has developed in *Theileria* transformed leukocytes.

IT06

Forward genetics in *Cryptosporidium* enabled by complete in vitro development in stem cell-derived intestinal epithelium

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Cryptosporidium parvum is a common cause of diarrheal disease in agricultural animals and it also infects humans either via zoonotic or direct transmission. Research on this important pathogen has lagged due to lack of small animal models and robust in vitro culture systems.
We recently described a new platform for complete life cycle development and long-term growth of *C. parvum* in vitro using "air-liquid interface" (ALI) cultures derived from intestinal epithelial stem cells. Transcriptomic profiling revealed that differentiating epithelial cells grown under ALI conditions undergo profound changes in metabolism and development that enable completion of the parasite life cycle in vitro. ALI cultures supported parasite expansion >100-fold and led to the production of viable oocysts that were transmissible in vitro and to mice. Transgenic parasite lines created using CRISPR/Cas9 were used to complete a genetic cross in vitro, demonstrating segregation of chromosomes during meiosis. We are presently using this system to explore the mechanism of action of novel compounds that offer promise for new therapeutics for cryptosporidiosis and to examine interactions with the microbiome.

IT07

Experimental models to study pathogenesis and control of apicomplexan parasites in ruminants

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Diseases caused by apicomplexan parasites in livestock have a deep impact on their welfare and the productivity of the farms. Among these diseases, toxoplasmosis, neosporosis and besnoitisios are of special concern for ruminants. Toxoplasmosis is a relevant disease of small ruminants caused by *Toxoplasma gondii*. The clinical consequences of the infection depend on the time of gestation when sheep are infected for the first time. Although initial investigations established the basic pathogenesis of this disease, there are still knowledge gaps regarding those precise mechanisms responsible for the abortion. Furthermore, recent studies based on pregnant sheep as experimental model have shown that relevant variables, such as time of gestation or parasite isolate or dose used in the inoculum, have a great impact of the consequences of infection and require further investigation. As an example, the occurrence of early abortion soon after inoculation is a little known clinical presentation in ovine toxoplasmosis which pathogenesis, different to that of late abortions, remains mostly unknown. Neosporosis, caused by *Neospora caninum*, is one the main infectious cause of reproductive failure in cattle and against which there is no treatment of vaccine commercially available. Several studies have successfully investigated the pathogenesis of this condition using both cases of natural infection and experimental bovine models. However, bovine experimental models are highly expensive, regarding both the cost in labour and animal maintenance. The susceptibility of sheep to the infection by *N. caninum* has been proven in different experimental studies, especially regarding the exogenous vertical transmission. Furthermore, the parasite shows a similar transmission in both species, sheep and cattle, as exogenous and endogenous vertical transmission have been described to occur under natural conditions. Besides entailing a significant finding as a relevant disease in sheep, this fact opens new opportunities for the experimental investigation in neosporosis, as sheep constitute an easier and more affordable experimental model. *Besnoitia benoitii* is the aetiologic agent of bovine besnoitiosis, a reemergent disease of cattle. Its increasing prevalence during the last decades, the lack of treatments or vaccines against this disease and the deep knowledge gaps regarding the cycle of transmission of the parasite makes the development of an experimental model an urgent need for the animal health field. Although an elusive achievement since the first description of this disease, more than a century ago, recent studies show promising results regarding the standardization of a bovine experimental model for besnoitiosis.

IT08

Current research in *Eimeria* species affecting chicken

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Seven species of the parasite *Eimeria* cause coccidiosis in chickens, an enteric disease transmitted between birds by the faecal-oral route, characterised by malabsorption, diarrhoea and/or haemorrhage with estimated costs of more than US$3 billion each year. Prophylactic anticoccidial drugs are routinely used to control *Eimeria* spp. and oral commercial vaccines based on live parasites are available, although their high costs of production limit their use, especially in the broiler (meat) sector. With poultry becoming the main source of animal protein in many developed and low-and-middle income countries (the world"s chicken flock is >22 billion, producing 1.4 trillion eggs and 100 million tonnes of meat each year) and new regulations to reduce usage of in-feed drugs, the poultry industry needs to explore alternative approaches to avoid losses caused by endemic and re-emerging diseases. Regardless of their high impact and wide prevalence, there are many aspects of the biology and host-parasite interaction completely unknown in *Eimeria* spp. In consequence, there are different lines of research open with the aim of generating new knowledge in basic biology of *Eimeria* as well as improving its control. Some of these investigations include: study of new morphological features and improvement of genome annotations; research on next-generation recombinant anticoccidial vaccines (identification of new candidates, evaluation of new delivery systems, analysis of populations), use of *Eimeria* as vaccine vector (for other *Eimeria* species and additional avian pathogens), improvement of molecular tools and *in vitro* systems for the identification and evaluation of new vaccine candidates and chemoprophylactic compounds.

IT09

A risk based surveillance programme for Toxoplasma gondii in pigs using a combination of farm auditing and serological screening

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Toxoplasma gondii is recognized as one of the major foodborne pathogens with a high human disease burden. In the Netherlands, pork contributes to about 11 % of the meatborne *T. gondii* infections. To control *T. gondii* infections in pigs, EFSA has advised to perform serological testing of pigs and audits of pig farms on risk factors for *T. gondii* infection. In the Netherlands, a program was started to translate the EFSA advice into a practical risk based surveillance system. In first instance, a large scale serological monitoring of fattening pigs was started and seroprevalence over time was determined. Next, the association between within-herd seroprevalence and risk factors for *T. gondii* on fattening pig farms in the Netherlands was determined. For this, a questionnaire for auditing farms for the presence of risk factors of *T. gondii* was developed and used on 25 case and 50 control farms. Results show that there is a significant association between seroprevalence and risk factors as cats present on farms, use of unheated feed products and feeding wet feed. Moreover, on-farm presence of rats and mice also increases Toxoplasma transmission risks. Subsequently, a study was started on farms to quantify the effectiveness of interventions on farms. A cross-over clinical trial was set up in which case farms were their own control and the cross-over moment is the implementation of interventions on risk factors to change farm management. Farms with a high within-herd seroprevalence were followed for at least during a year and monitored periodically for seroprevalence and implementation of interventions to eventually reduce the disease burden. The break-even point was calculated for which the intervention cost at fattening pig farms equal averted human disease burden and averted cost-of-illness minus cost of the surveillance program. The results shows favourable economic perspectives for interventions to control pig meat-born transmission of *T. gondii*.

AT01

Prevalence of potentially zoonotic assemblages of Giardia duodenalis in wild deer in Scotland

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Giardia duodenalis is a flagellated intestinal protozoan parasite (causative agent of giardiasis) which is of public health interest as two assemblages (A and B) are known to be zoonotic. The aims of this study were firstly to determine the prevalence of *Giardia* DNA in wild Scottish deer and secondly to identify the assemblages of any parasites found. Faecal samples (n=883) were collected from four species of wild deer (red (n=441), roe (n=327), sika (n=86), fallow (n=8) and from deer where no species identification was made (n=22)). DNA was extracted from each sample and nested PCRs were performed in duplicate to detect *Giardia* β-giardin DNA. The results from this study showed positive *G. duodenalis* DNA samples distributed widely across the country, with all four species of deer testing positive in at least one sample. The overall prevalence of *Giardia* DNA in wild deer sampled was 7.6% (67/883), within the different deer species prevalence ranged from 1.7% (1/86) in sika deer to 15.0% (49/326) in roe deer. Infections appear evenly distributed between both male and female deer with prevalence of 8.0% and 7.4% respectively. Sequence data was obtained from 63/67 positive samples. The majority of the samples 52/63 (82.5%) showed high levels of sequence identity (>99.7-100%) to Assemblage A parasites (MH765442, MK573339 and EU621373), assemblages B, D and E were also found, but in much lower numbers ((4/63) 6.4%, (2/63) 3.2% and (5/63) 7.9% respectively). Potentially zoonotic assemblages (A or B) were identified in all four species of deer more research is needed to determine whether wild deer could be contributing to the zoonotic and/or sylvatic transmission of the parasite in Scotland.

AT02

Monitoring Toxoplasma gondii infection in lactating goats: Antibody detection in serum and milk samples and parasitic DNA detection in milk

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Toxoplasma gondii is one of the major zoonotic agents and abortive pathogens in small ruminants. In goats, data on the infection during lactation and the presence of *T. gondii* in milk are lacking. A longitudinal study in a naturally infected caprine herd from northern Italy was planned with the following aims: i) evaluating the variation during the lactation of anti-*T. gondii* antibodies in blood and milk; ii) identifying the optimal phase during lactation for *T. gondii* monitoring; iii) detecting the presence of *T. gondii* DNA in the milk. Thirty Alpine goats were fortnightly visited seven time during the lactation period and sampled for blood (n=210) and milk (n=151). Age, information on previous reproductive disorders and the day of lactation (DL) were included in the statistical analysis performed with generalized linear mixed models (GLMMs). Anti-*T. gondii* antibodies were detected in blood and milk using a validated commercial ELISA kit. From nine seropositive animals, milk samples (n=63) were analysed by nested-PCR to amplify a sequence within the ITS1 region of *T. gondii*. A seroprevalence of 63.3% was calculated; a high agreement was obtained between serum and milk results (Spearman's coefficient=0.793, Kendall's tau=0.624), particularly in the period comprised between the 15th and the 60th DL, the optimal period for antibody detection in milk. Indeed, in the GLMMs the variable "phase of lactation" resulted strongly associated to ELISA values obtained in both serum (p-value=0.0001, F=5.197) and milk (p-value=0.016, F=2.755). Parasitic DNA was found in 20.6% of milk samples, with a discontinuous parasite DNA excretion during lactation. Milk was confirmed as a valid alternative to blood for screening and monitoring *T. gondii* infection in goat herds. *T. gondii* DNA in milk could enhance the possibility for raw goat's milk consumption to be considered as a risk to public health.

AT03

Detection of Toxoplasma gondii oocysts in fresh vegetables and fruits

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Toxoplasma gondii is the third most important contributor to health burden caused by foodborne illness. Ingestion of tissue cysts from raw or undercooked meat has been recognized as an important source of transmission to humans. In addition, there is an increasingly awareness for the consumption of fresh vegetables and fruits, as a source for oocyst transmission, since this stage of the parasite can persist and remain infective in soil and water.

Hence, this work aims to estimate the prevalence of *T. gondii* in vegetables and fruits that are raw consumed, and to assess the risk of oocysts transmission to humans. The food samples were collected from local producers and supermarket suppliers. Oocysts removal from the washing water was carry out in accordance with method 1623 (EPA 816-R-12-001- Jan 2012). *T. gondii* detection was performed by PCR, using specific primers for *T. gondii* repetitive DNA region. All positive-PCR DNA samples were purified and sequenced. Restriction enzyme digestion with EcoRV endonuclease was also used to confirm *T. gondii* DNA fragment. In addition, the presence of the parasite was observed by fluorescence immunolabeling, using a rabbit polyclonal antibody that recognize *T. gondii* oocyst wall protein TgOWP1. Forty-three (43%) of the analysed samples (28.0% - 59.2% CI) presented the expected PCR and digested fragments. These fragments were confirmed by sequencing. Moreover, compatible *T. gondii* oocyst structures, with positive expression of FITC-TgOWP and autofluorecence, were also observed. Experimental contamination of lettuces with oocysts (ME-49 strain) revealed recovery rates of 78 ± 20%. New approaches for oocyst detection were developed, providing relevant evidences of fresh vegetables and fruits contamination with *T. gondii.* Consumption of raw vegetables and fruits is a potential source of *T. gondii*, and a considerable risk for consumers since oocysts removal by washing is not completely effective.

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AT04

Comparative detection of Toxoplasma gondii in red deer, roe deer and wild boar in Germany

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The consumption of game in Germany has increased during the last ten years. Wild boar, roe deer, and red deer are the most frequently shot and consumed game animals in Germany, yet information on the prevalence of *Toxoplasma gondii* in these species is scarce. Additionally, little is known about the correlation of direct and indirect detection methods for *T. gondii* in game or about the suitability of meat juice as alternative sample type in serological testing. Aim of this study was to assess the prevalence of *T. gondii* in wild boar, roe deer, and red deer as well as the comparability of different methods for indirect and direct detection of *T. gondii* in these game species.

In this study, a total of 352 animals shot during the hunting seasons 2017/2018 and 2018/2019 in the German Federal State Brandenburg and intended for human consumption were examined. The highest seroprevalence of 23.9% was identified in wild boar (43/180, 95%CI: 17.9-30.8%) using the Multi-species ID Screen Toxoplasmosis Indirect ELISA Kit (IDvet, Montpellier, France), followed by 12.8% in roe deer (16/125, 95%CI: 7.5-20%) and 4.3% in red deer (2/47, 95%CI: 0.5-14.5%).

Analysis of 5 g heart muscle by qPCR targeting the 529 repeated element revealed the presence of tissue cysts in 29.1% of tested seropositive animals (7/24). One out of 114 seronegative animals (0.9%) was identified as positive using qPCR.

Comparative analysis of sera and heart meat juice showed substantial to almost perfect agreement in all three game species (Cohen's kappa coefficient κ = 0.79-0.95), demonstrating that heart meat juice can serve as an alternative sample type to detect *T. gondii*-specific antibodies using the commercial ELISA kit. In contrast, agreement between sera and meat juice derived from foreleg muscle of wild boar (κ =0.46) was only moderate.

The observed high seroprevalence of *T. gondii* in game animals in the studied hunting areas implies that game could represent a relevant source for human infection.

AT05

Quantitative microbial risk assessment for meatborne *Toxoplasma gondii* infection in the Netherlands

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Meat containing viable *T. gondii* tissue cysts is considered one of the main sources of human infection. The relative importance of the different types of meat depends, not only on the prevalence of *T. gondii* infection in the different livestock species, but also on consumed volumes and preparation habits. To take these factors into account a quantitative risk assessment model for meat-borne *T. gondii* infection was previously developed. At the time, the effect of salting on parasite viability was estimated based on a single experiment. In recent years, more data has become available, allowing to fit a new predictive model. In addition to the new salting model, a lower concentration of bradyzoites in cattle, more specific

heating profiles, and more recent consumption data were implemented in the QMRA model for meat-borne *T. gondii* infection in the Netherlands. Results show that beef remains the most important source, as it contributed 84.0% to the total number of predicted infections in the Dutch population, followed by pork (12.0%), mutton (3.7%), lamb (0.2%) pork/beef mixed products (0.1%), and veal (0.01%). The predicted number of *T. gondii* infections is reasonably in line with epidemiological data. At the product level, filet americain (a raw beef spread) alone contributed 79.8% to the total predicted infections in the base model, but scenario analyses demonstrate that its contribution is highly dependent on the salting parameters. A clear identification of the most risky meat products is important, as interventions focussing on these products could have a great impact on reducing *T. gondii* disease burden in the Netherlands. For that reason, it is important that the effects of salting and other processing methods are evaluated in line with industrial processing and incorporated in quantitative risk assessment models for meatborne toxoplasmosis.

AT06
Genetics of Eimeria populations: New findings from a familiar foe

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Eimeria species parasites can cause the disease coccidiosis, most notably in chickens where the annual global cost is thought to exceed US$3 billion. The majority of the >66 billion chickens produced each year are likely to be exposed to *Eimeria* and control is essential. Farmers have traditionally relied on chemoprophylaxis, although public pressure to reduce the use of antimicrobials in food production is increasing and legislative changes now influence the application of anticoccidial drugs. Recent developments have included the inception of "No Antibiotics, Ever" branding, a move which has resulted in dramatically increased use of live anticoccidial vaccines in North American poultry systems. However, while live and subunit anticoccidial vaccines are available, their relative cost and limited production capacity continue to restrict uptake, especially in Europe where live attenuated vaccines are required.
Seven *Eimeria* species have long been recognised to infect chickens, all with a distinct biological niche and a global enzootic distribution. However, the description of three cryptic strains or species, termed "Operational Taxonomic Units" (OTUs) X, Y and Z, circulating in South American, sub-Saharan, south Asian and Australian chicken populations has revealed unexpected complexity. The development of morphological and genomic resources for each OTU will be reported, revealing unexpected signatures of plasticity and genetic diversity, evolving from a small number of distinct ancestors with evidence of hybridisation. The risk posed by these novel genotypes is unknown, but it is clear that an understanding of eimerian population structure is now essential, with appropriate diagnostic tools required to facilitate the assessment of escape from current anticoccidial vaccines and possible future vaccine development.

AT07
A comparison of the early immune responses and parasite tissue distribution in mice experimentally infected with oocysts of either archetypal or non-archetypal genotypes of Toxoplasma gondii

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Toxoplasma gondii comprises mostly three archetypal genotypes, Types I, II and III, but South America is dominated by non-archetypal strains with genotypes BrI, BrII and BrIII being the most frequently isolated from animal reservoirs. The aim of this study was to assess the differences in mRNA expression in mice of cytokines, immune compounds and cell surface markers after experimental infection with oocysts of three *T. gondii* genotypes. Swiss Webster mice were divided in four groups, control (G1) (n=5 mice) and three challenge groups (n=35 mice each). Group 2 (G2) received an oral dose of 50 oocysts (strain M4), groups 3 and 4 received the same dose of BrI or BrIII oocysts, respectively. Mice (n= 5) from groups 2, 3 and 4 were culled at 24 hours (h), 48h, 72h, 96h, 7 days post infection (dPI), 14dPI and 21 dPI. All mice in G1 were culled at 21 dPI. Spleen and mesenteric lymph nodes (MLN) were analyzed using qPCR to evaluate levels of mRNA production of interferon gamma (IFN-γ), interleukin 12 (IL-12), T-cells surface markers CD8, CD4

and CD25, and receptor adapters MyD88 and CXCR3. Several tissues were tested with immunehisto-chemistry. Only two mice from G3 (BrI) survived until 14 dPI and it was observed significant increase in IFN-γ expression (P<0.0001) from them. The expression of CD25 was highest at 8-11 dPI in the mice from G3 culled earlier due to severe clinical symptoms. IL-12 expression in MLN was significant at 14 dPI (*P*=0.031) in G4 (BrIII) as the expression of MyD88 in splenic samples at 21 dPI (*P*=0.027). The pathological examination found mild lesions in G2 (M4) mice. Tachyzoites and tissue lesions were abundant from 7 dPI in G3 (BrI) mice. Tissue cysts appeared to be more common in G4 (BrIII) mice from 14 dPI and decreasing at 21 dPI comparing to the other experimental groups. The results show that different genotypes of *T. gondii* appear to elicit different immune responses in mice and non-archetypal strains demonstrate higher pathogenicity *in vivo*.

AT08

Genome-wide single nucleotide variation of Toxoplasma gondii type II isolates from Europe

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Toxoplasma gondii is a highly prevalent protozoan parasite, infecting a very broad range of animals and humans worldwide. A better knowledge of *T. gondii* genetic diversity and population structure may help to understand the transmission routes and sources of infection in livestock and humans.
There is limited data on genome-wide comparisons of field isolates representing the same clonal lineage. The aim of the present pilot study was therefore to assess genome-wide genetic diversity among *T. gondii* clonal lineage II isolates from Europe, where this lineage is predominant. To this end, four type II isolates obtained from Germany, the Czech Republic, Greece and France were used and DNA samples from these isolates were submitted to whole genome sequencing using the Illumina NextSeq platform.
For each of the *T. gondii* isolates, at least 95% of the reads were mapped to reference genomes. The mapped reads covered over 99% of the type II reference genome (ME49, ToxoDB, v36) with a read depth of > 20 per base. In total, 10185, 10462 and 11003 Single Nucleotide Variations (SNVs) were detected in isolates from Germany, the Czech Republic and Greece. The lowest number of SNVs (n = 4212) was observed in the isolate from France.
The highest number of unique/isolate-specific SNVs was found in the isolate from Germany (n = 2249) followed by the Czech (n = 2165) and Greece (n = 1921) type II isolates. The type II strain from France revealed the lowest number of unique SNVs (n = 765).
This pilot study demonstrates considerable genetic variation among four European clonal type II isolates and provides new chances to study the population structure of *T. gondii* parasites.

AT09

Whole genome sequencing to identify genetic variations in isolates of Sarcocystis neurona

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Sarcocystis neurona is the etiologic agent of equine protozoal myeloencephalitis. This parasite also causes neurologic disease in marine mammals. Despite a clonal population structure, like its close relative *Toxoplasma gondii, S. neurona* isolates exhibit antigenic variation, specifically in the major surface antigens (SAGs) expressed during the acute phase of infection. To determine the genetic basis for differential SAG expression and to identify additional variations that exist between isolates of *S. neurona*, whole genome sequence (WGS) data were obtained from three isolates of *S. neurona* (SN4, SN138, and SN-OT1). Comparison of these data to the reference genomes produced previously from the SN3 and SOSN1 strains of *S. neurona* revealed numerous indels, which extended up to more than 100 kb in size. Many of the sequence variants observed between the reference genomes of *S. neurona* and the WGS obtained from the other wild-type isolates likely represent natural variation. This includes the antigenic variation documented previously in different *S. neurona* isolates, which we found to be due to indels that encompass the major SAG genes. However, some of the largest interstitial deletions were unique to the SN3 reference genome and might be attributable to long-term cell culture passage of this strain (>20 years). Given the selective pressures inherent with *in vitro* propagation, it is conceivable that these regions have been lost from the SN3 genome because they contain sequences that slow asexual parasite growth *in vitro* but are potentially important during other stages of the parasite's life cycle (i.e., bradyzoites and/or sporozoites). Collectively, a more thorough understanding of the genetic variants that we

have documented may yield new information regarding *S. neurona* genome evolution and genes important for parasite development and survival.

AT10

Transcriptional changes in sexual-stage related genes of Cystoisospora suis in cell culture

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Coccidia including *Cystoisospora suis* are characterized by a complex life cycle involving asexual and sexual reproduction, but especially sexual development is not well characterized and the process of fertilization has not been described in detail. Here we analyzed the RNA levels of four genes related to sexual stages, four genes related to cellular division and meiosis and one related to merozoites of *C. suis* during its development *in vitro*. We hypothesized that genes are differentially regulated during the development and can be linked to the appearance of different stages. Dynein light chain 1 and the male gamete fusion factor HAP2 proteins are presumably restricted to microgametes. The oocyst wall protein 1 is a part of the wall-forming bodies and CSUI_001473 is an orthologue of GAM56 of *Eimeria tenella*, highly transcribed in macrogametocytes. The RAD51 family assists in repair of DNA double strand breaks during meiosis, a cell division process of gametogenesis. The NIMA-related protein kinases genes play a role in cell cycle regulation and are associated with male (NIMA1) or female (NIMA 2 NIMA4) gametocytes of *Plasmodium*. Whole RNA was extracted, transcribed to cDNA and subjected to quantitative real-time PCR using primers deduced from published data on apicomplexan genes and the *C. suis* reference genome in ToxoDB with GAPDH and actin as housekeeping genes for normalization. Transcription levels were compared from day 6 to 15 after infection of IPEC cells with sporozoites. Genes related to the sexual stages showed an upregulation with a peak on day 13 after which they declined. Merozoite gene transcription peaked on day 10 and then declined. Our results indicate that parasites differentiate from asexual to sexual stages in *in vitro* cultures. We could identify genes related to developmental changes of *C. suis* stages and cell cycle progression *in vitro*. Further work will involve the analysis of their role during the fertilization process and oocyst formation.

AT11

Isolation and genetic characterisation of Toxoplasma gondii in Spanish sheep livestock

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Toxoplasma gondii is a major cause of abortions in sheep and presents a remarkable zoonotic character being consumption of undercooked meat the main source of human infection. The aim of the present study was to investigate the genetic variability that exists in the *Toxoplasma gondii* population circulating throughout ovine livestock in Spain. Foetal and neonatal dead lamb brains and placental tissues (n = 242) along with adult sheep myocardium (n = 50) were collected from abortion outbreaks and authorized slaughterhouses, respectively. Selected PCR-positive tissues were subjected to mouse bioassay aiming parasite isolation; further on, both clinical samples and isolates were subjected to genotyping by multi-nested-PCR-RFLP and PCR-seq. As a result, 30 isolates were obtained from 6 Spanish regions; overall, three different genotypes were found, to note: ToxoDB#3 (type II-*PRU* variant) in 90% (27/30) of isolates, #2 (clonal type III) in 7% (2/30), and #1 (clonal type II) in 3% (1/30). When *T. gondii*-positive clinical samples (*n* = 151) were directly subjected to genotyping, up to 95% of the specimens belonged to type II-*PRU* variant, a foetal brain showed clonal II pattern, and four specimens showed unexpected type I alleles in *SAG3* marker, including a foetal brain that showed I+II alleles as a co-infection event. These results support the hypothesis of the existence of polymorphic and overlapping strains within ovine livestock in Spain, and point out the necessity of higher genotyping and sampling effort to accurately estimate *T. gondii* intraspecific genetic diversity.

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AT12

Dynamics and within-host competition of Theileria lestoquardi and T. ovis among naive sheep in Oman

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Mixed species infection of livestock by *Theileria spp*. is common in nature. Experimental as well as epidemiological data suggest that interaction between mixed parasites elicits cross-immunity that can modulate pathogenicity and disease burden at the population level. The present study examined within host interaction and disease outcome of the two prevalent ovine *Theileria* spp (pathogenic [*T. lestoquardi*] and nonpathogenic [*T. ovis*]) in Oman among a cohort of naive sheep, over a period of 11 months. The animals were raised in a tick-free farm and then transferred into a *Theileria* spp. endemic area. Species-specific qPCR was used to quantify *T. lestoquardi* and *T. ovis*. In the first two months, there was a high rate of morbidity and mortality among animals infected with *T. lestoquardi* alone. Levels of disease then subsided, and concurent infection (*T. lestoquardi* and *T. ovis*) prevailed. Overall densities of both parasites species were significantly higher as single infection vs mixed infection, suggesting a competitive interaction between the two parasite species. The pathogenic *T. lestoquardi* had a competitive advantage in a mixed infection. However, the density of both *T. lestoquardi* and *T. ovis* fluctuated significantly over time, with no difference in densities between the very hot (May to August) and warm seasons (Sep to April). A high level of genotype multiplicity was detected in *T. lestoquardi* infections, which increased with rising parasite density. Our results illustrate a competitive interaction between ovine *Theileria* spp (*T. lestoquardi* and *T. ovis*) in co-infected animals that results in a substantial reduction in the risk of mortality due to presumed heterologous protection.

AT13

Eimeria tenella ROP kinase EtROP1 induces G0/G1 cell cycle arrest and inhibits host cell apoptosis

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Context: Coccidia are obligate intracellular protozoan parasites responsible for human and veterinary diseases. *Eimeria tenella*, the etiologic agent of cecal coccidiosis, is a major pathogen of chickens. In *Toxoplasma gondii*, some kinases from the rhoptry compartment (ROP) are key virulence factors. ROP kinases hijack and modulate many cellular functions and pathways, allowing *T. gondii* survival and development. *E. tenella*'s kinome comprises 28 putative members of the ROP kinase family; most of them are predicted, as pseudokinases and their functions have never been characterized. One of the predicted kinase, EtROP1, was identified in the rhoptry proteome of *E. tenella* sporozoites. Here, we demonstrated that EtROP1 is active, and the N-terminal extension is necessary for its catalytic kinase activity.
Methods and results: Ectopic expression of EtROP1 followed by co-immunoprecipitation identified cellular p53 as EtROP1 partner. Further characterization confirmed the interaction and the phosphorylation of p53 by EtROP1. *E. tenella* infection or overexpression of EtROP1 resulted both in inhibition of host cell apoptosis and G0/G1 cell cycle arrest.
Conclusions: This work functionally described the first ROP kinase from *E. tenella* and its non-canonical structure. Our study provides the first mechanistic insight into the inhibition of host cell apoptosis by *E. tenella*. EtROP1 appears as a new candidate for coccidiosis control.

AT14

The Toxoplasma TgGRAx effector is involved in modulation of host autonomous innate immune response

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Toxoplasma gondii has the propensity to infect any nucleated cell and to reside inside a non-phagocytic parasitophorous vacuole membrane (PVM), that acts as protective but permeable and interactive barrier. Pivotal to restriction of *T. gondii* dissemination upon

infection in murine cells are the autophagy proteins regulated by IFN-gamma. The recruitment of these Immunity Regulated GTPases (IRGs) and Guanylate Binding Proteins (GBPs) to the PVM leads to pathogen elimination. To counter this host cell attack, *T. gondii* secretes the rhoptry proteins ROP5, 18 and 17 and the dense granules protein 7 (GRA7) that are embbeded in the PVM. These effector proteins synergistically inhibit the recruitment of IRGs to the PVM.

Here, we describe a new dense granules protein, (TgGRAx), which localizes to the PV space and to the host Golgi apparatus. Whilst generally succumbing to the virulent RH *T. gondii* strain within 7 days, mice infected with RH-GRAx-KO parasites survived for an additional 7-14 days. A comparable reduction in virulence was previously reported for RH-ROP18-KO acute infection studies, suggesting that TgGRAx could participate in the subversion of host cell defense mechanisms.

To test this hypothesis, we explored the localization of IRGs (Irga6) at the PVM as well as that of the host Golgi membrane associated regulator IRG(Irgm1) and effector (Irgm2) in INF-gamma activated mouse embryonic fibroblast. Irga6 was efficiently recruited around the PVM of RH-GRAx-KO as observed with RH-ROP18-KO but not with RH wild type . Remarkably the presence Irga6 at the PVM of RH-GRAx-KO dramatically dropped in mouse cell lines lacking Irgm1 or Irgm2. These findings point towards a role of TgGRAx in parasite resistance against the host cell autonomous defense mechanisms, preventing IRG recruitment to the PVM by a yet unknown mechanism. In parallel, we have investigated how TgGRAx traffics and resides at the host Golgi and are also probing for TgGRAx interaction partners.

Figure

AT15

Comparative proteomic analysis of bovine placentas infected with high and low virulence Neospora caninum isolates

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Understanding host-pathogen interactions is crucial for obtaining a systems-level overview of infectious diseases. In neosporosis, high-throughput technology has lately been used to explore this area using *in vitro* models, thus losing information on environment influence where host-pathogen interactions are happening. Recently, we used a bovine *in vivo* model of infection at mid-gestation to study the early infection dynamics (10 and 20 days post infection, dpi) after experimental challenge with high and low virulence isolates of *Neospora caninum* (Nc-Spain7 and Nc-Spain1H, respectively). Herein, we compared the proteomic profiles of the cotyledon and caruncle samples of our previous experimental infection at 20 dpi using label-free liquid chromatography tandem-mass spectrometry analysis. We found that 173 caruncle and 176 cotyledon proteins were more abundant in animals infected with the high virulence isolate, whereas 3 caruncle and 4 cotyledon proteins were more abundant in animals infected with the low virulence isolate. Gene ontology term enrichment analyses highlighted complement and coagulation routes, oxidation-reduction processes and extracellular matrix reorganization, among other biological processes, as key points in the pathogenic mechanism of *N. caninum* abortion. These findings corroborate the validity of previous *in vitro* models for pathogenesis studies, and provide new insight into the host-pathogen interactions in bovine neosporosis.

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AT16

Dissecting the trancriptional landscapes of Theileria annulata-infected host cell identifies novel tumor suppressor genes relevant to host cell transformation and human cancer

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Tropical theileriosis is a parasitic disease of calves caused by the apicomplexan parasite *Theileria annulata* and transmitted by Hyalomma ticks. *T. annulata* infects and transforms bovine leukocytes and macrophages into a cancer-like phenotype characterized by immortalization, dissemination and uncontrolled proliferation. The comprehensive understanding of the underlying mechanisms that cause host cell transformation necessitates the identification of the genes and pathways differentially regulated after infection. We investigate the transcriptome of *T. annulata*-transformed bovine lymphocytes and macrophages to define the genes transcriptionally perturbed upon host cell infection and parasite attenuation. We have identified four genes (*MMP9, GZMA, RASGRP1* and *SEPP1*) that likely play key roles in host cell transformation and virulence – one of which (*MMP9*) has been already functionally implicated in *Theileria*-mediated host cell transformation and in human carcinomas. We functionally characterized the role of *GZMA*and *RASGRP1*, via CRISPR/CAS9-mediated gene knock-down and activation of expression. The knockdown of both *GZMA* and *RASGRP1* in attenuated macrophages leads to a regain in adhesion and invasion, both *in vitro*and *in vivo*, and a decrease in the oxidative stress level in *T. annulata*-transformed leukocytes. In an attempt to extend our conclusions to human cell lines and investigate the role of *GZMA* and *RASGRP1* in human cancer invasiveness, we queried the publicly available transcriptomes of 934 human cancer cell lines and clustered them with *T. annulata* host cells. Using this approach, we identified 3 lymphoma cell lines as being the transcriptionally closest to *T. annulata* -transformed host cells. The up-regulation of *GZMA*and *RASGRP1*l evels in the human lymphoma cell lines results in a decrease in their invasive capacity, confirming the obtained results on the tumor suppressor role of *GZMA* and *RASGRP1*and extending our observations to human lymphoma.

AT17

Actin dynamics, regulation and function in Toxoplasma gondii

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The acto-myosin system of apicomplexan parasites is critically involved in parasite gliding motility, host cell invasion and intracellular parasite development. With the adaptation of chromobodies in apicomplexan parasites it is now possible to study the highly complex regulation and dynamics of F-actin during the life cycle of these parasites.
Surprisingly, *Toxoplasma gondii* forms an impressive intravacuolar F-actin network during intracellular parasite growth, consisting of short, highly dynamic F-actin filaments. This intravacuolar network defines the so called residual body (RB), which acts as a recycling hub for maternal organelles. Furthermore, our imaging approaches allowed us to determine the dynamics of F-actin during host cell invasion and led to a novel model for this essential process, where the parasite nucleus is the major obstacle that needs to be squeezed into the host cell by way of F-actin dependent forces.
Intriguingly, apicomplexans possess only a reduced set of (conserved) F-actin binding proteins, leading to the hypothesis that these diverged eukaryotes evolved unique, apicomplexan-specific actin regulatory proteins that cannot be identified via standard bioinformatic analysis. Given the importance of F-actin throughout the apicomplexan life cycle, we hypothesised that some of these proteins are essential.
Based on the identification of critical genes in a recent, genome-wide screen (Sidik et al., 2016), we designed a curated library containing all gRNAs targeting essential genes conserved in apicomplexans. In order to perform a phenotypic screen on known phenotypes caused by disruption or stabilisation of F-actin, we adapted a conditional Cas9-system (splitCas9) and expressed actin-chromobodies as indicator. We identified several candidate genes, causing similar phenotypes upon there disruption and are currently localising the respective proteins and characterise their function in detail.

AT18

The predicted T. gondii rhoptry kinase ROP39 indirectly affects PVM accumulation of Irga6

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Following infection of an IFNg-stimulated cell of a laboratory mouse, IRG and GBP proteins begin to accumulate at the *T. gondii* PVM in high densities very early after invasion and both families of GTPases contribute to membrane disruption and parasite clearance. To defend the PVM against the deleterious action of the IRG resistance system, *T. gondii* virulent strains utilize the kinase complex ROP18/ROP5/GRA7 for phosphorylation and thereby inactivation of Irga6. The pseudokinase ROP5 plays a major role for *T. gondii* virulence suggesting that ROP5 assists other kinases – above ROP18 – in terms of IRG protein inactivation.
We identified direct interaction of another rhoptry protein, ROP39, with ROP5 isoform B, the predominant isoform for *T. gondii* virulence in laboratory mice. Deletion of *rop39* in a type I genetic background results in elevated numbers of Irga6-positive vacuoles. No direct binding of ROP39 to Irga6 or any other IRG protein could be observed so far indicating that ROP39 is not an IRG kinase but rather indirectly affecting the PVM accumulation of Irga6.
The necessity of two families of GTPases (IRG and GBP proteins) for *T. gondii* control in mice and their functional inhibition by the same parsite effectors infers a potential interdependence. Identification of a direct Irgb10:GBP6 interaction will allow us to clarify such an interdependence in terms of parasite control. Irgb10:GBP6 might represent only one of a number of pairs crucial for control of *T. gondii* and eventually other pathogens prone to IRG/GBP-mediated resistance.
Under the premise of a heterologous protein complex, a GBP protein that is interacting with Irga6 could be the target of ROP39 explaining the indirect effect on Irga6 accumulation at the PVM.

AT19

Generation and characterisation of selected virulence factor knockout strains of C. parvum using CRISPR/CAS method

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Introduction
Cryptosporidium parvum is an intracellular yet extra-cytoplasmatic residing parasite, that infects the intestinal mucosa of animals and humans. It causes acute, persistent and chronic diarrhoea and can become a life-threatening illness for immune-suppressed persons and malnourished infants. Despite being known as an important pathogen for many decades, research on this protozoon has been challenging, due to the lack of molecular tools, as well as the lack of an appropriate in vitro system that enables a continuous culture of the parasite. Several putative virulence factors of C. parvum have been described in the past, but their final role for infection remains to be proven. In this project we will use the CRISPR/Cas-method to knock-out or tag specific genes encoding for virulence factors, especially genes involved in locomotion and adhesion. The generated transgenic strains will be characterized using different cell line-based in vitro systems as well as in vivo using the IFN-γ receptor knockout mouse model of cryptosporidiosis.

AT20

First evidence of interferon-gamma response in *besnoitia besnoiti* naturally infected animals

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Besnoitia besnoitii (Bb), an apicomplexan parasite, is the cause of bovine besnoitiosis. Cell mediated immune response (CMI) play an important role in *Bb* related parasites, such as *Toxoplasma gondii* (Tg) and *Neospora caninum (Nc)*. In besnoitiosis, CMI plays a major role as a T-cell response was already described.
In this study, the CMI to Bb was investigated by IGRA (Interferon Gamma Release Assay tests) in naturally infected animals. Heparinized whole blood and serum samples were taken from cattle from a high prevalence infected herd.
Whole blood samples were stimulated using either *Bb* or *Nc* semi-purified antigens, respectively considered as

specific and non-specific antigens, as well as Nil (PBS) and pokeweed mitogen as controls. The presence of interferon Gamma (IFNg) in stimulated plasmas was further tested using the ID Screen® Ruminant IFN-g ELISA. Antibody (Ab) responses against *Bb, Tg* and *Nc* were evaluated using the ID Screen® IDvet ELISA tests.
Out of the 97 cattle in the herd, 54 were tested in parallel in serology and IFNg. A specific interferon gamma secretion in response to Bb antigens was observed in some animals[PJ1] , whereas the Nc extracts did not generate any IFNg response. 39/54 were Ab and IGRA positive, and 11 were Ab and IFNg negative. Only 2 animals were Ab positive and IGRA negative. Interestingly, 2 animals were Ab negative but IGRA positive; 1 was giving moderate signal and 1 a very strong response.
The high and specific release of IFNg in naturally infected animals seems to indicate that Bb elicit a strong CMI response, globally correlated to the antibody response (k=0.797;[0.531-0.998]). However, the discrepant results between the two techniques might require further research, and might raise the question to what extent the humoral immune response might reflect the disease protection status. In conclusion, IGRA tests could have a certain interest to explore the immune response to *Besnoitia besnoiti*.

AT21

Comparative tachyzoite proteome analyses among six Neospora caninum isolates with different virulence

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The molecular basis that determines biological variability among *Neospora caninum* isolates, such as differences in virulence, remains unknown. Due to the scarce polymorphism revealed in candidate genes associated to virulence, the goal of our study was to compare the proteome of a large population of *N. caninum* isolates that differ in virulence. Label free LC-MS/MS was used to investigate the proteomic differences among Nc-Bahia, Nc-Spain4H and Nc-Spain7, representing high-virulence isolates and Nc-Ger6, Nc-Spain2H and Nc-Spain1H, representing low-virulence isolates.
Forty one and 48 proteins were more abundant in high- and low-virulence isolates, respectively. Thirty percent of these proteins were hypothetical, which highlights the importance of generating a well-annotated genome to make real progress in this area. The microneme protein MIC15 and the rhomboid protease ROM5, involved in parasite invasion and egress, the bradyzoite marker, NcSAG4, the dense granule protein GRA42 and the rhoptry proteins ROP12 and SUB2 were more abundant in low-virulence isolates. In contrast, GRA25, GRA16 and GRA32 and three rhoptry proteins (subfamily BPK1, family ROP20 and subfamily ROP37), as well as proteins involved in energy and redox metabolism and DNA/RNA processing were more abundant in high-virulence isolates.
In a second analysis, pairwise comparisons were performed between all isolates. The results obtained corroborated results of the above approach and of previous transcriptome and proteome comparative studies. Thus, these differences in protein abundance levels could play central roles in the phenotypic and virulence differences observed among isolates. The role of these candidate in parasite virulence deserves further investigations.
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AT22

Experimental toxoplasmosis in pigs inoculated with different parasite stages and different genotypes of Toxoplasma gondii

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Pork is considered an important source of human *Toxoplasma gondii* infection and the course of human and porcine toxoplasmosis is much more comparable than that of human and another animal toxoplasmosis. The objective of this study was to compare clinical signs, pathology, and evolution of the specific IgG humoral response in pigs experimentally inoculated either with oocysts or tissue cysts of a type II or a type III *T. gondii* isolates, obtained from a Siberian tiger and a domestic cat, respectively. Twenty four weaned 35-40 kg pigs seronegative to *T. gondii* were divided into four groups of six animals. Pigs of the first and second groups were orally inoculated with either 400 oocysts or 10 tissue cysts of a type II. Similarly, pigs of the third and fourth groups were orally inoculated with either 400 oocysts or 10 tissue cysts of a type III. All inoculated pigs had transient fever (> 40.0° C) during first week post inoculation (wpi), accompanied by inappetence and occurrence of mild diarrhoea in some animals. These clinical signs continued in pigs infected with oocysts and tissue cysts of a type III during second wpi only. Two of the pigs inoculated with oocysts of a type III died 12 days post inoculation. The histopathological lesions were similar in both animals with involvement of all examined lymphatic tissues and consisting of foci of necrosis, neutrophilic inflammatory reaction, reactive hyperplasia of lymphatic follicles and haemorrhages All animals seroconverted (PrioCHECK *Toxoplasma* Ab porcine ELISA, Prionics, Switzerland). The antibody levels were higher than the cut-off (PP 20) by 2-3 weeks post inoculation (wpi) and remained at high levels until the end of the experiment. Higher levels of anti-*T. gondii* antibodies were found in pigs inoculated with a type III. In conclusion, we found differences in the course of experimental toxoplasmosis in pigs inoculated with low numbers of oocysts or tissue cysts of different genotypes of *T. gondii*.

AT23

Physiopathology of Eimeria tenella infection and integrity of the intestinal barrier: Influence of the microbiota

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Eimeria tenella infection leads to a severe intestinal disease leading to high economic impact in poultry industry. Coccidiosis is frequently associated with a high prevalence of opportunistic infections. Our objective was to study during *E. tenella* infection the importance of immune cells and mediators of the inflammatory response on the severity of caecal lesions and on the potential dissemination of commensal bacteria. An original model of germ-free and conventional broilers chickens was developed to study the impact of the caecal microbiota on the physiopathology of the infection. Our results show that, at the same parasite load, lesions caused by *E. tenella* are dependent on the microbiota. Seven days post-infection, an increase of inflammatory mediators (interferon-g, colony stimulating factor, CSF-1, 2) are detected at the caecal tissue level in conventional chickens and germ-free chickens whereas interleukin-17A (IL-17A) is increased only in conventional chickens. Moreover, administration of microbiota to germ-free chickens 4 days post-*E. tenella* infection restored the caecal lesions and the expression of IL-17A to a similar level to conventional chickens suggesting that inflammatory cells producing IL-17A could play a role in the physiopathology of the infection. The lesions represent a rupture of the intestinal barrier, which, at homeostasis, protects the chicken against invasion of commensal bacteria. Our hypothesis is that intestinal lesions caused by the infection would lead to commensal bacterial translocation. This was confirmed with a non-pathogenic *E. coli* strain administered by oral route that presented a dissemination in the spleen during *E. tenella* infection. In conclusion, during infection, the microbiota is responsible for the inflammatory response *via* IL-17A and the formation of

lesions. The loss of the intestinal integrity leads to commensal bacterial translocation that could contribute to the development of opportunistic diseases.

AT24

Recrudescence and vertical transmission of persistent infection of Neospora Caninum in sheep

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The aim of the current study was to investigate recrudescence of natural chronic infection and vertical transmission of *Neospora caninum* in sheep. Thirteen naturally infected sheep were oestrus synchronized and serologically followed up during gestation. Two non-infected sheep were used as control. Ewes were euthanized when an increase of specific serological antibodies, measured through ELISA, was detected or, when this did not occur, at 135 days of gestation. Lesions were studied through histological analysis of samples from placentomes (*n*=9/sheep) and foetuses (brain, liver, lung, skeletal muscle and heart). Presence of the parasite in same samples was analysed by PCR. Immune response of the host was analysed in placentomes throughout IHC (CD4, CD8, CD3, CD21 and CMH-II) and qPCR (IFNγ, IL10, IL4, TNFα, IL12, IL2, TGFβ and IL18). Recrudescence was detected between days 80 and 120 of gestation in ten out of thirteen ewes. Most of foetuses (75%) from those ten sheep were alive at the time of euthanasia. While lesions at the placenta from these ten animals, characterized by foci of necrosis and infiltration of mononuclear cells, were generally scant and mild, lesions were found in 75% in the foetuses from these ewes, affecting mainly the brain (multifocal non purulent encephalitis). Regarding immune response, the immunehisto-chemical characterization showed a high number of T lymphocytes CD3+ (CD4/CD8<1) and macrophages (CMH-II+) mainly in those sheep with death lambs, which also showed an up-regulation of IFNγ, IL10, TNFα and IL18. Vertical transmission of the parasite occurred in all the sheep where an increase of specific serological antibodies was found. These findings are similar to those described in bovine neosporosis, suggesting that this disease could also be very relevant in sheep and should be included in the differential diagnosis of ovine reproductive failure.

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AT25

Phenotypic characterisation of Spanish Toxoplasma gondii isolates in a murine model

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Toxoplasma gondii is a major abortifacient agent in sheep flocks, and its zoonotic character entails around 30% of seroprevalence in human population worldwide. Virulence was evaluated in 8 *T. gondii* isolates representative of the genetic diversity (ToxoDB#1 and #3) of strains circulating in sheep flocks in Spain (isolates TgShSp1, 2, 3, 7, 8 and 10 originated from abortion outbreaks and TgShSp11 and 16 from chronically infected adult sheep) by intraperitoneal inoculation of tachyzoites in a standardized murine model. Accumulated morbidity varied between isolates from 56% to 100%, and accumulated mortality was 0% in 5 isolates, and 4.5%, 8.0% and 20.8% in the remaining 3 strains, so all of them can be considered as avirulent. Two additional groups per isolate were inoculated at doses of 1000 tachyzoites and sacrificed at 7 and 30 days post inoculation (dpi), representative of acute and chronic phases of the infection, respectively. Parasite tissue tropism (nPCR), load (qPCR), and histological evaluation, demonstrated differences among isolates. TgShSp1, 2 and 3 isolates stood out due to its low parasite burdens and lesions detected in all tissues studied. By contrast, TgShSp16 showed enhanced cystogenic capacity in brain (10 to 100 times more parasites/mg of tissue than the rest of isolates; $p<0.01$ in all cases), and ability to proliferate in acute-phase organs such us lung (up to 35 times more parasites/mg of lung tissue detected than less proliferative isolates; $p<0.01$) or heart (up to 20 times more parasites/mg of heart tissue detected in comparison with less proliferative isolates; $p<0.01$). Results evidence phenotypical differences among strains with low genetic diversity using a well-established panel of markers. Besides, observed differences between isolates obtained from adult sheep and abortion-derived tissues deserve further studies.

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AT26

Lessons from BKIs: Why it may be hard to get a drug that treats cryptosporidiosis and cystoisosporosis but also toxoplasmosis, sarcocystiosis, besnoitiosis and neosporosis

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Bumped kinase inhibitors (BKIs) have antiparasitic properties in vivo against some apicomplexan parasitic organisms. BKIs have the best activity against *Cryptosporidium, Cystoisospora, Toxoplasma, Neospora, Besnoitia,* and *Sarcocystis* species; parasites with a glycine-gatekeeper in their calcium-dependent protein kinase 1 (CDPK1) ortholog. The site of infection varies for these different apicomplexans. *Cryptospori-dium parvum, C. hominis,* and *Cystoisospora suis* are generally limited to gut epithelial cells, while *Toxoplasma*, *Neospora*, *Besnoitia,* and *Sarcocystis* species (*Sarcocystidae* family) are widely and systemically distributed including the central nervous system. While evaluating *in vivo* efficacies of different BKIs in animal models of infection against these apicomplexan parasites, we noted examples of compounds with good *Cryptosporidium* efficacy but poor efficacy against *Toxoplasma.* Conversely, other BKIs gave good efficacy against *Toxoplasma* but generally poor efficacy against *Cryptosporidium.* Physiologically based pharmacokinetic (PBPK) models were generated and identified correlations between gut enterocyte levels of BKIs and good efficacy outcomes with *Cryptosporidium* therapy; high systemic or extraintestinal tissue levels did not correlate with good efficacy. The same pattern was not true for *Toxoplasma* therapy. BKIs which are highly absorbed in the proximal GI tract and have high systemic tissue distribution are more likely to be effective against *Toxoplasma*. We hypothesize that *Cystoisospora* infection has similar pharmacokinetics/ pharma-codynamics (PK/PD) to *Cryptosporidium*, while *Neospora, Sarcocystis* and *Besnoitia* PK/PD is more similar to *Toxoplasma* PK/PD. We conclude it is unlikely we will find one BKI, which is efficacious and safe in both GI and systemic/CNS apicomplexa infections, and probably two BKIs need to be developed for the different sites of infection. This issue for BKIs may be generalizable to other apicomplexa drug candidates.

Figure

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AT27

Vaccination with transgenic Eimeria tenella expressing vaccine candidates from Eimeria maxima confers partial protection against high level E. maxima challenge in a broiler model of coccidiosis

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Coccidiosis, caused by *Eimeria* parasites, has been ranked among the ten most economically significant enzootic livestock diseases in the UK. Routine chemoprophylaxis and/or vaccination with live parasite vaccines are effective to control *Eimeria*, although the emergence of drug resistance and the relative cost and production capacity of vaccine formulations, consisting of up to eight *Eimeria* species/strains, can prove limiting. Genetic manipulation of *Eimeria tenella* sporozoites has now allowed the generation of stable populations expressing vaccine candidates from other *Eimeria* species. Previously, we have demonstrated that vaccination of inbred chickens with transgenic *E. tenella* expressing *Eimeria maxima* antigens such as apical membrane antigen 1 (EmAMA1) was able to induce significant levels of immune protection against low level *E. maxima* challenge (300 oocysts). In the present work we aimed to assess the relevance of the *E. tenella* [EmAMA1] vaccine in a commercial setting, using commercial broiler chickens exposed to higher *E. maxima* challenge doses (10,000 oocysts). The efficacy of vaccination was defined by production performance and the severity of pathology. In addition, three *E. tenella* lines expressing the vaccine candidates

Immune-Mapped Protein 1 (EmIMP1), Lactate Dehydrogenase (EmLDH) and Microneme protein 2 (EmMIC2) from *E. maxima* were developed, combined with the *E. tenella* [EmAMA1] line, and evaluated under field conditions to analyse potential synergy. Herein we describe the effect of vaccination with up to four different *E. tenella* transgenic lines on parasite replication, chicken body weight gain and lesion scores after high-level *E. maxima* challenge; developing a powerful tool with the potential to streamline vaccine formulations available in the market.

AT28

Metal-captured inhibition of pre-mRNA processing activity by CPSF3 controls very efficiently Cryptosporidium infection

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Cryptosporidium parvum is one of the most important diseases of young ruminant livestock, particularly neonatal calves. Infected animals may suffer from profuse watery diarrhoea, dehydration and in severe cases death can occur. Chemotherapy is very limited and there is a need for more effective treatments. Here we show inhibition of cleavage and polyadenylation specificity factor 3 (CPSF3) as a new strategy to control *Cryptosporidium* infection. Remarkably, we find that oxaborole-mediated inhibition of CPSF3 reduces intestinal parasite burden in both immunocompromised and neonatal mouse models with far better efficacy than nitazoxanide. We present crystal structures (1.6 to 2.0 Å) revealing an unprecedented mechanism of action, whereby the mRNA processing activity of CPSF3 is efficiently blocked by the binding of the oxaborole group at the metal-dependent catalytic center. Our data provide insights to accelerate the development of next-generation anti-*Cryptosporidium* therapeutics. Given the high structural similarity of the CPSF3 drug site of other parasites, it is very likely that the CPSF3 inhibition mechanism proposed here is shared across parasite species, such as Plasmodium, Toxoplasma and trypanosomatids.

AT29

Optimisation of a mucosal immunisation approach against Neospora caninum infection

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Background: We developed a mucosal immunization approach using *Neospora caninum* membrane proteins (NcMP) plus CpG adjuvant that conferred IFN-γ-dependent long-term protection to mice challenged with this parasite [1, 2]. Here we assessed, in the bovine host, the immunogenicity of this antigenic preparation using CpG as well as alternative adjuvants. **Results**: In vitro stimulation of bovine monocyte-derived dendritic cells with the immunogenic preparation induced a marked up-regulation of antigen-presenting and co-stimulatory molecules and production of IL-12/23 p40. Moreover, heifers immunized with NcMP plus CpG presented elevated levels of parasite-specific IgG and IgA antibodies and peripheral blood mononuclear cells collected from the immunized animals responded by producing IFN-γ upon restimulation with *N. caninum* antigens. To improve the immunogenicity and mucoadhesiveness of this experimental vaccine, a polymer-based adjuvant was used in combination with CpG. This immunogenic preparation also resulted in protection in mice. The lower parasite-specific IgG1/IgG2c ratios detected in the immunized animals indicated preferential IFN-γ-induced isotype switching. These features suggest that the novel formulation may also induce a protective response in bovines, and is currently being assessed in this relevant host. **Conclusions**: These results altogether indicate that our previously developed immunization strategy is effective in inducing a humoral and cellular immune response in the bovine host, involving the stimulation of the IL-12/IFN-γ axis, a key protective mechanism against neosporosis.

[1] *Vaccine*. 2016; 34: 6250-8.; [2] *Vaccine*. 2018; 36: 4890-6.

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AT30

Novel generation of arene Ruthenium complexes based compounds for specific targeting of Toxoplasma gondii

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Current chemotherapy of human toxoplasmosis is still challenging; available medications are often associated with severe side effects and ineffective against tissue cyst stages.
Recently, we reported promising activities of dinuclear arene ruthenium complexes against *Toxoplasma gondii* (*T. gondii*), however further refinement of these molecules is needed to increase their parasiticidal effect. In this perspective, paths to explore could be that of (i) exploiting auxotrophies and metabolic peculiarities of *T. gondii* to increase drug uptake, (ii) applying the structure activity relationship (SAR) concept to optimize ideal structure and (iii) linking bioactive fluorescent dyes derivatives that exhibits anti-parasitic properties to the ruthenium complexes.
Based on these strategies, large series of therapeutic molecules were designed and synthetized by conjugating arene ruthenium moieties to (i) metabolites that are essential for the parasite, (ii) coumarin, bodipy derivatives and (iii) various small molecules to create "Like"-compounds for SAR study. Initial screening of compounds was performed *in vitro* at 0.1 and 1µM and consisted in determining anti-*T. gondii* tachyzoites activity and cytotoxicity in host cells.
Thus, we identified forty-two hit compounds showing all a potent action on tachyzoites at low concentration without damaging host cells. Determined IC50 values were in the nanomolar range. Transmission electron microscopy revealed ultrastructural alterations in the mitochondrial matrix of treated parasites.
Additionally, some of these compounds were also highly active against *Trypanosoma brucei* with a significant decrease in mitochondrial membrane potential. Now, these compounds are being tested for efficacy against Theileria parasites.
In the next step of this work we will refine our selection to very few compounds for further *in vivo* studies and identification/validation of drug target candidates.

AT31

Endochin-like Quinolones exhibit promising efficacy against Neospora caninum in vitro and in experimentally infected pregnant mice

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We report on the efficacy of selected endochin-like quinolones (ELQs) against *N.caninum* tachyzoites grown in human foreskin fibroblasts (HFF) and in a pregnant BALB/c mouse model. Fourteen ELQs were screened against transgenic *N. caninum* tachyzoites expressing β-galactosidase (Nc-βgal). Drugs were added concomitantly to infection and the values for 50% proliferation inhibition (IC_{50}) were determined after 3 days. Three compounds exhibited IC_{50} values below 0.1nM, 3 ELQs had IC_{50}s between 0.1-1nM, 7 ELQs had values between 1-10nM, and one ELQ had an IC_{50} of 22.4nM. Two compounds, namely ELQ-316 and its prodrug ELQ-334 with IC_{50}s of 0.66 and 3.33nM respectively, displayed previously promising activities against experimental toxoplasmosis and babesiosis caused by *Babesia microti* in mice, and were further studied. They were assessed in long-term treatment assays by exposure of infected HFF to 0.5µM of ELQs, starting 3h after infection for up to 17 days followed by release of drug pressure. Results showed that the compounds delayed parasite proliferation, but did not exert parasiticidal activities. TEM of drug treated parasites detected alterations within the parasite mitochondria, but not in other parasite organelles. Potential effects of ELQs on the mitochondrial membrane potential are currently investigated. Assessments of safety of ELQ-334 in the pregnant mouse model showed that the compound did not interfere in fertility or pregnancy outcome. In parallel, ELQs were assessed for interference in embryo development in Zebrafish (*Danio rerio*) embryos. In *N. caninum* infected pregnant mice treated with ELQ-334 at 10mg/kg/day for 5 days, neonatal mortality was found in 7 of 44 pups (15.9%), but no postnatal mortality was noted. Vertical transmission was reduced

by 49% compared to the placebo group, which exhibited 100% vertical transmission.Neonatal mortality in the placebo group occurred in 15 of 34 pups (44%), and 18 of the residual 19 pups died in the next 4 weeks. These findings encourage more research on the use of ELQs for therapeutic options against *N. caninum* infection.

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AT32

Wildlife as environmental sentinels and potential sources of novel genotypes of Cryptosporidium parvum for neonatal calves

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Cryptosporidium parvum infection is a significant problem for the beef and dairy sector, where infection in neonatal calves can lead to inappetence, severe diarrhoea, dehydration, death and other economic losses. Relatively little is known about how *C.parvum* establishes in the calf population and how it is maintained within a herd with seasonal calving.

Three separate studies in different geographical locations were conducted to look for *Cryptosporidium* in rodents; rabbits and pheasants using molecular speciation and genotyping techniques. The aim was to investigate if these animals could be either sentinels of environmental contamination with *C.parvum* or be potential sources of *C. parvum* infection for livestock.

Of 156 rodent samples 50 tested positive for *Cryptosporidium* but only one was *C.parvum*, while the other 49 samples were positive for as yet unnamed *Cryptosporidium* species/genotypes, which have not been seen in livestock. For the rabbits, 134 out of 359 tested positive for *Cryptosporidium* and 78 were positive for *C.parvum*. Of the *C. parvum* genotypes, 57.7% were are associated with infections in cattle, while 22 rabbits shed a genotype that has previously only been seen in humans (IIcA5G3). Out of 50 pheasant samples 24 were positive for *Cryptosporidium*. Sequencing and genotyping has shown that the majority of the *Cryptosporidium* infected pheasants (n=23) were shedding *C. parvum* that is potentially infectious to cattle and some of these genotypes were also found in adult cattle and the calves on the same farm.

In this study we found that *C.parvum* was very rare in the sampled rodent population (1/156) and it was common in the tested rabbits and pheasants. This work raises questions whether these wildlife species were actively infected or just mechanically transmitting the parasite. The study shows that rabbits and pheasants can be sentinels for *C. parvum* in the environment and that their movement between farms may spread *C.parvum* genotypes between farms.

AT33

Multigenome sequence-based genotyping and morphotyping of Eimeria species infecting commercial sheep and goat

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Coccidiosis, caused by *Eimeria* species, can produce severe clinical signs including weight loss, bloody diarrhea and, in severe cases, death. Each *Eimeria* sp. varies in its ability to cause clinical disease; therefore, it is important to identify individual species. Traditionally, *Eimeria* spp. were differentiated using oocyst morphometrics but overlapping features and measurements made identifications difficult. Sequence-based genotyping using loci from the mitochondrial (mt) and nuclear (nu) genomes have been exploited for distinguishing *Eimeria* spp. infecting poultry. This study tested the hypothesis that these loci could be used to differentiate *Eimeria* spp. of small ruminants. *Eimeria*-positive fecal samples from small ruminants were collected from various geographic locations. Morphometric data were recorded from ~50 oocysts per sample using light microscopy. Initial genotype data were collected by sequencing loci within the mt (i.e. COIII, COI) and nu genomes (i.e. 18S rDNA) using Sanger sequencing of PCR amplicons to identify samples with one *Eimeria* sp. predominantly. For "single-species" samples, whole mt genome and nu 18S rDNA sequences were generated. For mixed-species samples, next generation sequencing (NGS) of amplicons was used to estimate species diversity following PCR of mt loci. We were able to link morphometric features used to describe each *Eimeria* sp. with a complete mt genome sequence and nu 18S rDNA sequence to provide unequivocally identification of individual *Eimeria* spp. infecting small ruminants. With these morphotypes linked to specific genotypes, multi-locus sequence data were used to infer the phylogenetic relationships among *Eimeria* spp. using Bayesian inference (MrBayes). Morphometric data were then mapped onto the resulting phylogenetic tree. Sequences from these phylogenetically informative loci will support the creation of molecular assays (i.e.

species-specific PCR primers, ddPCR) to identify *Eimeria* spp. for accurate diagnostics.

AT34

Differentiating mixed Cryptosporidium infections in calves

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Cryptosporidiosis is one of the most common parasitic infections that causes diarrhea in young calves as well as in humans. Currently, diagnostic methods and treatment options of this economically important disease are restricted. To analyze the present situation in Switzerland, which *Cryptosporidium* species and *C. parvum* subtypes occur, 120 fecal samples (out of 57 farms) of diarrheal calves aged 4 days to 4 weeks were microscopically examined for *Cryptosporidium* oocysts by Ziehl-Neelsen staining and by ELISA. Overall, 50% were tested positive for *Cryptosporidium* spp. and further differentiated into species and *C. parvum* subtypes by performing nested PCR and Sanger sequence analysis of the 18S ribosomal RNA gene (*18S rRNA)*and the 60kDa glycoprotein gene (*gp60)*. *C. parvum*, which is the common infective species in this age group, was found in 96.7% of all positive calves. All isolates belonged to the subtype family IIa with IIaA15G2R1 as the most common subtype (in 69.5% of the cases). Interestingly, all gp60 and several 18S sequence electropherograms showed composite sequence traces after well-defined break points. Utilizing a bioinformatic algorithm for decomposition suggested underlying mixed population structures. Cloning of the amplified PCR products confirmed these findings, identifying up to five different gp60 subtypes in a single sample. To be able to identify possible mixed infections on the species level, we developed a new nested PCR method for the 28S ribosomal RNA gene region *(28S rRNA)*. With this newly established PCR, which has a high analytical specificity for general *Cryptosporidium* spp., the five most common *Cryptosporidium* species of cattle (*C. parvum, C. bovis, C. ryanae, C. andersoni, and C. ubiquitum),* as well as *C. hominis,* can be detected without sequencing. By testing all samples collected in this study with this new 28S PCR tool, a novel 28S gene variant was discovered in samples from several independent animals.

AT35

Serological diagnosis of besnoitiosis: A multi-species competitive enzyme linked immunosorbent assay overcoming the problem of false-positive reactions

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Serological cross-reaction represents a serious problem in the currently available tests to diagnose *Besnoitia* infections in many species including cattle, caribou and donkeys. False-positive reactions are often due to the low positive-predictive values (PPVs) of most serological tests for besnoitiosis. These tests are therefore of limited value in screening large herds in areas with low prevalence, since increased numbers of false-positive reactions require confirmatory testing by alternative, time consuming serological methods causing additional costs, e.g. by immunoblotting.
To overcome this problem, we aimed at developing highly sensitive and specific competitive enzyme linked immunosorbent assays using monoclonal antibodies produced against the tachyzoite stage of *Besnoitia besnoiti*. A competitive ELISA set up with one of these antibodies was screened with a large panel of *Besnoitia besnoiti*-positive bovine sera to estimate the diagnostic sensitivity of the test. Sera from herds with *Neospora caninum*- and *Sarcocystis* spp.-infected cattle were used to estimate its diagnostic specificity. Relative to a previously established highly sensitive and specific ELISA, the new competitive ELISA revealed a diagnostic sensitivity and specificity of >99%.
Furthermore, this novel assay was tested on a variety of proven *Besnoitia*-positive sera from other species, including *B. besnoiti*-infected cats, rodents, rabbits or *B. bennetti*-infected donkeys or *B. tarandi*-infected caribou. Also in these animal species, the results obtained with the new competitive *Besnoitia*-ELISA corresponded perfectly with those of the reference tests, which included immunoblots and immunofluorescence antibody tests.

In conclusion, the novel competitive *Besnoitia*-ELISA represents a valuable tool for the control of bovine besnoitiosis and for studies on the epidemiology of *Besnoitia* infections in a variety of host species, including also wild living natural and experimental hosts.

AT36

Sarcocystis falcatula-like derived from opossum in Northeastern Brazil: In vitro propagation in avian cells, molecular characterization and bioassay in birds

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Most isolates of *Sarcocystis* spp. derived from Brazilian opossums are genetically distinct from the known species of *Sarcocystis*, but behave similarly as *S. falcatula*, as they are infective to birds and do not cause infection in mice. In previous studies *Sarcocystis falcatula*-like isolates were originated from the South and Southeast regions of Brazil. In the current work, we aimed to culture and to perform multilocus sequence analysis of *Sarcocystis* sp. derived from an opossum (*D. aurita*/*D. marsupialis*) from Salvador, Northeastern Brazil. The parasite was isolated in Vero cells, referred here as Sarco-BA1, and propagated in avian cells (DF-1). Molecular analysis of Sarco-BA1 revealed that the nucleotide sequence of the internal transcribed spacer 1 (ITS1) of the rDNA was identical to all isolates (n = 19) of *Sarcocystis* spp. reported in two studies from the South and Southeast regions of the country. Nucleotide sequences related to three surface antigens (SAG2, SAG3 and SAG4) showed a typical variation described for *S. falcatula*-like organisms in Brazil. Two budgerigars were inoculated with sporocysts of Sarco-BA1 and developed acute sarcocystosis, showing that the parasite behaves like *S. falcatula*. Sarco-BA1 had almost identical ITS1 and SAG sequences to all 16 isolates of *S. falcatula*-like recently described in Magellanic penguins (*Spheniscus magellanicus*) rescued on the coast of Espírito Santo state, Brazil. Our results suggest that Sarco-BA1 and *S. falcatula*-like may represent a single species of *Sarcocystis*. Propagation of the parasite in a permanent avian cell line significantly improved the yield of merozoites in cell culture. To our knowledge, this is the first molecular study and *in vitro* isolation of *S. falcatula*-like derived from Northeastern Brazil. Studies are under way to determine the infectivity of Sarco-BA1 to other animal species, as well as to investigate serological cross-reactivity among Sarco-BA1, *S. neurona* and related species.

P01

Do all seropositive cattle in a farm contribute to bovine besnoitiosis transmission in the same way?

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Bovine besnoitiosis is a re-emerging protozoan disease in Europe caused by the cyst-forming apicomplexan parasite *Besnoitia besnoiti.* Bradyzoites and Tachyzoïtes are responsible for the chronic scleroderma phase of the disease. Biting flies are able to penetrate bradyzoite filled cysts with their mouthparts. When blood feeding is interrupted and continued on other bovine hosts, the parasite is transmitted. Thus, bovine with a dermis rich in bradyzoites are likely "high spreaders" of *B. besnoiti* for the herd. Cyst-filled dermises occur following clinical infection; culling of these animals would potentially lower the selection pressure on the herd. However, seropositive cattle that have never had obvious clinical signs may also carry cysts. The aim of this preliminary study was to identify seroprevalent cattle with high quantities of parasite DNA in their skin biopsies using Real-Time PCR, and consider herd sanitation strategies. A total of 471 skin biopsies were performed across 6 farms (A, B, C, D, E, F) known to have a high seroprevalence of *B. besnoiti* infection (50 at 92%). A significant qPCR signal was detected in 13% of the biopsies, 17% had a questionable result, the rest were negative. These three categories were found in the six farms where the proportion of individuals with a Ct ≤ 36 varied from 9 to 22%, depending on the farm. The qPCR results were immediately communicated to the breeders so that they could take the necessary steps to remove animals with Ct ≤ 36 from the rest of the herd. Where removal was carried out rapidly (farms A and D), serological incidences over the next 12 – 24 months were very limited, and reinforce the merits of this strategy. In farms B and C, where no action was taken, the serological incidences were very high. Monitoring for farms E and F is still in progress.

P02

Molecular characterisation of *Cryptosporidium* spp. from domestic pigs in Argentina

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Cryptosporidium spp. can cause neonatal morbidity in farmed livestock. Pigs are infected by different species of *Cryptosporidium*, however, studies revealed the presence of two major porcine specific species: *Cryptosporidium suis* and *Cryptosporidium scrofarum*. *C. suis* was reported more frequently in pre-weaners and *C. scrofarum* in weaners. There have been a few reports of mixed *Cryptosporidium* infections in pigs, may be due to the use of unspecific molecular tools. The aim of this study was to identify the presence of *Cryptosporidium* spp. in pigs and perform the molecular characterization. Faecal samples (n=520) were obtained from animals under intensive breeding system. The study was conducted in 13 farms. Two categories were studied: piglets pre-weaners (n=400) from birth to 3 weeks of age and weaners (n=120)of a 4 week of age. Samples were concentrated by sedimentation-flotation and stained by modified Ziehl-Neelsen technique. DNA was extracted from samples with *Cryptosporidium*-like oocysts at microscopy by a ZR fecal DNA kit(Zymo,USA).DNA amplification was performed by a nested-PCR protocol which amplified a fragment of the 18S rRNA gene of *Cryptosporidium* spp. First, a set of genus-specific primers were used for the primary nested-PCR protocol. The second step was to identify*C. suis* and *C. scrofarum* with species-specific primers pairs. PCR products were analysed on 1,5% agarose gel and sequenced.Out of 520 faecal samples, 45 were microscopically positive, however, only 15 samples were PCR positive, 3 samples were positive for *C. suis*, 8 for *C. scrofarum* and 4 were identified as mixed infection. These PCR results were confirmed by sequencing and BLAST comparison.The two *Cryptosporidium* species were detected both in pre-weaners and weaners piglets.Results of the present study did not show age-specificity of pig specific *Cryptosporidium* spp. Nevertheless, the study suggests specie-specific nested-PCR protocol as an useful tool for *Cryptosporidium* diagnosis in pigs.

P03

First report of Neospora caninum vertical transmission in human being in Brazil

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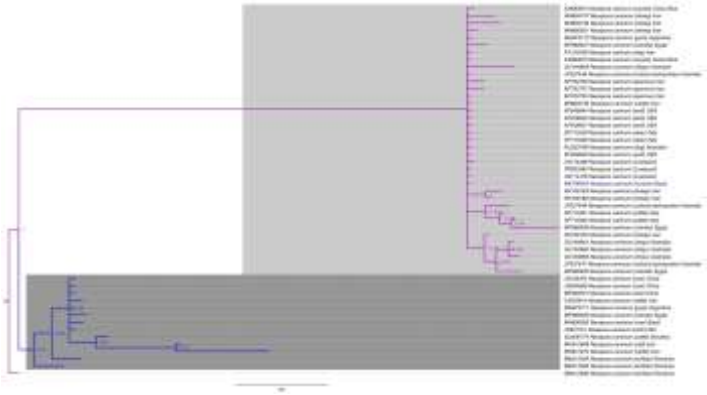
Neospora caninum is a mandatory intracellular parasite belonging to the phylum Apicomplexa, has a wide range of hosts, the canids being their definitive hosts. The objective of this work was to evaluate by means of the serological and molecular diagnosis of *N. caninum* in umbilical cord blood and placental tissue the possibility of vertical transmission in the human being. Approved by the Human Ethics Committee of the UFMS, under Number 1.804.047. Samples of placenta and umbilical cord blood from pregnant women and newborns were collected, respectively, in the Cândido Mariano Maternity Hospital in Campo Grande-MS, Brazil. To perform the IFAT, antigens (Immunodot diagnósticos®) were used to detect IgG/ IgM antibodies. For the molecular tests, DNA extraction and PCR were performed using primers NP7-NP4, which amplify a fragment of 275 bp. A Bayesian Phylogenetic analysis was performed using the MrBayes 3.2.6 program. For the data set used in this study, approximately 10 million generations were found to be sufficient for topology and were plotted using the FigTree 1.4.2 program. The samples were evaluated in parallel to *Toxoplasma gondii* as control in the assays. Of the 201 serum samples analyzed 24.3% were positive for the presence of anti-*N. caninum* IgG antibodies. In the molecular assays, a sample of umbilical cord blood was positive and presented 100% identity sequencing with *N. caninum* Liverpool strain. Positive serology suggests human exposure to the parasite, also reported by other authors. *N. caninum* (GenBank: MK790054), has identity (98-100%) and with several strains of the database, suggesting that it belongs to the species *N. caninum*. The data suggest the possibility of a potential vertical transmission, that is, presence of the parasite in the fetus from the intrauterine contact.
Figure: Phylogenetic tree of *Neospora caninum* (GenBank: MK790054)

Figure



P04

Identification of *TOXOPLASMA GONDIi* in a horse with glanders

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Toxoplasma gondii is a zoonotic parasite of mammals and birds, and worldwide distributed. Naturally infected horses seem to be more resistant to development of clinical toxoplasmosis, and present low antibody titer but, tissue cysts may persist. Glanders is an emerging zoonotic disease of Equidae caused by the bacterium *Burkholderia mallei,* with no effective veterinary treatment and leading animals to death. The occurrence of glanders must be notified to the official animal health services, that will euthanize the infected animal after confirmation. This study aimed to verify for any concomitant disease in horses with glanders from outbreaks recently occurring in Brazil. The experiment was carried out with the Ministry of Agriculture and the Sao Paulo State Animal Health Department; procedures were approved by the Committee of Ethics from the Instituto Biologico (CETEA n°156/2017). One horse from Sao Paulo State seropositive in the Western-Blot (WB) and in PCR for the *Flip* gene for glanders, presenting nasal fistula and rattling with purulent hemorrhagic discharge was euthanized. The serum was analyzed for antibodies anti-*T. gondii* using Indirect Fluorescence Antibody test (IFAT), (cut-off = 64) and resulted reagent to the titration 1:256. Digested brain was used for bioassay in VERO cells and tachyzoites were visualized in cell culture. Cell suspension and brain

were tested using a PCR protocol targeting the 529bp RE repetitive fragment of *T. gondii* genome and DNA was detected in both samples. Pathology analysis showed a mild non-purulent meningoencephalitis. Opportunistic agents as *T. gondii* might occur along with immunosuppressive diseases. In this report, toxoplasmosis was not confirmed, but its surveillance is suggested when in an epidemic of glanders, both being hazards to human health.

Keywords: Toxoplasmosis, equines, isolation, zoonosis, PCR, pathology.

P05

Comparison of Cystoisospora suis developmental stages in cell culture and a novel cell-free culture system

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Cystoisospora suis, an apicomplexan parasite of pigs, causes severe economic losses in the livestock industry worldwide. It is characterized by a complex life cycle, during which asexual multiplication, with sporogony and merogony, is followed by sexual development with two morphologically distinct cell types, the micro and macrogametes. These fuse to form a zygote and continue their life cycle. We hypothesize that the fusion of micro- and macrogametes is crucial to continue the whole life cycle and that this fusion occurs outside of the host cell. Sporozoite-infected IPEC-cell cultures already provide an established method for producing all stages of *C. suis in vitro*. Merozoites are found from day 6 post infection (dpi) onwards. Sexual stages occur between 10-12 dpi and occur mainly outside of the host cell. The first oocysts appear on 12 dpi. Continuing the life cycle of the parasite in a cell-free environment is a novel approach for studying the advanced stages of *C. suis.* To start the PBS-based cell-free cultivation, the supernatant of cell cultures including free merozoites is used at 6 dpi. From the supernatant of infected cell cultures, $1.2*10^4$ to $1.2*10^6$ merozoites per well were harvested, washed and further incubated in PBS at 40°C. Although merozoites cannot infect more host cells in the cell free culture, the life cycle of *C. suis* proceeds as the first unsporulated oocysts can be found on day 4 after transfer (dat). Sporulated oocysts can be found from 5 dat onwards. We conclude that potentially sexually committed *C. suis* merozoites might not require host cells to continue with gamogony and further development to oocysts. In further steps, the cell-free culture will be used for single cell mounting for electron microscopy and real-time quantitative PCR to quantify the transcription factors of *C. suis* development in vitro in small amounts of parasite cells.

P06

Investigation of Sarcocystis species infection in farmed fallow deer (Dama dama) from Lithuania

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Protists of genus *Sarcocystis* are apicomplexan parasites with an obligatory prey-predator two-host life cycle. *Sarcocystis* parasites employ various members of Cervidae as intermediate hosts by forming sarcocysts in their muscles. Reindeer, red deer, moose, roe deer and sika deer were exhaustively examined for *Sarcocystis* infection in Europe. No less than six *Sarcocystis* species were identified in these hosts. By contrast, *Sarcocystis* species diversity in fallow deer is not revealed due to the lack of researches. Fallow deer is introduced species in Lithuania. Currently these animals are bred for meat production and ornamental purposes as well as some animals are found in the wild. In 2018, diaphragm muscles of 48 farmed fallow deer were examined for the presence of sarcocysts. By light microscopy, *Sarcocystis* spp. infection prevalence was 81.3%. Overall, 36 sarcocysts were isolated and examined using 18S rDNA and *cox1* sequences analysis. By the means of molecular analysis, two species, *S. morae* (n=33) and *S. entzerothi* (n=3), were confirmed. The dominant species, *S. morae,* was recently found in one red deer from Spain. Meanwhile, *S. entzerothi* was identified in roe deer and sika deer. Results of the present study show relatively small *Sarcocystis* species variety while comparing to other cervids in the research area. Previously five *Sarcocystis* species were characterised in moose, six species were identified in roe deer and seven species were established in sika deer from Lithuania. Interestingly, *S. entzerothi* was confirmed only in the area of investigations. Further researches of *Sarcocystis* in fallow deer from broader geographical region are needed.

P07

Progress on the diagnosis of bovine besnoitiosis: Added value of IgM detection and low avidity index as indicators of acute disease

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Early *in vivo* diagnosis of bovine besnoitiosis is crucial for the success of control programmes. Diagnosis is particularly important in acutely infected animals but it is hindered by the low sensitivity available serological tools.
A novel ELISA to detect specific anti-*Besnoitia besnoiti* IgM antibodies was developed. Next, the usefulness of this tool and avidity ELISA were studied with a well-coded sera panel from experimentally and naturally infected cattle. First, the kinetics of specific IgM levels were determined in experimentally infected calves during the acute and chronic infection. Next, IgM levels were determined in naturally infected cattle with either acute or chronic infection. Finally, the IgG avidity index was monitored in both experimentally and naturally infected cattle.
Specific IgMs were detected prior to specific IgGs (7-19 days *vs.* 17-26 days post-infection). A prompt IgM response was associated with an onset of fever in experimentally infected calves. Naturally and acutely infected animals with clinical signs showed IgM-positive and IgG-negative results, followed by IgG seroconversion 2-3 weeks later. Chronically infected cattle showed IgM- and IgG-positive results. Moreover, a progressive increase in the avidity index (AI) was observed in all experimentally infected calves during the course of the experimental trials. However, low avidity values coexisted with visible tissue cysts until 90 days pi. In naturally and acutely infected cattle, low AIs were detected when animals seroconverted, in contrast to a high AI detected in chronically infected cattle.
In summary, IgM and avidity ELISAs improved the early *in vivo* diagnosis of bovine besnoitiosis. IgM-positive and IgG-negative results were indicative of an acute infection, whereas IgG positive results accompanied by low avidity values confirmed a recent infection. Further longitudinal studies are needed in infected herds to define IgG avidity usefulness in the design of control programmes.

P08

Endothelial injury and inflammation are key pathogenic mechanisms responsible for early azoospermia during acute besnoitiosis in bulls

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Bovine besnoitiosis, caused by the apicomplexan parasite *Besnoitia besnoiti*, is a chronic and debilitating cattle disease that notably impairs fertility. Acutely infected bulls may develop respiratory signs and orchitis, whereas sterility has been reported in chronic infections. The pathogenesis of the acute disease and its impact in reproductive function remain unravelled.
Herein we studied the microscopic lesions as well as parasite presence and load in the testis (pampiniform plexus, testicular parenchyma and scrotal skin) of seven bulls with an acute *B. besnoiti* infection. Bulls presented clinical signs compatible with acute besnoitiosis (e.g. fever and orchitis). Acute infection was later confirmed by serological techniques (IgM seropositive results and IgG seronegative results) and parasite detection by PCR and histological techniques.
The most parasitized tissue was scrotal skin. Moreover, tachyzoite presence evidenced by immunohisto-chemistry was associated to vasculitis and three bulls had already developed juvenile tissue cysts. Severe endothelial injury was evidenced by marked congestion, thrombosis, necrotizing vasculitis and angiogenesis, among others, in pampiniform plexus, testicular parenchyma and scrotal skin. Vascular lesions coexisted with lesions characteristic of a chronic infection: hyperkeratosis, acanthosis and a marked diffuse fibrosis in the dermis of the scrotum. An intense inflammatory infiltrate was also observed in testicular parenchyma accompanied by different degrees of germline atrophy in the seminiferous tubules with disappearance of various strata of spermatids in four bulls. We hypothesized that the azoospermia might be a consequence of: i) thermoregulation failure induced by the tachyzoite stage and vascular lesions; and ii) blood-testis barrier damage induced by an inflammatory response.

This study confirmed that severe acute besnoitiosis leads to an early sterility that might be permanent supported by the severe lesions observed

This study gives molecular clues on *B. besnoiti*- BAECs interactions and shows the progression of endothelial cell activation upon parasite invasion and proliferation.

P09

RNA-Seq analyses reveal that endothelial activation is associated with Besnoitia besnoiti host cell invasion and proliferation

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The pathogenesis of bovine besnoitiosis and the molecular bases that govern disease progression remain to be elucidated.
Thus, we have employed an *in vitro* model of infection based on primary bovine aortic endothelial cells (BAEC), a target cell culture of acute infection. Next, host-parasite interactions were investigated by RNA-Seq at two time points post-infection (pi): 12 hpi, when tachyzoites have already invaded host cells, and 32 hpi, when tachyzoites have replicated for at least two generations. All analyses were performed with four biological replicates and, in each replicate samples were collected for validation by RT-qPCR.
Up to 446 differentially expressed genes were found between both time points. Of these, 249 genes were upregulated and 197 genes were down-regulated at 32 hpi. The results showed an activation of endothelial cells with an upregulation of different leukocyte adhesion molecules, cytokines and chemokines predominantly at 12 hpi, and molecules involved in angiogenesis and extracellular matrix organization at both time points. NF-κB and TNF-α signalling pathways appeared to be mainly modulated upon infection, which would coordinate the expression of several effector proteins with a proinflammatory and pro-fibrotic phenotypes. These mediators would be responsible for macrophage recruitment setting the basis for chronic inflammation and fibrosis characteristic of chronic besnoitiosis. Angiogenesis regulation predominated and this multistep process was evidenced by the upregulation of markers involved in both early (eg. growth factors and matrix metalloproteinases) and late steps (eg. integrins and vasohibin). Key molecules with the highest fold change values and representative of the pathways mentioned above (eg. ICAM1, VCAM1, SELE, CCL2, IL6, ADAMTS1) were validated by RT-qPCR.

P10

Molecular characterisation and in vitro virulence phenotypes of Argentinean Neospora caninum bovine isolates

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Neospora caninum is an apicomplexan parasite responsible for cattle abortions worldwide. We have obtained two *N. caninum* isolates (i.e. NC-Argentina LP1 and NC-Argentina LP2) from dairy and beef herds, respectively. The aims of this study were to characterize these isolates by multilocus-microsatellite analysis and to assess their virulence phenotype *in vitro*. For *in vitro* studies, 10^5 VERO cells were grown/well of a culture plate and 10^4 purified tachyzoites of each isolate were added to each well. NC-Spain7 was used as high virulent control. The parasites were detected using an anti-SAG1-Alexa 488-conjugated monoclonal antibody. Invasion rate (IR) and tachyzoite yield (TY) were estimated. The IR was estimated at 4 h post-infection (p.i.) (IR_{4h}) (after medium change) and at 48 h pi (without medium change) (= total tachyzoite IR [IRT]), both determined as the total number of parasitophorous vacuoles/total well area. For TY studies, $2x10^5$ tachyzoites were cultivated for 48 h. Tachyzoites DNA was extracted and quantified by real-time PCR. Significant differences were detected in pair-wise analysis between IR_{4h} and IRT for the three isolates ($p<0.0001$), indicating that under the evaluated conditions, invasion continued after 4 h p.i. The IR_{4h} and IRT values were significantly

different (p=0.002 and p=0.042, respectively), where only LP1 showed lower IR_{4h} and IRT compared to control. Regarding TY, significant differences were found for LP1 and LP2, demonstrating lower values compared to control (p=0.028). The microsatellite pattern from both Argentinean isolates showed slight differences at MS5 and MS10; MS2 and MS10 of LP2 were reported in GenBank (MK674397 and MK674398). We showed that LP1 has very low IR and TY, being characterized *in vitro* as a low virulent strain. Despite having a high IR, LP2 isolate, showed a very low TY. Our results indicate that both parameters (IR and TY) are useful for strain characterization and may potentially predict *in vivo* behavior.

P11
Identifying the mechanism of action of Tartrolon E: A broad spectrum anti-apicomplexan compound

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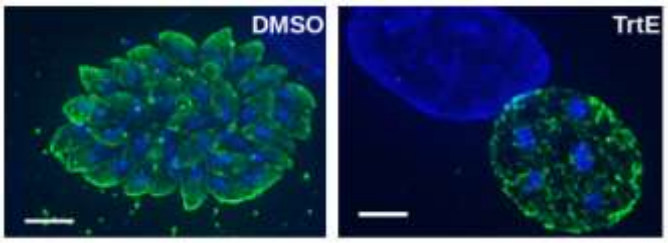
The apicomplexan parasite *Cryptosporidium* is the cause of the severe diarrheal disease, cryptosporidiosis. Cryptosporidiosis is one of the most important diseases of young ruminant livestock, particularly neonatal calves. Currently there is no treatment for cryptosporidiosis in neonatal ruminants, thus new therapeutics are a high priority. We have identified Tartrolon E (TrtE), a secondary metabolite derived from shipworm symbiotic bacteria to have broad-spectrum anti-apicomplexan parasite activity. TrtE inhibits *Cryptosporidium* at nM concentrations *in vitro* and is highly effective inhibiting infection *in vivo*. As *Cryptosporidium* is difficult to manipulate, we elected to investigate the mechanism of action of TrtE using the model apicomplexan parasite *Toxoplasma gondii*. Since repeated attempts to generate TrtE-resistant *T. gondii* mutants were unsuccessful, we investigated changes in the transcriptome of TrtE treated *T. gondii*. RNA-sequencing data revealed that the conserved gene TGME49_272370 is significantly upregulated within four hours of treatment, which suggested a role of TGME49_272370 in the parasite"s response to TrtE treatment. The gene TGME49_272370 encodes for a hypothetical protein product with multiple predicted transmembrane domains and phosphorylation sites. Antibodies reactive with TGME49_272370 gene product localize the protein to the apical region of the parasite. To examine the effect of TGME49_272370 on parasite susceptibility to TrtE, we produced a luciferase-expressing TGME49_272370 deletion mutant. Characterization of this mutant and its response to TrtE drug treatment is ongoing. In uncovering the mechanism by which TrtE inhibits *T. gondii* parasites, we may identify a shared pathway critical to apicomplexan parasite survival and advance the search for a new treatment for cryptosporidiosis.

Figure



P12
Eimeria bovis macromeront formation: The role of LDL

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Question: *Eimeria bovis* macromeront formation in bovine endothelial host cells is a highly cholesterol-demanding process. Apicomplexan parasites are generally considered as defective in cholesterol synthesis, consequently they scavenge cholesterol from their host cells either by enhancing the uptake of extracellular cholesterol sources or by up-regulating host cell *de novo* biosynthesis.
Methods: We here examined host cellular uptake of different low density lipoprotein (LDL) variants: non-modified (LDL), oxidized (oxLDL) and acetylated (acLDL) LDL. Furthermore, expression of oxidized lipoprotein receptor 1 (OLR1) mediating acLDL and oxLDL internalization was examined throughout first merogony. In addition, effects of inhibitors blocking exogenous sterol uptake (ezetimibe, poly-C, poly-I, sucrose) or intracellular transport and release from endosomes (progesterone, U18666A) on *E. bovis* macromeront formation were studied. As read-out, the size and number of macromeronts as well as merozoite I production were estimated.
Results: All LDL variants were internalized into *E. bovis*-infected BUVEC. Supplementation with oxLDL and acLDL boosted parasite replication and resulted in an increase of macromeront numbers and size. Furthermore, OLR1 expression was found significantly

enhanced in *E. bovis*-infected BUVEC when compared to non-infected controls. Ezetimibe treatments led to a highly significant blockage of macromeront formation and merozoite I production proving the relevance of sterol uptake for parasite development. Non-specific inhibition of LDL internalization via sucrose, poly-I and poly-C induced less prominent effects. In addition, the blockage of cholesterol transport via progesterone and U18666A treatments resulted in significant inhibition of parasite replication.

Conclusions: The current data underline the relevance of exogenous sterol uptake and intracellular cholesterol transport for adequate *E. bovis* macromeront formation.

P13

Transcriptomic analysis of mice brain infected with two Neospora caninum isolates from goats in Brazil

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Neospora caninum is an abortifacient protozoan that infect mainly cattle, and other ruminants, causing major economic losses in livestock production worldwide. The study of new strains can greatly contribute to the discovery of new approaches to control the parasite in animals. This study presents partial results of a PhD. research that describes the behaviour of two new isolates of *N. caninum*, from naturally infected goats, in a mouse model of infection. Previously results shown that the two isolates (NC-goat1; NC-goat2) causes encephalitis in mice. NC-goat1 showed to be more virulent than NC-goat2, with infected mice presenting higher count of CD3+ T lymphocyte infiltrates in brain. Mice were inoculated with 106 tachyzoites (IP) of each isolate. At 30dpi, total RNA was extracted from brain, processed for RNAseq (Illumina TruSeq RNA Sample Prep Kit), sequenced in a NextSeq 550-high throughput. 88.7% of readings had a Q score >30. Samples were analysed individually and in groups against control samples, using the Galaxy platform. Reads were aligned to mouse genome (mm9) with Hisat2, counted with FeatureCounts and differential expression analysis was made using the package Deseq2. The analysis revealed 256 up regulated genes for NC-goat1 infected animals and 226 for NC-goat2 infected animals. A gene onthology analysis revealed an enrichment of genes related to inflammatory response including: response to interkeukins, response to interferon gamma, leukocyte adhesion molecules, Tcell activation (including cytotoxic T cells), nitric oxyd byosythesis and others. The up regulation of genes related to inflammatory processes were more significative in animals infected with NC-goat1 comparing with those infected with NC-goat2. This study shows the importance of studying *N. caninum* encephalitis in mice, since the brain is the main target organ for parasite encystation and CD8+ T cells could be involved to cyst elimination during *N. caninum* encephalitis.

P14

Seroprevalence of Toxoplasma gondii and Neospora caninum in livestock in Tanzania

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Toxoplasma gondii and *Neospora caninum* are important causes of abortion and reproductive failure in small ruminants and cattle, respectively, worldwide. Little is known about the epidemiology and impact of these parasites on livestock production systems in Northern Tanzania. Serum samples were collected between January and December 2016 across six districts in Arusha region and four districts in Manyara region. Sera from 1010 sheep and 1420 goats were screened for antibodies to *Toxoplasma gondii* using an in-house ELISA incorporating RH antigen, and sera from 3007 cattle were screened for antibodies to *Neospora caninum* using an in-house ELISA utilising a recombinant SRS2 protein. Sample to positive (S/P) ratios were calculated for each test sample and cut-off values were determined by fitting a bi-modal normal distribution model to the observed S/P values. Antibodies to *T. gondii* were detected in 107 (10.6%; CI 8.8-12.6%) sheep and 211 (14.9%; CI 13.1-16.8%) goats. Antibodies to *N. caninum* were detected in 379 (12.6%; CI 11.4-13.8%) cattle. As part of a larger study, risk factors for seropositivity were identified using multi-level logistic regression. Restricted grazing and increasing cover with shrub or forest land were significantly associated with *N. caninum* seropositivity

in cattle. Identification of risk factors for *T. gondii* seropositivity is currently underway. Results of the study indicate that *N. caninum* and *T. gondii* infections are common in livestock in Northern Tanzania and could impact on production. There is also a potential public health risk of *T. gondii* infection if meat from infected sheep and goats is handled or consumed undercooked.

P15

Targeting Theileria effector proteins at the schizont membrane

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Theileria is a genus of tick-borne parasites. The two most important species, *Theileria annulata* and *Theileria parva* cause Tropical Theileriosis and East Coast Fever (ECF) in cattle, respectively. *T. annulata* and *T. parva* are equipped with the unique ability among eukaryotic microorganisms to convert their mammalian host cell into a transformed malignant stage. A striking difference between *Theileria*-transformed cells and cancer cells is that the cancerous phenotype seems not to be based on genome instability or DNA mutations. This implies that a multitude of parasite proteins must be altering host signaling pathways in parasite-infected leucocytes. Only very few effector proteins and their mode of action have been characterized so far, although several, including subtelomeric SVSPs and the secreted TashATs, have been hypothesized to interfere with the host cell. To investigate potential *Theileria* effector proteins passing the schizont membrane and to identify candidate proteins constituting a so far elusive export machinery, the TurboID proximity labeling technique was adapted to *in-vitro* cultivated *T. annulata* cell lines. For this, we utilized the promiscuous biotin ligase BirA* and fused it to different known host proteins hijacked by the parasite to the membrane surface. Affinity purification and liquid chromatography-tandem mass spectrometry (LC-MS/MS) coupled to a bioinformatic approach, identified several novel *Theileria* proteins, including several TashAT-like and SVSP proteins. Results of this approach will be presented, focusing on novel candidate proteins that might help explain the transformative nature of*Theileria*schizonts.

P16

Luminex-based serological determination of Toxoplasma gondii infection in chickens

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The detection of *Toxoplasma gondii* infections in animals relies primarily on serological assays. Here, we aim to establish a bead-based multiplex assay using Luminex technology for the specific and sensitive detection of *T. gondii* infections in chickens. Since assays based on this methodology enable the simultaneous analysis of one single biological sample with regards to multiple analytes the described assay may represent one component of future multiplex assays for avian serological monitoring.
Commercially available magnetic Luminex beads were coupled with streptavidin which subsequently bound recombinant biotinylated *T. gondii* surface antigen TgSAG1. Chicken serum antibodies recognizing TgSAG1 were detected by a fluorophore-coupled secondary antibody. Beads of a different color code were conjugated with anti-chicken IgY or chicken serum albumin and served as positive and negative control, respectively. The assay was validated with several hundred sera from naturally or experimentally infected chickens. Results were subsequently compared with those from previous reference testing methods, including other serological tests and bioassay in mice.
The vast majority of chickens that appeared seropositive in the Luminex test were shown to be also positive in a previously established ELISA. Moreover, examination of sera from chickens that appeared seronegative after mouse bioassays and in reference serological tests (immunofluorescence assay, modified agglutination assay) in general exhibited only background reactions in the Luminex TgSAG1 assay.
Taken together, the described Luminex TgSAG1 assay provides a suitable method for the detection of *T. gondii* infections in chickens with sensitivity and specificity comparable to other testing methods and exhibits the potential to be involved in future multiplex assays for avian serosurveillance.

P17

Besnoitia besnoiti-induced NETs damage host endothelial cells but do not alter parasite proliferation

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The obligate intracellular parasite *Besnoitia besnoiti* replicates *in vivo* mainly in endothelium and represents the causal agent of besnoitiosis in cattle.
Polymorphonuclear neutrophils (PMN) and endothelium are key players of host innate immune responses interacting with *B. besnoiti* stages during acute infection. Classical effector mechanisms of PMN include reactive oxygen species (ROS) production, phagocytosis and degranulation. In addition, PMN are able to extrude neutrophil extracellular traps (NETs) composed of chromatin (DNA + histones) and enzymes of granular origin that are able to ensnare and eventually kill pathogens.
Direct interactions of PMN or NETs with endothelium at physiological conditions indicated a critical role of PMN in the pathophysiology of endothelium impairment. However, information on NET-derived effects on parasite-infected endothelium *in vitro* and *in vivo* is scarce. Our previous data showed that *B. besnoiti* infections induced the following early innate immune reactions in primary bovine umbilical endothelial cells (BUVEC): *i)* increased gene transcription of adhesion and inflammatory molecules (ICAM-1, CXCL1, CXCL8, CCL5 and COX-2), *ii)* augmented PMN adhesion to BUVEC layers and *iii)* NET release under physiological flow conditions.
Current data show that *B. besnoiti*-triggered NETs, A23187-induced NETs and histone H2A induced cytotoxicity and damage in *B. besnoiti*-infected BUVEC. With respect to parasite intracellular development, *B. besnoiti* meront diameter and number per host cell were found diminished in treated BUVEC. However, the total tachyzoite proliferation over time was not significantly affected by NET-derived treatments, thereby denying a direct effect of NETs on intracellular *B. besnoiti* replication.

P18

In vitro overexpression of Babesia bovis hap2 protein induces morphological changes in transfected parasites

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Babesiosis is a tick-borne disease that infects animals and humans worldwide. Bovine babesiosis, mainly caused by *Babesia bovis* and *B. bigemina,* has an important economic impact in livestock due to the weight loss, abortions and lately death, which brings the development of an attenuated vaccine desired and much needed. *Babesia bovis* is an intra-erytrocytic protozoan parasite that requires two hosts to complete the life cycle: a mammalian host where asexual replications occur and a tick vector where a sexual replication occurs. The development of sexual stage forms in the midgut of the tick vector requires the differential expression of a set of specific proteins including hap2.
Question: Will a stable transfection of *B. bovis* overexpress hap2, a potential candidate for a transmission blocking vaccine, in in vitro erythrocytic culture?
Methods: A transfection construct was designed, containing the enhanced *green fluorescent protein-BSD* (*egfp-bsd*) fusion gene under control of the *B. bovis elongation factor-1α* (*ef-1α*) promoter and the *hap2* gene under the control of the actin promoter targeted for integration in the *ef-1α* locus of the parasite.
Results: Reverse transcriptase-quantitative PCR and sequencing were also performed to demonstrate that the *hap2* gene was stably integrated into the *ef-1α* locus of *B. bovis*. The expression of hap2 protein was demonstrated in transfected green fluorescent parasites by immunofluorescence using polyclonal specific antibodies against the hap2 protein. Transfected parasites overexpressing hap2 protein show morphological changes compatible with previously defined sexual forms.
Conclusions: These transfected parasites will be important tools for demonstrating the functional role of hap2 protein in the life cycle of *B. bovis*, and for the development of novel vaccines.

P19

Simultaneous and positively correlated bovine NET formation and autophagy in Besnoitia besnoiti tachyzoite and bradyzoite-exposed polymorphonuclear neutrophils

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Given that *B. besnoiti* infects host endothelial cells of vessels *in vivo*, they become potential targets for professional phagocytes [e. g. polymorphonuclear neutrophils (PMN)] when in search of adequate host cells or in case of host cell lysis. It was recently reported that *B. besnoiti* tachyzoites can efficiently be trapped by neutrophil extracellular traps (NETs) released by bovine PMN. So far, the potential role of autophagy in parasite-triggered ET formation is unclear. Thus, we here analyzed autophagosome formation in potentially ET-forming innate leukocytes being exposed to *B. besnoiti* tachyzoites and to bradyzoites obtained from a chronically infected animal.
Blood was collected from healthy adult dairy cows and bovine PMN were isolated via density gradient centrifugation. Scanning electron microscopy showed PMN to undergo NET formation upon contact with *B. besnoiti* tachyzoites and bradyzoites. Nuclear area expansion (NAE) and cell-free/anchored NET-related analyses confirmed parasite-triggered NETosis on a quantitative level. Interestingly, *B. besnoiti* tachyzoites and bradyzoites both induced LC3B-related autophagosome formation in parallel to NET formation in bovine PMN. In addition, isolated NETs induced by *B. besnoiti* tachyzoites failed to trigger autophagy thereby suggesting independence between both cellular processes. Finally, significantly enhanced phosphorylation of AMP activated kinase α (AMPKα), a key regulator molecule of autophagy, was observed within the first minutes of interaction in tachyzoite-exposed PMN thereby emphasizing that *B. besnoiti*-triggered NETosis indeed occurred in parallel to autophagy.

P20

Establishing a skin immune alarm to prevent tick-borne apicomplexan parasite transmission

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Ticks are main vectors of highly relevant apicomplexan parasites such as those in the genera *Babesia* and *Theileria*, representing major constraints to animal health and production worldwide.
Vaccines against the vectors, the pathogens or combining both would be cost-effective and more sustainable alternatives to anti-tick and anti-parasitic drugs. Furthermore, tick control by immunization can surpass acaricide resistance.
An important obstacle for the efficacy of these vaccines is the impressive capacity of ticks to influence the immune environment at the biting site. Very early after entering the host skin, ticks secrete molecules that block host defence mechanisms and favour their attachment and persistence in the host. For an anti-tick or anti-tick-born pathogen vaccine to be effective, it is thus important to impose an effective immune response before the tick-secreted immunomodulatory factors take control of the skin immune milieu. In this work, we explore immunization strategies to establish a fast and early alert of the immune system upon tick bite by localizing specific immune memory in the skin directed towards early-exposed tick antigens.
Tick-cement proteomics at sequential timepoints identified proteins expressed at early attachment and we are now cloning these antigens in a plasmid vector developed by our group for the expression of antigens in fusion with a bacterial lipoprotein, a TLR2/1 ligand.
In parallel, we are screening a panel of ligands of the innate immune system receptors to activate dendritic cells and evaluate their potential to imprint skin-homing properties to the specific lymphocytes.
By combining early exposed tick antigens with immunomodulators that can localize memory immune mechanism in the skin we expect to improve the efficacy of induced anti-tick immunity. Apicomplexan antigens will then be included in these formulations with the objective of effectively block transmission at entry site in the host.
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P21

Electron microscopical and immunocytochemical characterisation of multinucleated complexes formed upon exposure of Neospora caninum tachyzoites to the bumped kinase inhibitiors BKI-1294 and BKI-1748

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Bumped kinase inhibitors (BKIs) are a class of compounds that target calcium-dependent protein kinase 1 (CDPK1), which is involved in host cell invasion and egress in several apicomplexan parasites, including *Neospora caninum.* When BKI-1294 or BKI-1748 are added to infected host cell monolayers a few hours after infection has taken place, the compounds induce the formation of multinucleated complexes (MNCs). These MNCs were studied by SEM, TEM and immunofluorescence labeling employing antibodies directed against different parasite antigens, including an antiserum directed against CDPK1. TEM shows that MNCs are located within a parasitophorous vacuole (PV) and are enclosed by a parasitophorous vacuole membrane (PVM). The matrix of the PVs containing MNCs was composed of electron-dense granular material, in contrast to the matrix of PVs containing untreated tachyzoites, which was comprised of a tubulovesicular network. Immunofluorescence labeling of *Neospora* MNCs using specific antibodies showed that they exhibited a SAG1-positive outer membrane, and remained viable for extended periods of time during which they increased in size, and DNA-synthesis and nuclear division took place. However, newly formed zoites, which expressed an inner membrane complex (detected by anti-IMC1-antibodies) but no outer surface membrane (not stained by anti-SAG1 antibodies), remain trapped within the host cells, and the disjunction of these novel zoites from each other was inhibited. TEM also showed that zoites formed an anterior conoid and exhibited structurally intact mitochondria, and typical secretory organelles such as micronemes, rhoptries and dense granule organelles, whichwere detectable by immunofluorescence using antibodies directed against specific markers for these secretory organelles. In intracellular *N. caninum* tachyzoites, CDPK1 exhibited an even cytoplasmic localization. However, upon egress, the localization of CDPK1 shifted towards the anterior end of the tachyzoites. In MNCs treated with BKI-1294 for 6 days or longer, the expression of CDPK1 was downregulated, resulting in diminished fluorescence staining.

P22

Molecular Phylogeny and Diversity of Apicomplexans: An Aquatic Perspective

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Apicomplexans are obligate protist parasites that cause disease in a wide variety of aquatic and terrestrial hosts. While there has been extensive research on terrestrial apicomplexans, marine apicomplexans have received far less attention. Historically, sporocyst morphology has been used to identify these parasites, but recent studies suggest that molecular analysis is also needed for accurate identification. Recent phylogenetic analyses show that coccidians such as *Goussia* and *Eimeria* spp. from both terrestrial and marine hosts are polyphyletic, due to sporocysts morphology being used as a sole criterion for generic assignment in the absence of DNA data. In addition, recent molecular studies have found similarities between apicomplexans found in corals and blood parasites of fish. The present study uses DNA sequences of marine apicomplexans to clarify the evolutionary history of the group. Results confirm that some coccidian genera are polyphyletic, and that the parasitism of terrestrial hosts has likely evolved separately on multiple occasions. In addition, some coccidians appear to have returned to marine hosts from terrestrial groups. We have also confirmed the existence of two new clades of apicomplexans consisting of blood parasites of fish and apicomplexans found in corals. These new clades form as a robustly supported sister group to members of the suborder Adeleorina, which include many important veterinary parasites, such as the tick-borne apicomplexans *Hepatozoon* spp. that cause hepatozoonosis in numerous mammals.

P23

Risk factors associated with the occurrence of various tick-borne pathogens on livestock farms in the arid and semi-arid agro-ecological zones of Pakistan

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Question
Tick-borne diseases have a damaging impact not only on the animal health but also poses serious risks to human health resulting in heavy burden on national economy particularly in developing countries like Pakistan where livestock has a significant share in GDP. Although climatic and ecological conditions in Pakistan may favour the transmission of tick-borne pathogens (TBPs), to date, there is no study where the authors tried to identify the risk factors associated with the occurrence of TBPs.
Methods: A total of 3,807 ticks were collected from ruminant species on 108 livestock farms in the arid and semi-arid agro-ecological zones in Pakistan. Ticks were identified and screened for the presence of various tick-borne pathogens. The data on potential risk factors associated with the occurrence of TBPs on livestock farms was also collected on a predesigned questionnaire and was analysed using a univariable analysis followed by a multivariable logistic regression model with backward step-wise removal of non-significant variables in R.
Results: Risk factors analyses showed that presence of other TBPs on farm (OR=9.12, P=0.031), not using any acaricide for ticks control (OR=7.81, P<0.001), rural traditional housing system (OR=5.38, P=0.025), large herd size (OR=3.85, P=0.005), presence of livestock farm in the arid agro-ecological zone (OR=3.16, P=0.0341), presence of vegetation on farm (OR=1.65, P=0.002) and farm distance with the neighbouring livestock farms (OR=1.01, P=0.035) were found to be positively associated with the occurrence of *Anaplasma*, *Ehrlichia*, *Babesia* and *Theileria* species on livestock farms in Punjab, Pakistan.
Conclusions: The study reveals that there were several important risk factors associated with the occurrence of TBPs on livestock farms in Punjab that must be considered while designing a comprehensive control strategy to reduce the incidence of tick-borne diseases in the country.

P24

Bumped kinase inhibitor 1369 is effective against porcine cystoisosporosis at reduced treatment frequencies

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Repeated doses of bumped kinase inhibitors (BKIs) targeting *Cs*CDPK1 have been shown to be safe and highly effective in reducing oocyst shedding and diarrhea as well as improving body weight gain in piglets experimentally infected with *Cystoisospora suis*. However, repeated applications of BKI 1369 therapy against cystoisosporosis is not practical in the field. In the present study, the most appropriate time point for treatment and the efficacy of reduced treatment frequencies with BKI 1369 *in vitro* and *in vivo* were determined compared to the previous treatment regimen. Pre-incubation of sporozoites with BKI 1369 *in vitro* completely failed to inhibit the infection, unless treatment was prolonged post infection. The number of total merozoites detected 7 days post infection (dpi) did not differ in cultures treated immediately after infection or 3 dpi, suggesting that the molecular effects of BKI 1369 are not limited to inhibition of initial host cell invasion. Notably, a single treatment of infected cultures 2 dpi resulted in a significant dose-dependent reduction of total merozoites 7 dpi with drug concentrations ≥200 nM. In an experimental infection model, treatment of piglets 2 and 4 dpi with 20mg/kg BW BKI 1369 completely suppressed oocyst excretion. A single treatment on the day of infection or 2 dpi suppressed oocyst excretion in 50% and 82% of the piglets, respectively, and reduced the quantitative excretion in those that shed oocysts to 95.2% and 98.4% oocysts per gram feces. Moreover, significantly increased body weight gains and reduced numbers of diarrhea days were observed in BKI 1369 treated piglets compared to the mock-treated control piglets, irrespective of time points and frequencies of treatment. Given that the efficacy of reduced treatment frequencies with BKI 1369 are comparable to repeated applications, this could be considered as a practical therapeutic alternative against porcine cystoisosporosis, also under field conditions.

P25

Seasonal variation of Anaplasma marginale in Brangus breed in midwest Brazil

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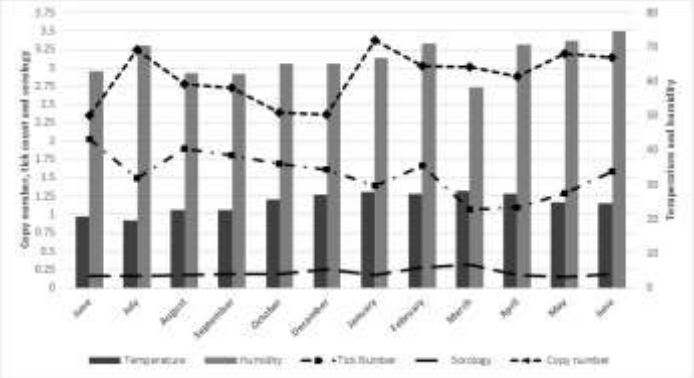
The *Rhipicephalus microplus* tick is an important vector of pathogens in the bovine cattle industry, being responsible for the transmission of diseases that can lead to serious economic losses. Among these pathogens are the *Babesia bigemina* and *Babesia bovis*, as well as the *A. marginale* that together are responsible for cattle fever tick complex, which can cause severe anemia, abortions and even death. This study related the number of ticks, number of circulating *A. marginale* and serology in Brangus cattle in the Brazilian savannah. At the Sanyo farm, in the Água Clara County, MS, Brazil (20 ° 46'24 'S latitude, 52 ° 32'24' W longitude, 309 m altitude) 11 Brangus animals aged 8 to 10 months infested naturally in the density of 0.6 animal units per hectare. The survey occurred from June 2016 to June 2017 with the interval of 18 days for tick counts, 36 days for blood collection and subsequent DNA extraction. The tick count was performed according to (WHARTON & UTECH, 1970). As screening, the presence of *A. marginale* was confirmed with the PCR technique (ECHAIDE et al., 1998) using the msp5 gene and later the qPCR technique was used (CARELLI et al., 2007) for quantification using the gene msp1b, and 95 bp Gblock as internal control. From the qPCR result, the number of DNA copies was calculated according to Ke et al. (2006). The ELISA test was performed according to Machado et al. (1997). There was a great variation throughout the year in the number of circulating copies, but without great alteration in the immune response and the absence of clinical signs of disease, suggesting enzootic stability. The profile of the relationship between number of ticks and number of copies of *A. marginale* suggests that the importance of tick presence is important in the process of transmission of the pathogen throughout the year.
Figure: Seasonal variation in number of ticks, number of DNA copies of *A. marginale*, Serology, Ambient temperature and Humidity during the year.

Figure



P26

Differential effects of trypsin and chymotrypsin under reducing or non-reducing conditions on Eimeria excystation and release of viable sporozoites – Relevance to intestinal site specificity

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Release of sporozoites from Eimeria oocyst/sporocysts is an essential step in the intracellular development of the parasite in its host. Little is known about this process except that elevated temperature (~ 40oC) plus trypsin and bile salts are required to for sporozoite discharge. In this study, it was found that adding the reducing agents, DTT or TCEP, was necessary for release of viable *E. maxima* sporozoites. While the addition of DTT or TCEP affected the apparent molecular weight of trypsin, it did not interfere with excystation of *E. maxima*, but rather had a temporal positive effect on sporozoite viability. This effect was time-dependent in that the number of intact sporozoites at 15 and 30 min after excystation was similar between untreated and DTT- or TCEP-treated sporocysts. However, by 45 – 60 min, virtually no sporozoites were observed in excystation fluid not containing DTT or TCEP. Of interest is that this effect appeared to be *Eimeria* species-dependent. *Eimeria acervulina* and *E. tenella* sporozoites remained viable for at least 60 min after excystation in the absence of DTT or TCEP. The effect of DTT and TCEP on chymotrypsin was also studied because there is some evidence that chymotrypsin is an effective excystation enzyme. Indeed, *E. maxima* sporozoites excysting with chymotrypsin in the presence of DTT or TCEP remained viable for at least 60 min after release, unlike excystation done in the absence of these reducing agents. Chymotrypsin was capable of excysting *E. acervulina* in the presence or absence of DTT or TCEP.

Of interest, is that chymotrypsin was ineffective in the excystation of *E. tenella*. These findings suggest that trypsin and chymotrypsin have differential effects on sporozoite excystation and that reducing agents may alter sites on the enzyme that affect sporozoite viability, but not release from sporocysts. These findings may reflect avian intestinal physiology and how *Eimeria* may have adapted to invading specific regions of the gut.

P27
EtROP2 is an early expressed kinase localisated in the rhoptry compartment of Eimeria tenella

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Coccidia are obligate intracellular parasites responsible for human and veterinary diseases. *Eimeria tenella* is an apicomplexan protozoan which is responsible for avian coccidiosis. This pathology induces a major economic loss for poultry industry worldwide. The parasite invades the digestive epithelial cells, which cause intestinal lesion that can lead to death. Until today, prophylaxis permitted to fight against coccidiosis by using chemotherapy and vaccination. But, with the apparition of resistance against anticoccidian molecules and with the high cost of vaccines, it appears necessary to improve these means of control.
During invasion, the parasite releases the content of rhoptries, apicomplexan specific secretory organelles. *Toxoplasma gondii* rhoptry protein repertoire includes kinases that are key virulence factors. Kinases are involved in the molecular dialogue between the parasite and host cell. This involvement allows them to modulate cellular functions and pathways allowing *T. gondii* development. *E. tenella* kinome is predicted to contain 28 putative ROP kinases. Among them, two predicted kinases were identified in the rhoptry proteome of *E. tenella* sporozoites. The first kinase EtROP1 is described as an active kinase that phosphorylates the p53 inhibiting the apoptosis of parasited cells and thus promoting the parasite development. The second kinase EtROP2 is an active kinase whose functions need to be characterized. This work shows the apical localization of EtROP2 by microscopy techniques and his early expression in the parasite life cycle. The EtROP2 study may reveal it as a good candidate in the race for the improvement of the means of coccidiosis control.

P28
Detection of antibodies to Toxoplasma gondii in oral fluid from pigs

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T.gondii infected pigs play a major role as a source of infection for humans. Detection of high-risk herds is essential to implement control measures and reduce seropositivity at the farm level. Oral fluid (OF) has been successfully used for detection of pathogens and antibodies in pigs and can be easily collected by hanging a cotton rope on the box for the animals to chew. The aim of this study was to detect antibodies against *T. gondii* in OF from pigs by immunoblot (IB) and to state if this method could replace standard serology. OF from experimentally infected sows (*n*=8) and naïve sows (n=3) (serial samples), and from group-housed fatteners (n= 42 groups, 1 sample/group) were analysed for IgG and IgA against *T. gondii*-SAG1 (P30) by IB. As standard of comparison, parallel collected serum samples from each animal were tested for anti-*T. gondii* IgG by ELISA. Specific IgG was detected in serum of all inoculated sows since 2 or 3 wpi; in 3.4 to 13% of the pigs in 11 groups and in 91 and 92% of the fatteners in 2 groups. The remaining 29 groups contained 100% of seronegative pigs. Inoculated sows showed positive IB results (IgG and IgA) at most time points from 1.5 to 4 wpi; however, at later time points positive results were intermittent. Interestingly, consecutive daily samplings (4 days) at 13 or 30 wpi showed inconsistent results. OF from groups with 91 and 92% of seropositive pigs yielded positive IB results (IgG and IgA). Naïve sows and fattener groups with ≤13% of seropositive pigs gave negative IB results. OF does not seem to be a robust matrix for detection of *T. gondii* antibodies. Variable sensitivity may depend on time after infection, % of seropositive animals/group and timely variations of antibody concentration in OF. Therefore, although OF may be used in epidemiological studies at the farm level, it is not equivalent to serum. In case of negative IB results on OF, a serological examination should be performed to rule out contact of swine with *T. gondii*.

P30

Abortion during a subsequent pregnancy due to endogenous transplacental infection of natural Neospora caninum postnatally infected cattle in the dry period

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Experimental studies suggest that postnatally infected adult cows fail to lead to endogenous transplacental transmission in subsequent pregnancies (Williams et al., 2000; Innes et al., 2001). However, in a herd without an obvious increased incidence of abortions, postnatally infected dams inseminated after the infection period, give birth to seropositive offspring indicating endogenous transplacental transmission (Dijkstra et al., 2008).
In the current study, cows calved in the period from 25-10-2004 to 12-02-2005 and calves born in the period from 05-01-2005 to 05-05-2005 the prevalence was respectively 74% (17/23) and 88% (7/8). This was in contrast of the 15% (15/99) prevalence of the other animals in the herd. Indicating that the cows in the dry period were postnatally infected. In the subsequent pregnancy in total 10 cows aborted of which 9 where Neospora seropositive.

Dijkstra T, Lam TJ, Bartels CJM, Eysker M, Wouda W. 2008. Natural postnatal *Neospora caninum* infection in cattle can persist and lead to endogenous transplacental infection. Vet Parasitol. 152:220-225.
Innes, E.A., Wright, S.E., Maley, S., Rae, A., Schock, A., Kirvar, E., Bartley, P., Hamilton, C., Carey, I.M., Buxton, D., 2001. Protection against vertical transmission in bovine neosporosis. Int. J. Parasitol. 31, 1523-1534.
Williams, D.J.L., Guy, C.S., McGarry, J.W., Guy, F., Tasker, L., Smith, R.F., MacEachern, K., Cripps, P.J., Kelly, D.F., Trees, A.J., 2000. *Neospora caninum*-associated abortion in cattle: the time of experimentally-induced parasitaemia during gestation determines foetal survival. Parasitol. 121, 347-358.

P31

Molecular identification of Sarcocystis hominis and other three Sarcocystis species in cattle meat from Lithuania

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The members of genus *Sarcocystis* are broadly distributed parasites of mammals, birds and reptiles. These apicomplexan organisms are characterised by two-host prey-predator life cycle. Up till 2015-2016 three *Sarcocystis* species, *S. cruzi*, *S. hirsuta* and *S. hominis* were known to form sarcocysts in cattle muscles. Nowadays, six species are characterised in these hosts, including *S. bovini*, *S. bovifelis* and *S. heydorni*. It should be noted that humans are definitive hosts of *S. hominis* and *S. heydorni*. Out of these two species, *S. hominis* was identified worldwide, causing nausea, vomiting, diarrhea and other intestinal disorders. Due to changes in the classification of *Sarcocystis* species in cattle, high *S. hominis* infection prevalence recorded is likely misleading. *S. hominis* might be misidentified because of morphological similarity with *S. bovini* and *S. bovifelis*. Furthermore, molecular data on *S. hominis* is still lacking. In 2018-2019, 102 diaphragm muscle samples of cattle bred in Lithuania were examined for *Sarcocystis*. Using meat trypsinisation, species-specific PCR targeting *cox1* and sequencing 100% of samples were positive for *Sarcocystis*. Four *Sarcocystis* species, *S. cruzi* (96.1%), *S. bovifelis* (71.6%), *S. hirsuta* (30.4%) and *S. hominis* (13.7%) were identified. Co-infections were frequently observed (75.5%): mostly, two species were diagnosed in the same sample (44.1%), followed by three (26.5%) and four (4.9%). Further researches on *S. hominis* epidemiology in various regions are needed.

P32

Serologic cross-reactivity between Sarcocystis neurona and Sarcocystis falcatula-like in experimentally infected Mongolian gerbils

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Sarcocystis neurona is the major cause of the equine protozoal myeloencephalitis (EPM) and has opossums of the genus *Didelphis* as definitive hosts. Most Brazilian isolates of *Sarcocystis* spp. that use opossums as definitive hosts behave similarly as *Sarcocystis falcatula*, which causes sarcocystosis in birds, and for this reason have been classified as *Sarcocystis falcatula*-like. Genes coding for the immunodominant surface antigens SAG2, SAG3 and SAG4 of *S. falcatula*-like are similar to those from *S. neurona*. The aims of the current study were to test the susceptibility of gerbils (*Meriones unguiculatus*) to experimental infections with *S. neurona* and S. *falcatula*-like, and to investigate potential serologic cross-reactivity to these parasites by immunofluorescent antibody test (IFAT) and Western blot (WB). A total of 27 gerbils, distributed in five groups (G1-G5), were employed in this work (G1: 4 negative controls; G2: 6 infected with *S. neurona* merozoites, G3: 6 infected with *S. falcatula*-like merozoites; G4 and G5: 5 and 6, respectively, infected with different doses of *S. falcatula*-like sporocysts). None of the 17 animals that seroconverted for the parasites presented any visualized organism or *Sarcocystis* DNA in the examined tissues. No serologic cross-reactivity was observed using IFAT. However, sera from animals infected with *S. falcatula*-like and *S. neurona* presented a similar pattern of antigenic recognition when *S. neurona* merozoites were used as antigen in WB, including reactivity to proteins of 30 and 16 KDa, regarded as specific markers for *S. neurona*-infected animals. Gerbils did not sustain infection by these parasites, although seroconverted after inoculation. These results indicate that gerbils inoculated with *S. falcatula*-like present serologic cross-reactivity to *S. neurona* in WB. IFAT was demonstrated to be more specific that WB for the detection of antibodies to *S. falcatula*-like and *S. neurona* in the experimental conditions of this study.

P33

Placental immune response and extracellular matrix organisation during the early stages of Neospora caninum infection in pregnant heifers inoculated with high (Nc-Spain7)- or low (Nc-Spain1H)-virulence isolates at mid-gestation

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Bovine neosporosis represents one of the most important causes of abortion in cattle. Early infection dynamics (at 10 and 20 days post-infection, dpi) have been recently investigated in heifers inoculated with high (Nc-Spain7)- and low (Nc-Spain1H)- virulence isolates of *Neospora caninum* at mid-gestation. Results revealed wider dissemination, higher parasite burdens and more severe lesions in placental and foetal tissues as well as foetal death in Nc-Spain7-infected animals. In the present work, placental samples from the previous experiment were studied by immunohisto-chemistry and qPCR in order to investigate elements of the innate and adaptive immune responses, endothelial adhesion molecules genes and components of the extracellular matrix (ECM). In summary, upregulation of Th1, Th2 and Treg cytokines and an extensive inflammatory response characterized by an inflammatory infiltrate of CD4 + and CD8 + T cells were found in all infected samples although the increase was more marked at 20 dpi. At 10 dpi, a general upregulation was observed only in Nc-Spain1H, triggering an immune response that may help to control the infection. Nc-Spain7 induction of immune responses was delayed until 20 dpi, which may facilitate the parasite earlier multiplication and dissemination causing an exacerbated immune response (mainly IFN-γ, iNOS, TNF-α and IL-12p40) that could contribute to foetal death. Moreover, IL-8, TNF-α and iNOS were upregulated in placental tissues from non-viable foetuses, suggesting that they may be directly related to *N. caninum* abortion. ECM components were upregulated only by Nc-Spain1H infection, suggesting the induction of tissue remodelling by this isolate.

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P34

Can an in vitro assay replace experimental infections to evaluate toltrazuril efficacy and resistance in Cystoisospora suis?

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The recent detection of resistance of *Cystoisospora suis* against toltrazuril (TOL), the only currently registered and effective anticoccidial for the use in piglets, has raised concerns about the sustained use of the drug and the lack of adequate methods to detect resistance in the field. So far, detection of TOL resistance is only possible in a controlled trial *in vivo* with experimental infections of piglets, which is time-consuming, expensive and requires the use of animals in large numbers. To address the requirement of improved resistance testing, a quantitative *in vitro* test was established which determines multiplication of *C. suis* in IPEC-cells during the asexual development (merozoite development assay, MDA). Seven days after infection of cells with sporozoites, parasites were harvested and subjected to quantitative real-time PCR to determine the number of copies of a reference gene in relation to a solvent control and a plasmid standard curve. A dose titration was carried out to determine the 95 and 50% inhibitory concentrations (IC95 and IC50) in a susceptible laboratory strain of *C. suis* for TOL and its metabolites, TOL sulfoxide and TOL sulfone. All three substances had similar IC95 values (20 µM). The IC50 was about 5 µM with considerable inter-test variations. Preliminary tests on a TOL-resistant strain showed reduced efficacy of all three compounds *in vitro*. Similar to *in vivo* studies, the dose-dependency of toltrazuril and its metabolites appears to be poor. However, for resistant isolates a "break-point" of efficacy at which a susceptible strain shows growth reduction by at least 90% while efficacy against resistant strains is 50% or less is conceivable. With sufficient material, the test can give hints at reduced efficacy of TOL in field isolates. Strain differences and isolates with suspected or confirmed resistance in the field will be evaluated further to validate the test.

P35

Improvement of propagation and reverse genetics on Cryptosporidium baileyi in ovo

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The Apicomplexan parasite Cryptosporidium is one of the world`s leading causes of diarrheal disease in many different species including humans. Serious infections are especially dangerous for immunocompromised hosts, which show severe diarrhea with a prolonged period of recovery and even chronic disease.
In veterinary medicine, cryptosporidiosis plays a crucial role as a perpetrator of diarrhea in neonatal calves and lambs. Besides C. baileyi, an avian specific Crypto species, which mainly affects young poultry, is causative for respiratory and gastrointestinal symptoms, reduced weight gain and growth rates. Moreover, there are indications that the immunosuppression during avian cryptosporidiosis limits the efficiacy of vaccinations against ubiquitous viral infections like Marek Disease or Avian Infective Bronchitis.
The most common systems for investigating Crypto infections, with C. parvum leading the way, still represent immunocompromised mouse models, as the parasites tends to show a premature termination of its life cylce in many in vitro systems used so far.
It was shown that C. baileyi can successfully be cultivated in chicken embryos and that the parasites fulfills its complete life cycle in ovo. Therefore, our aim is to elaborate this system to further investigate host pathogen interactions and crucial virulence genes.
Recently, draft genome sequences of C. baileyi have been annotated. The pioneering work of the Striepen Lab, where reporter genes could be successfully integrated into the C. parvum genome, gives hope to adapting several reverse genetic tools in C. baileyi, which allow the characterization of essential genes, such as protein degradation systems, inducible Cre-Lox-technologies and CRISPR/Cas9.
By refining the in ovo cultivation of C. baileyi we will be able to establish a highly effective, reliable, low cost and low maintenance model system for Crypto in general.

P36

Toxoplasma gondii atypical genotypes from synanthropic rodents in Argentina

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Toxoplasmosis is caused by the protozoan parasite *Toxoplasma gondii* that can infect a wide range of warm-blooded animals including humans. Synanthropic rodents as intermediate hosts of *T. gondii* can act as environmental sentinels of toxoplasmosis in urban and peri-urban areas. Rodents could contribute to the spread of this parasitosis in farm and wild animals with carnivorous habits, including the felids (definitive hosts). This is a report of molecular diagnosis and genotyping of *T. gondii* from 2 urban synanthropic rodents from Argentina. The rodents were one *Mus musculus* (IS 22, serologically negative) and one *Rattus norvegicus* (IS 24, serologically positive: IFAT titer 800). *Toxoplasma gondii* DNA was detected in both rodent central nervous system samples and were genotyped by multilocus nPCR-RFLP (eleven markers). The *T. gondii* genotype from IS 24 resulted type III allele for all markers except for L358 that was type II (genotype not registered in Toxoplasma Data Base). The genotyping from IS 22 showed both type II and III alleles from most markers, except for C29-2 and Apico thaw were type III. These results suggest a potential infection with 2 *T. gondii* genotypes in this rat. The results of this report revealed the presence of atypical isolates and mixed infections of *T. gondii* in synanthropic rodents from the studied area. The predominance of type III alleles is frequently observed in *T. gondii* isolates obtained from animals and humans from the same region.

P37

First evidence of clinical besnoitiosis in donkeys in Italy

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Besnoitiosis in equids, caused by *Besnoitia bennetti*, was historically limited to donkeys and horses in Africa. However, reports have suggested that besnoitiosis may be an emerging disease of donkeys in the United States (Ness et al. 2012, J.Am.Vet.Med.Assoc. 240:1329-37) and clinical cases were recently reported in Belgium (Lienard et al. 2018, Parasit. Vectors 11:427). Furthermore, the presence of *Besnoitia* spp. specific antibodies was detected in equids in Spain (Gutierrez-Exposito et al. 2017, BMC Vet.Res. 13:128) and in Italy (Villa et al. 2018, Parasitol.Int. 67:640-3), in areas where outbreaks of bovine besnoitiosis were previously reported.

A case of clinical besnoitiosis in donkeys, for the first time in Italy, was described. Two donkeys reared in Northern Italy (Brescia suburbs) and showing suspected skin lesions and poor body condition were clinically examined and endoscopy of upper respiratory tract was performed. Blood samples and skin biopsies were collected. Western Blot was performed to detect anti-*Besnoitia* spp. antibodies. A PCR targeting ITS-1 region and sequencing were performed on DNA extracted from skin biopsies. Furthermore, microsatellite analyses are in progress to characterize *Besnoitia* spp. species infecting these animals.

Clinical examination revealed the presence of numerous scleral pearls in both animals and also several skin nodules in region of neck, hind leg and on the pinnae. No cysts were detected in the nares and in the upper respiratory tract. Both animals resulted seropositive according to Western Blot results. Skin biopsies collected from both donkeys resulted positive for the presence of parasitic DNA. Sequencing demonstrated a homology of 100% with *Besnoitia* spp. sequences deposited in GenBank.

This is the first confirmed clinical case of besnoitiosis in donkeys in Italy and confirms the circulation of *Besnoitia* spp. in Italian equids. Further studies are needed to infer the relevance of the disease in equids in Europe.

P38

Experimental infection with Toxoplasma gondii in broiler chickens (Gallus domesticus): Seroconversion and distribution of the tissue cysts in brain, heart and skeletal muscle and prophylaxis

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Toxoplasma gondii is a widespread zoonotic protozoan that infects most species of mammals and birds. The aims of this study were to investigate the course of infection with *T. gondii* in chickens and the efficacy of Diclazuril and *Artemisia annua* in preventing *T. gondii* experimental infection. Seventy-five one-month-old chickens were randomly divided into 5 groups: NC (negative control, uninfected), group DC (infected with *T. gondii* cysts isolated from a domestic cat – genotype II), group WC (infected with *T. gondii* cysts isolated from a wild cat – genotype II/III), group DC-D (infected with *T. gondii* domestic cat strain, and treated with Diclazuril) and group DC-A (infected with *T. gondii* domestic cat strain, and treated with *A. annua).* The weight gain (WG), feed conversion ratio (FCR), clinical signs, body temperature, hematology and mortality rate were recorded in all groups. Seroconversion was evaluated by MAT on day 0, 3, 7 and 28 pi. On day 28 pi 5 chickens/group were euthanized and *T. gondii i*nfection evaluated by mouse bioassay and PCR (brain, heart, skeletal muscle). No clinical signs related to the experimental infection were observed throughout the study period, none of the chickens died following the infection with *T. gondii* and the recorded temperatures were within normal range. No statistical significance was recorded between the groups with regards to weight gain and feed conversion ratio, still the feed conversion ratio was lower in group DC-A on day 28. The hematological profile of the individuals revealed lower numbers of total leucocytes in the infected groups, and an increased percentage of monocytes in groups DC and DC-A. *T. gondii* specific antibodies were detected on day 28 pi in all infected groups. *T. gondii* DNA was detected in 80% of examined tissues in the infected and non-treated chickens and in 30% of examined tissues in the treated groups.
Keywords: *chickens, T.gondii, experimental, infection*

P39

Investigation of the Theileria parva sporozoite surface glycan repertoire and binding C-type lectin receptors

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East Coast Fever is one of the most important diseases in sub-Saharan Africa and kills annually more than one million cattle. The causative agent, Theileria parva, is a tick-borne apicomplexan parasite with obligate differentiation steps in bovine blood cells. Successful treatment of the disease with the antiprotozoal drug buparvaquone is impaired by increasing resistances and the current prevention strategy, the so-called "Infection and Treatment Method", is not cross-protective between different T. parva strains. High genetic complexity of the parasite, immunodominance as well as MHC class I restriction of the cytotoxic CD8+T cell response significantly limit traditional vaccine development. One potential solution are glycoconjugate vaccines based on sugar moieties of T. parva sporozoites. A recent analysis of the parasite transcriptome has revealed a wide variety of glycosylated proteins and previous investigations of the cattle genome identified genes for more than ten different lectin receptors. One of these receptors seems to be essential for the development of experimental cerebral malaria, a closely related apicomplexan parasite. In contrast, almost nothing is known about potential carbohydrate-based targets of T. parva sporozoites and the C-type lectin receptors of bovine cells that are crucial for the initial infection process. Therefore, we will illustrate the glycan repertoire of T. parva sporozoites that we were able to identify and highlight the binding affinities of these

targets to different C-type lectin receptors of the mouse. Methods used to gain these results include the screening of sporozoites with C-type lectin receptor (CLR)-Fc fusion proteins in an ELISA-based glycan array and a subsequent flow cytometry-based binding study to verify the results. We will present the identified C-type lectin receptors that recognise surface glycan targets of T. parva sporozoites.

P40
Identifying essential host proteins for Theileria-induced cell transformation

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Theileria spp. are tick-transmitted apicomplexan parasites responsible for severe leukoproliferative disorders in cattle, causing a substantial economic impact. Among these parasites, the most medically important species are *Theileria annulata* and *Theileria parva*. *Theileria* is unique in biology as it is the only eukaryotic cell known to fully transform its eukaryotic host cell. However, despite advances in identifying signaling pathways that the parasite hijacks, we only have limited knowledge about the essential host proteins that *Theileria* effectors interfere with. We therefore used a bioinformatic approach to identify potential effector proteins secreted into the bovine host cell. To discover whether these parasite proteins interfere with specific host signaling pathways we utilize selected protein-activatable reporter constructs. To complement these experiments, we also leverage recent advances in CRISPR/Cas9-based genome editing to identify host proteins potentially modified by candidate parasite effectors. To this end, we employ a forward genetic screen making use of a novel bovine genome-wide CRISPR/Cas9 library containing 85,155 guide RNAs (gRNA) targeting more than 20,000 bovine protein-coding genes. Using this tool, we are currently performing drop-out screens in *T. annulata*-infected bovine cells. We expect that the combination of a targeted, as well as an unbiased screening approach, will yield a more complete picture of host genes essential for the transformative state of schizont-infected cells. By linking parasite effector proteins to the most critical biochemical pathways for parasite survival, we anticipate new insights to develop therapeutic strategies to specifically target the parasite.

P41
Coccidiostatic effects of tannin rich diets in rabbit production

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Eimeria infections is one of the major health problems in rabbit breeding causing important economic losses. Generally, the infections remain subclinical with production consequences as reduced growth or weight loss; but can also be deadly, especially in young rabbits. Furthermore, by reducing the immunity of the host, coccidian infections could favour other diseases. The potential anticoccidial effects of tannin containing resources such as sainfoin and carob in rabbits feed were tested when given to does at pre-weaning and to growing rabbits. The trial started at parturition (D0), when 24 does and their litters were assigned into three groups. They were fed either with a control (Group CO), a carob (containing 10% carob pods meal) (Group CP) or a sainfoin diet (containing 34% dehydrated sainfoin pellets) (Group SA). All diets were made isoproteic and isoenergetic and also balanced for crude fibre but differed by their tannins content. Weaning occurred at D37, and growing rabbits remained in the same cage until D51. Then, they were transferred to fattening cages until the end of the trial (D104) and slaughtering. Weight gain of young rabbits among the three groups (mean = 31.2 g/d) did not differ statistically. The mortality rates were respectively 10% (SA), 15% (CP) and 20% (CO) but the differences were not statistically significant. Economical feed conversion ratio (FCR)post-weaning was reduced between rabbits of group SA compared to CO and CP groups. Faecal oocyst count (FOC) in group SA was 60% lower than in CO and CP groups. Areas under the curve (AUCs) calculated between sampling days and FOC, after rabbit transfer in fattening cage was 62% lower in group SA than in CO and CP groups. The main *Eimeria* species identified (from D59 to D83) was *Eimeria magna*(53% of oocysts). AUCs for *E. magna* did not differ according to diet. In conclusion, a diet containing sainfoin can reduce by 60% oocyst excretion of *Eimeria*spp and improved the economical FCR.

P42

Cytokine expression during in-vitro infection of sheep trophoblasts with Toxoplasma gondii tachyzoites

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Toxoplasma gondii is a ubiquitous apicomplexan parasite. It can infect all warm-blooded animals but only members of the Felidae family can act as final host. Vertical transmission of *T. gondii* affects a wide variety of species, including humans and small ruminants. Toxoplasmosis is an important cause of reproductive maladies in sheep, which translates into considerable economic loses for the industry. *T. gondii* infection in ewes can cause abortion, still birth, foetal death and congenital problems in lambs. The mechanisms of infection however are still not understood. The placenta conforms the first barrier against foetal infections. Amongst other factors, placental immunomodulation could play a role in *T. gondii* vertical transmission. The objective of this preliminary study is to elucidate cytokine expression in ovine trophoblasts during *T. gondii* infection using an in-vitro model. For this, ovine trophoblasts (AH-1 cell line) were seed in 24-well plates, after 48 hours, they were infected with 104 or 105 tachyzoites of *Toxoplasma gondii* (ME-49 strain, type II). Incubation times were set at 3 hours p.i., 6 hours p.i., 12 hours p.i., 24 hours p.i., and 48 hours p.i. Afterwards, RNA purification was performed using the RNeasy Mini kit (Qiagen, Germany). Reverse-transcription was conducted following the manufacturer"s instructions from the RevertAid first cDNA Synthesis Kit (Thermo Fischer, Germany). Finally, IL-1, IL-6, IL-12, and TNF-alpha expression were measured according to Rutigliano et al. (2015). X-fold changes were calculated in comparison to a negative control group. The preliminary results of this study could increase knowledge regarding placental *T. gondii* infection. They can set the basis for further research in an in-vivo ovine model.

References:

Rutigliano et al. (2015) Cytokine gene expression at the maternal-fetal interface after somatic cell nuclear transfer pregnancies in small ruminants. Reproduction, Fertility and Development 29:649-657

P43

Activities of endochin-like quinolones against in vitro cultured Besnoitia besnoiti tachyzoites

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Endochin-like quinolones (ELQs), previously shown to exhibit profound activities against *Plasmodium, Toxoplasma* and *Babesia*, are quinolone derivatives that target the cytochrome *bc1* complex and interfere in oxidative phosphorylation. The activities of 12 different ELQs against *B. besnoiti* tachyzoites grown in human foreskin fibroblasts (HFF) were assessed by quantitative real time PCR. Drugs were added concomitantly to infection, initially at 0.01, 0.1 and 1 µM, and subsequently the values for 50% proliferation inhibition (IC50) of the two most promising compounds, ELQ121 and ELQ 136, were determined in three-day growth assays. The IC50 of ELQ 121 (0.495 nM) was roughly 5 and 80 times higher than the reported IC50 values for *N. caninum* and *T. gondii.* ELQ 136 exhibited an IC50 value of 2.634 nM, which was 130 times (*N. caninum*) and 20 times (*T. gondii*) higher. Additionally, ELQ 316 and its prodrug ELQ 334, which had previously shown great potential in *T. gondii* and *N. caninum*, exhibited IC50 values of 7.972 nM and 46.785 nM, both also approximately 10 times higher than the corresponding values for *N. caninum*. However, the Qi and Qo drug-binding sites on the *cytochrome b* genes of all three species are virtually identical, indicating that the differential susceptibility is probably not due to differences in the cytochrome b target, but could be caused by differential uptake of compounds, or differences in parasite metabolism, or alternatively these differences could also be caused by potential off-target effects. All four compounds were evaluated in long-term treatment assays by exposure of infected HFF to ELQs at 2.5 mM concentration, starting 4.5 h after infection and lasting for up to 20 days, followed by the release of drug exposure. In these assays, the compounds caused a substantial delay in parasite growth/proliferation, but they did not exert parasiticidal activities. TEM of drug-treated parasites detected alterations within the parasite mitochondria as well as numerous vesicular structures within the tachyzoite cytoplasm. These findings encourage further research on the use of ELQs as therapeutic options against *B. besnoiti* infection.

P44

Serological survey on Neospora spp. and Besnoitia spp. in wild rabbits in Portugal

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Neospora caninum and *Besnoitia besnoiti* are closely related obligate intracellular cyst-forming coccidia. Both parasites are important pathogens of cattle, causing significant economic losses. While there is evidence that rabbits may be a reservoir species for *N. caninum*, their role in the sylvatic cycle of this parasite is poorly understood. As for *B. besnoiti*, though several species are experimentally susceptible to infection, in particular rabbits, no other hosts besides cattle have yet been identified. A serological survey was carried out to evaluate the presence of specific antibodies to *Neospora* spp. and *Besnoitia* spp. in naturally exposed rabbits in Portugal. The study involved a total of 385 wild rabbits (*Oryctolagus cuniculus*) hunted in different geographical areas. Sera were screened for both parasites by the Indirect Fluorescent Antibody Test (IFAT). Samples with a titre ≥ 50 were considered positive. The seroprevalence of *Neospora* spp. was 3.1% [95% confidence interval (95% CI): 1.8–5.4%]. The vast majority of *N. caninum* IFAT positive samples tested negative in *T. gondii* IFAT. Though a few sera showed some degree of fluorescence when tested for *Besnoitia* spp., none of them could be undoubtedly classified as positive. The present results show that rabbits are unlikely natural hosts for *B. besnoiti,* but suggest that that they can act as wild reservoir hosts of *N. caninum* for dogs and wild carnivores. Moreover, since infection in rabbits reflects environmental contamination with oocysts, this means that cattle grazing in the same areas may be exposed to horizontal transmission.

P45

Genotypes of *Toxoplasma gondii* circulating in free-range chickens, pigs and pregnant women in Benue state, Nigeria.

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The population structure of Toxoplasma gondii was initially described as highly clonal, but many isolates from Africa do not fit into the three major lineages. The present study was designed to establish the genotype, genetic diversity and phylogeny of T. gondii in free-range chickens (thigh muscle, n=173), pigs (thigh muscle, n=368) and pregnant women (blood, n=261) in Benue state, Nigeria. The presence of T. gondii DNA was ascertained using a published nested polymerase chain reaction (nPCR) approach, targeting the 529 bp multicopy gene element. DNA samples positive for *T. gondii* were subjected to quantitative-PCR (qPCR) to determine parasite load. Samples with the highest parasitaemia were selected for PCR-restriction fragment length polymorphism (PCR-RFLP) and sequencing, targeting the surface antigen 3 (SAG3), SAG2 (5' and 3'), beta tubulin (BTUB) and dense granule protein 6 (GRA6) genes, and the apicoplast (Apico). Sequences were subjected to in-silico digestion to assign RFLP genotype where PCR-RFLP results were ambiguous. SAG3 and BTUB sequences generated from the study were aligned with reference sequences to define genetic diversity and for phylogenetic analysis. *Toxoplasma gondii* DNA was detected in all three of the populations sampled, presenting 30.6, 31.3 and 25.3% prevalence in free-range chickens, pigs and pregnant women, respectively. Quantitative-PCR results indicated a low parasitaemia in most positive samples, limiting some further molecular analyses. PCR-RFLP results indicated that *T. gondii* circulating in the sampled populations were primarily of type II, with evidence of some hybrid type I/II and II/III haplotypes. Genetic analysis revealed limited diversity within the SAG3 and BTUB loci with four haplotypes identified in free-range chickens. Humans shared 2 and 1 haplotypes with free-range chickens and pigs respectively. Africa remains under-explored for *T. gondii* genetic diversity

and this study provides the first detailed definition of genotypes circulating in Nigeria.

P46

5-Aminopyrazole-4-carboxamide-based Bumped-Kinase Inhibitors 1770 and 1708: Moving to a preclinical candidate for cryptosporidiosis

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Effective treatment of diarrhoea and associated stunting caused by *Cryptosporidium parvum* and *C. hominis* requires new agents with improved efficacy and toxicity compared with current therapies. We previously described a series of low nanomolar 5-aminopyrazole-4-carboxamide bumped kinase inhibitors (AC-BKIs) of Calcium Dependent Protein kinase 1 that potently inhibit parasite growth and are effective in reducing oocyst shedding in *C. parvum* infected IFN-γ KO mice. The AC-BKIs were further optimized, using *in vitro* and *in vivo* PK and toxicity models, to improve clinical efficacy and safety. Two AC-BKIs, BKI-1708 and BKI-1770, emerged as potential preclinical leads for cryptosporidiosis therapy, with confirmed *in vivo* efficacy in mouse and calf models. Clinical health significantly improved in infected calves treated with either compound relative to untreated controls as well as better daily fecal consistency scores, reduced severity of diarrhoea and oocyst shedding. BKI-1770 also demonstrated efficacy in a gnotobiotic piglet model of *C. hominis* infection, recapitulating the clinical improvements observed in calves. When screened against a representative panel of human kinases, only MEK, PKD3 and RIPK2 were inhibited. BKI-1708 and BKI-1770 are non-toxic to mammalian cells, safely administered orally in mice up to 200 mg/kg body weight for 7 days, and lack hERG activity at therapeutic doses. Additional advanced biorelevant studies to determine cardiac safety in anesthetized rats and dogs resulted in no observable effects on mean arterial pressure, heart rate, systemic vascular resistance, cardiac output and contractility, and the ECG at tested concentrations. Metabolites of the two preclinical leads have been identified and are currently being analyzed for activity and safety. These preclinical compounds have pharmacokinetic properties for effective treatment outcomes and safety parameters to be considered for both veterinary and human health use.

P47

Cryptosporidium parvum infection alters glucose transport in infected enterocytes

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Cryptosporidium parvum causes enteropathies in humans and animals all over the world. Although a lot of work has been conducted on cryptosporidiosis in recent years, the parasite-host interactions are still not completely understood. The intestinal brush-border membrane is crucial for the uptake of nutrients, e.g. glucose and galactose. The parasite is able to destruct parts of this membrane, so we were interested in investigating whether glucose transport mechanisms of the host cells are influenced by the presence of *C. parvum*. IPEC-J2 cells were infected with *C. parvum* oocysts and the success of infection was ensured by quantification of the hsp70 gene expression using qPCR and immunofluorescent staining of *C. parvum*. The gene expression levels of glucose transporter (GLUT) 1 and 2 and Na+-coupled glucose transporter (SGLT) 1 were compared in infected and uninfected cells 24, 48, 72 and 96 h p. i. by RT-qPCR. Additionally, the protein expression of SGLT 1 was quantified in Western blot studies. While the protein expression of SGLT 1 was not altered in infected cells, gene expression of SGLT 1 and GLUT 1 was significantly increased 24 h p. i. The gene expression of GLUT 2 was significantly decreased at nearly all time points measured and this decrease correlated significantly with the infection dose. Our results indicate that an adaptation of the host cells is taking place in the acute phase of the infection. To answer the question, whether this is initiated by host cells in unspecific response to infection or by modulatory stimuli of the parasite, further investigations need to be conducted. However, in animal husbandry, an early onset of therapeutic oral administration of high glucose solutions is of imminent importance. A better understanding of the mechanism

involved in regulation of glucose transport during cryptosporidiosis could help to design new therapeutic options.

P48

Zoonotic Cryptosporidium species and subtypes in lambs and goat kids in Algeria

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Question: Little is known on the occurence and identity of *Cryptosporidium* species in sheep and goats in Algeria. This study aimed at investigating the occurrence of *Cryptosporidium* species in lambs and goat kids younger than 4 weeks.
Methods: A total of 154 fecal samples (62 from lambs and 92 from kig goats) were collected from 13 sheep flocks in Médea, Algeria and 18 goat flocks across Algiers and Boumerdes. They were screened for *Cryptosporidium* spp. by nested-PCR of a fragment of the small subunit (SSU rRNA) rRNA gene, followed by RFLP and sequence analyses to determine the *Cryptosporidium* species present. *C parvum* and C. *ubiquitum* were further subtyped by sequence analysis of the 60 kDa glycoptrotein gene.
Results : C*ryptosporidium* spp. were detected in 17 faecal samples (11.0%): 9 from lambs (14.5%) and 8 from goat kids (8.7%). The spaecies identified included *C. parvum* in 3 lambs, *C. xiaoi* in 6 lambs and 6 goat kids, and *C. ubiquitum* in 2 goat kids. C*ryptosporidium* infections were detected mostly in animals during the first two weeks of life (7/8 for goat kids and 7/9 for lambs) and in association of diarrhea occurrence (7/17 or 41.2% goat kids and 7/10 or 70% lambs with diarrhea were positive for C*ryptosporidium* spp.). Subtyping of *C. parvum* and C. ubiquitum isolates identified the zoonotic IIaA13G2R1 and XIIa subtype families, respectively. Minor differences in the SSU rRNA sequences were observed between C. xiaoi from sheep and goats.
Conclusion: Results of this study indicate that C*ryptosporidium* infection occurred in lambs and goat kids in Algeria including zoonotic species. Cryptosporidial infection seemed to be a potential cause of neonatal diarrhea in these animals within the country.

P49

Assessment of a yeast based vaccine system for oral delivery of Eimeria tenella antigens in commercial layer chickens

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Oral vaccines are relevant to the control of enteric diseases such as coccidiosis, caused by *Eimeria* parasites, as they can deliver specific antigens to the gastrointestinal tract and stimulate an appropriate mucosal and systemic immune response. A number of antigens have been identified that are protective against *Eimeria tenella* challenge when delivered by recombinant protein or DNA vaccination; these include apical membrane antigen 1 (AMA1) and immune mapped protein 1 (IMP1). Now, a major challenge is to find an effective method for flock-level delivery. The aim of this study was to use a yeast based system to express recombinant protein for oral vaccine delivery and assess vaccine efficacy *in-vivo*. AMA1 and IMPI coding sequences were cloned and expressed in *Saccharomyces cerevisiae*, prior to inactivation and delivery by oral gavage to commercial layer chickens at 7, 11, 15 and 19 days of age. Chickens were challenged with 250 *E. tenella* oocysts at 22 days of age and samples collected five days later. Parasite replication in the caeca was assessed by qPCR for *E. tenella* gDNA, normalised against chicken β-actin. Parasite replication was reduced in chickens immunised with *S. cerevisiae* expressing either AMA1 or IMP1, or a combination of both antigens, compared with those immunised with *S. cerevisiae* alone (negative control). Reduction in parasite replication was comparable with those vaccinated intramuscularly with recombinant IMP1 protein (positive control). The use of a yeast based system for delivering *E. tenella* antigens appears to be effective at reducing parasite replication in the caeca. If successful in larger trials, this system would provide a

scalable non-GMO oral vaccination strategy with no requirement for a consistent cooling chain.

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P50

Sources of bovine sarcocystosis at farm: An innovative approach

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Sarcocystosis is a parasitic disease caused by protozoan coccidian parasites. Several experimental evidences have suggested that *Sarcocystis* species are responsible for bovine eosinophilic myositis (BEM) lesions.
This study has an innovative and exploratory nature and focuses on sources of cattle contamination in farms. The objective is to determine the different species of *Sarcocystis* in the farm environment, in relation with farming systems.
A case-control study has been conducted on 60 farms in six different areas of France. The first step consisted in a qualitative investigation based on a detailed questionnaire, to describe precisely the breeding management system in the selected farms. The second line of action consisted in taking environmental samples (pasture grass, water and soil) to detect and identify environmental cattle *Sarcocystis* species in different zones in farms at risk (positive for BEM) and control farms. For each sample, a Multiplex PCR protocol targeting the 18s RNA gene and mitochondrial COI gene was applied to determine the different *Sarcocystis* species.
A statistical analysis using multivariate models has been carried out to identify risk factors which significantly (at a 5% threshold) increase the probability for a farm to be a case.
The results have highlighted several significant risk factors, in particular linked to grazing/breeding activities.
Sarcocystis species differences found in the environmental was not correlated to the status of farms. However, the presence of *Sarcocystis* in pasture grass (irrespective to the species) was found to be a significant risk factor.
This exploratory study is to be considered a first step and it would be interesting to complete the results with additional investigations.
Acknowledgements

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Novel in vitro models of Besnoitia besnoiti infection based on primary bovine aorta endothelial cells, fibroblasts and BVDV coinfections in endothelial cells

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In vitro models of *Besnoitia besnoiti* infection in target cells are desirable to address the cellular and molecular basis of the host-parasite interactions, as well as to test safety and efficacy of potential drug or vaccine targets.
In the present work, two primary bovine cell cultures, bovine aorta endothelial cells (BAEC) and fibroblasts that are target cells during the acute and chronic infection, respectively, were obtained and characterized by flow cytometry to study *B. besnoiti* infection. Two key aspects were considered: i) the employment of primary cells and ii) the health status of the donor animals was carefully checked, verifying the absence of bovine viral diarrhoea virus (BVDV), a common cattle pathogen and frequent contaminant of foetal calf serum.
Morphology and cytometry results evidenced the endothelial and fibroblast origins, since each cell culture showed a distinct expression pattern of intracellular and surface markers. CD31 surface marker better discriminated between BAEC and fibroblasts, since fibroblasts lacked CD31 labelling. On the contrary, low or absence of CD34 labelling together with positive labelling for CD44, vimentin and cytokeratin was observed in both cell lines. Next, the lytic cycle of *B. besnoiti* tachyzoites was characterized in BAEC and fibroblasts. Despite low invasion rates (around 3-4%) found in both cell cultures, higher values were obtained in BAEC at 24 and 72hpi. In contrast, proliferation kinetics did not differ between BAEC and fibroblasts. When the influence of BVDV on *Besnoitia in vitro* behaviour was investigated, BVDV infection favored early *Besnoitia* invasion of host endothelial cells, which slightly influenced on proliferation kinetics.
In conclusion, we have provided two standardized *in vitro* models based on bovine primary target BAEC and fibroblasts. Moreover we have showed the relevance of

BVDV coinfections, which should be considered in further *in vitro* studies with other cattle pathogens.

P52

Anti-Toxoplasma gondii antibodies profile in female sheep of the State of Paraíba, Brazil: From birth to sexual maturity

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Toxoplasmosis is a zoonosis of worldwide distribution that affects man and homeothermic animals, caused by the protozoan *Toxoplasma gondii*. This parasite is responsible for congenital diseases, spontaneous abortions and stillbirth in some species, as in sheep. Although outbreaks of abortion are considered important, descriptions of these outbreaks in sheep in Brazil are rare. Studies have shown a strong association between the presence of antibodies in the mothers and the protection of the fetus to *T. gondii* infection during gestation. The objective of the study was to estimate the prevalence of *T. gondii* infection in sheep from the Northeast region of Brazil and to determine if these animals acquire the infection in the first year of life before the beginning of the reproductive phase. This was done by monitoring the dynamics of anti-*T gondii* antibodies, by the indirect immunofluorescence antibody test (IFAT ≥64) in the serum of the animals from birth to 12 months of age, obtained once a month. Fifty-six crossbred females" sheep and their offspring (61 offspring), belonging to 7 rural properties, with representative management of most farms in the region, were evaluated. Of the 61 lambs studied, 55.7% (34/61) were susceptible to infection at the end of the 12 months, since only colostral antibodies were detected or the samples remained negative throughout the study. The remaining 44.3% (27/61) or were born infected (3/61, 5%) or infected in the first year of life (24/61, 39.3%). Antibody titers ranged from 64 to 65,356. The analyzed variables (presence of cats, type of water provided, type of sheepfold, sanitary management and sewage inside the property) were not associated with the serum conversion of the sheep. The descriptive analysis also did not identify a more common period of serum conversion of the animals. The study concludes that around half of the ewes at the age of one year are susceptible to *T. gondii* infection during pregnancy.

P54

The Theileria annulata schizont recruits nuclear-pore containing membranes to its surface

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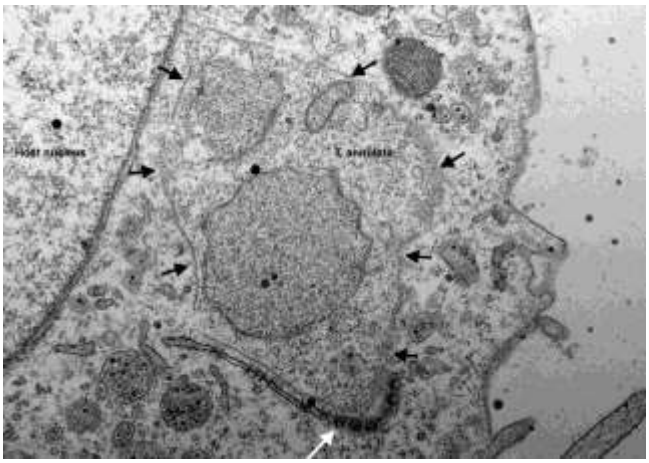
Theileria annulata, the causative agent of Tropical Theileriosis, is an intracellular parasite which modifies host cell signaling to such an extent that infected cells acquire cancer like properties. Following invasion of a leukocyte, sporozoites rapidly escape from the surrounding host membrane and differentiate into multinucleated schizonts. The schizont resides in the host cytoplasm where it interacts closely with microtubules and recruits several adaptor and signaling molecules to its surface. To date, little is known about the molecular mechanisms by which cellular transformation is achieved, and still less is understood about how the parasite and its host communicate with one another. We have previously defined several host-parasite protein complexes that occur at the schizont surface that likely play a role in regulating microtubule dynamics. Now, using transmission electron microscopy, we show that host derived membranes which are embedded with pores associate closely with the parasite surface during interphase. These pores morphologically resemble nuclear pores, and to confirm this we prepared cryosections according to the "Tokuyasu method" for immunoelectron microscopy. Using high resolution fluorescent microscopy and live cell imaging, we demonstrate that a large group of nuclear pore complex proteins and nuclear trafficking machinery accumulate in a cell cycle dependent manner at the lobes of the schizont surface. The function of these cytoplasmic porous membranes, referred to as annulate lamellae, remains unclear, and here we discuss several hypotheses that might explain their presence in *Theileria*-transformed cells.

Figure



P55

Effect of the microbiota on the development of the parasite Eimeria tenella

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Coccidiosis is caused by the parasite *Eimeria,* the first parasite in poultry farms. *E. tenella,* the most virulent specie colonize the caeca rich in bacteria. Our objective was to study the impact of intestinal microbiota on *E. tenella* infection. An original model of germ-free and conventional broilers was developed. While using the same dose of *E. tenella inoculum,* germ-free chickens excrete 4000 fold less oocysts than conventional chickens 6 days post-infection. This lower parasite load in caecal tissues is measurable as soon as 2 days post infection. Several biological processes in the parasite cycle may explain this result (excystation, invasion, development, …). As the excystation is the first step of the parasite cycle, *in vitro* capacity of excystation of the parasite using intestinal contents and biles of chickens was studied. When using duodenal contents and biles from germ-free chickens, the excystation rate of sporozoites was higher in contrast to those from conventional chickens. To shunt the excystation step *in vivo*, chickens were infected with sporozoites by cloacal inoculation. Parasite invasion after 24h infection was similar between germ-free and conventional chickens. However, after 7 days of infection, there was still less parasite excretion and parasite tissue load in germ-free chicken compared to conventional chicken (500 fold) suggesting that the microbiota and its metabolites may play an important role on parasite replication. We also studied the development stages of the parasite by using specific gene of different parasite stages. A delay of schizonte and gamete specific gene expression was observed in caeca of germ-free chicken compared to conventional chickens. Overall, these data suggest that the microbiota and its metabolites may play an indirect role on bile content and subsequently on the excystation rate and, also on the parasite development. Consequently, modulation of microbiota composition could influence coccidiosis severity.

P56

Adaptative changes in recently obtained Toxoplasma gondii isolates after in vitro cultivation

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Toxoplasma gondii is the causative agent of toxoplasmosis in humans and animals. Most research on *T. gondii* is based on isolates that have been maintained in tissue culture for long periods of time. Under these conditions, there are evidences that parasite may go experience an adaptation process with important changes in its phenotypic characteristics. Mechanisms involved in adaptation are poorly studied, but it could be of relevance to know the effect of passage-adaptation on isolates phenotype. In this study, we investigated the adaptation of 4 isolates recently obtained from sheep abortions (*n*=3) and adult swine myocardium (*n*=1) of the genotypes #3-type II-*PRU* variant (TgShSp1 and TgShSp3), #1-clonal type II (TgShSp2) and #2-clonal type III (TgPgSp1), respectively. We investigated the tachyzoite growth and their capacity to form cysts during 40 successive passages *in vitro* by means of direct microscopy counting. Also we studied the capacity to develop cysts by detection of BAG1 and CST1 bradyzoite antigens comparing passage 10 (*p*10) to *p*50, spontaneously and under stress conditions (pH). Results showed a rapid adaptation with significant phenotypic changes after only 20-30 passages. Tachyzoite yield (TY) increased in the majority of isolates whereas spontaneous tachyzoite to bradyzoite conversion (TBC) decreased with the passage number. TY and TBC rate were dependent on the genotype and isolate. Interestingly, adaptation also decreased TBC rate under stress conditions. Extensive BAG1 and CST1 (DBA) expression in the parasites also suggests the development of a "pre-bradyzoite" stage in low-passage isolates.

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Bioinformatic and experimental identification of a MEN/Hippo pathway in Toxoplasma gondii

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The Hippo pathway and the mitotic exit network are signaling vias that share homologous proteins and functions. In metazoans, Hippo is involved in mitosis, tissue growth and differentiation, while, in unicellular eukaryotes, MEN regulates cytokinesis and mitosis. As a unicellular eukaryote, T. gondii transitions between several cellular stages during its life cycle. Notably, its ability to interconvert between tachyzoite and bradyzoite stages is instrumental for its success as a parasite. It permits parasite survival by establishing a chronic infection and enabling recrudescence. This conversion, related to cell cycle regulation, involves, among other events, cell differentiation and division rate regulation, aspects common to those that are regulated by Hippo and MEN. We hypothesized that a MEN/Hippo pathway is present in T. gondii and participates in the tachyzoite-bradyzoite stage conversion process. To identify its members, we searched the T. gondii genome using BLAST and MEN/Hippo characterized proteins from S. cerevisiae and H. sapiens. This method identified nine probable homologs among eleven proteins searched. We also employed the STRING database to detect probable interactors of the T. gondii MOB1 kinase, which identified seven kinases, one protein with a TBC-domain and one phosphatase, previously identified through homology. To assess experimentally the T. gondii MEN/Hippo via, we developed an assay for the identification of the MOB1 interactome using BioID. We detected seven MOB1 partners: GRA5, ribosomal proteins L13, S18 and S23, two transmembrane proteins and one protein containing a structural maintenance of chromosomes domain. Our bioinformatic data show the presence of most MEN/Hippo core members, with one confirmed by STRING, suggesting this via is conserved and could be active in T. gondii. However, the BioID data points to a MOB1 role that also reflects the specificity of this parasite's biology. Funding: UID/CVT/00276/2019, SFRH/BD/101619/2014.

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Molecular detection of Toxoplasma gondii: Is one marker enough?

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The development of simple, sensitive and rapid methods for the detection and identification of *Toxoplasma gondii* is pivotal for the diagnosis and epidemiological studies of this zoonotic disease. The protocols targeting conserved repetitive elements of the genome, such as the 529 bp element and the B1 gene, are largely considered the most reliable. Recently different Loop Mediated Amplification (LAMP) protocols targeting SAG1 and SAG2 genes have been proposed. This latter, in particular, demonstrated to be a promising screening method, paralleling the nested PCR (nPCR) assay based on the amplification of the B1 gene. During a study on the presence of *T. gondii* in sheep milk, we were able to detect the parasite DNA (n=17) with the SAG2 LAMP assay, but only 4 samples resulted positive by B1 nPCR amplification and none with the 529 bp PCR assay. The positivity of the samples, however was confirmed by amplification and sequencing of the GRA6 gene. To further support the result, 529bp and B1 nPCR were repeated, on the same extract, by three different laboratories, with the same results. Therefore, a set of samples from wildlife tissues (n=87) tested negative by 529 bp and B1 nPCR assays in previous studies, was re-submitted to SAG2 LAMP and GRA6 PCR assays. Out of the 87 samples tested, 84% (n=73) resulted positive to the SAG2 LAMP assay, although only 30% (n=26) were confirmed by GRA6 fragment sequencing. LAMP technique has demonstrated to be highly sensitive and particularly resistant to inhibitors which can prevent standard PCR reactions. However, this technique is also known to be susceptible to false positives and downstream processing of the amplification products is somewhat

cumbersome. However, in at least 30% of the samples considered negatives after the 529 bp PCR and B1 nPCR assays was demonstrated the presence of *T. gondii* DNA. These results highlights the need of multiple target assays to improve the sensitivity of *T.gondii* detection.

P59

IFN-γ production by bovine adipose tissue stromal vascular fraction cells upon in vitro stimulation with Neospora caninum tachyzoites

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Neospora caninum is a worldwide etiologic agent of clinical infections in diverse animal hosts, assuming particular economic relevance in cattle. Resistance to neosporosis has been associated with the production of IFN-γ. In previous work we observed that adipose tissue (AT) stromal vascular fraction (SVF) cells isolated from mesenteric AT (MAT) of WT mice responded to *in vitro* stimulation with freeze-killed *N. caninum* tachyzoites (NcT) by producing IFN-γ. Moreover, higher IFN-γ levels were detected in culture supernatants of NcT-stimulated SVF cells isolated from MAT and subcutaneous AT (SAT) of one year-infected mice indicating parasite specific long-term memory. Therefore, we aimed here at determining if, in the bovine host, seropositivity to *N. caninum* could be associated with higher production of IFN-γ in AT. For this MAT and SAT SVF cells of animals seropositive and seronegative to *N. caninum* were cultured in the presence of NcT and IFN-γ quantitated in the culture supernatants. Higher levels of this cytokine were detected in cells isolated from MAT upon stimulation with NcT in only a fraction of the analysed animals. No difference was detected in IFN-γ levels between seropositive and seronegative animals. Overall our results show that in bovines, contrastingly to our previous observations in mice, MAT SVF cells of only a fraction of animals respond to *N. caninum* by producing IFN-γ.

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P60

Metabolic requirements of Besnoitia besnoiti tachyzoite-triggered NETosis

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Besnoitia besnoiti is the causative agent of bovine besnoitiosis, a disease affecting both, animal welfare and cattle productivity. NETosis represents an important and early host innate effector mechanism of polymorphonuclear neutrophils (PMN) that also acts against *B. besnoiti* tachyzoites.
So far, no data are available on metabolic requirements of *B. besnoiti* tachyzoite-triggered NETosis. Therefore, here we analyzed metabolic signatures of tachyzoite-exposed PMN and determined the relevance of distinct PMN-derived metabolic pathways via pharmacological inhibition experiments.
Overall, tachyzoite exposure induced a significant increase in glucose and serine consumption as well as glutamate production in PMN. Moreover, tachyzoite-induced cell-free NETs were significantly diminished via PMN pretreatments with oxamate and dichloroacetate which both induce inhibition of lactate release as well as oxythiamine, which inhibits pyruvate dehydrogenase, α-ketoglutarate dehydrogenase and transketolase, thereby indicating a key role of pyruvate- and lactate-mediated metabolic pathways for proper tachyzoite-mediated NETosis. Furthermore, NETosis was increased by enhanced pH conditions, however, blockers of MCT-lactate transporters (AR-C141900, AR-C151858) failed to influence NET formation. A significant reduction of tachyzoite-induced NET formation was also achieved by treatments with oligomycin A (inhibitor of ATP synthase) and NF449 (purinergic receptor P2X1 antagonist) thereby suggesting a pivotal role of ATP availability for tachyzoite-mediated NETosis.
In summary, the current data provide the first evidence on carbohydrate-related metabolic pathways and energy supply to be involved in *B. besnoiti* tachyzoites-induced NETosis.

P61

Do dendritic cell derived exosomes play a role in Toxoplasma gondii infection?

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Exosomes are extracellular vesicles of 30-100 nm released from vacuoles of the endocytic pathway, selectively packed with various molecules (miRNAs, proteins, lipids) and constitute themselves a vehicle of cell-cell communication. Exosomes have been shown to have various functions, namely in immune cell activation and suppression and recently in parasite-host cells communication. Dendritic cells (DCs) are pivotal players in host defense, able to detect pathogens and drive the adequate adaptative immune responses to counteract infection. Exosomes released by DCs (DEX) have been found to express functional MHC class I and II and T-cell co-stimulatory molecules on their surface, which built the idea that DEX could be used for vaccination purposes. On the other hand, DEX are selectively packed with different molecules (ex. miRNAs), depending on the DC maturation status, which raised the question whether DEX may act as a vehicle of DC-DC communication, namely in the context of infection. To test this hypothesis, we purified exosomes released by T. gondii pulsed DCs, visualised by atomic force microscopy and determined their protein content by western blotting. We are now evaluating if DCs pulsed with these exosomes have altered responses to different stimuli and how these exosomes influence DCs susceptibility to infection. In the future, the microRNA profile of the exosomes will be characterised and the impact of these exosomes in *in vivo* infection using the murine model will be evaluated.

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P62

Programming mucosal-homing lymphocytes to block Toxoplama gondii oral infection

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Toxoplasma gondii is one of the most widespread parasites worldwide and has an important impact in animal production, causing abortion in sheep and goats and neonatal mortality in pigs. With the exception of a live attenuated vaccine used in some countries to prevent abortion in sheep, there are no vaccines to prevent animal or human toxoplasmosis.

The development of novel and improved vaccines against *T. gondii* infection must rely not only on a more accurate identification of relevant target antigens, but also on the capacity to induce the correct protective immune mechanisms at the port-of-entry and/or at the sites of persistence of the parasite. Given that, with the exception of vertical transmission, *T. gondii* infection is universally acquired through the gut mucosa, strategies to localize there specific memory immune mechanisms are of particular relevance.

Recently, the activation of dendritic cells through pattern recognition receptors (PRR) has been shown to play a role in lymphocyte homing and the Toll-like receptor 2 (TLR2) has emerged as a key receptor for mucosal imprinting of immunity. In the past, we established a cloning system in *E. coli* to produce antigens in fusion with a TLR2 ligand, the OprI lipoprotein from *Pseudomonas aeruginosa*. In this work, we screened a panel of innate stimuli to address how they act in combination with the OprI lipoprotein to equip DCs with the potential to imprint mucosal-tropism in lymphocytes, and how those characteristics correlate with the potential to polarize lymphocyte differentiation. Through this screening, we identified two stimuli combinations for alternative lymphocyte polarization with mucosal-homing potential. Those molecules will be combined with *T. gondii* SAG1 expressed in fusion with OprI for immunization experiments in a *T. gondii* ME49 oocysts infection model developed by our groups to evaluate their potential to block *T. gondii* entrance at the gut mucosa.

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P63

Toxoplasma gondii strain and dose effect on body weight, serum antibodies response and systemic distribution in intraperitoneally infected domestic turkeys

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Domestic turkeys represent a very important food source for humans and they also serve as an important intermediate host of the zoonotic parasite *Toxoplasma gondii*. In the present study, 24 four-week old, female domestic turkeys were separated into four treatment groups. Each of the groups was intraperitoneally infected with either a virulent or avirulent strain at one of two doses ($1X10^5$ or $1X10^8$). In addition, 10 birds were injected with sterile PBS only and served as a negative control group. The study aimed to investigate the parasite tissue tropism, seroconversion time line, weight gain and feed conversion in relation to the parasite dose and strain. Birds were monitored twice daily throughout the experiment. Clinical signs, weight, and mortality were recorded. Serum samples were collected every week starting the day before the infection until day 42 of the experiment and then every other week until 95days post infection. Sera were tested with the modified agglutination test to detect *T. gondii* antibodies. At termination of the study, multiple tissue samples were collected and tested for *T. gondii* by qPCR and light microscopy using H&E staining. Clinical signs and weight gain of the birds were related to the dose and strain of *T. gondii* used to infect the birds. Birds infected with $1X10^8$ tachyzoites of either strain showed the most severe clinical signs and the highest and earliest antibody conversion. The group infected with the higher dose of the virulent strain showed lower weight gain compared to other groups. Brain tissues were the most commonly infected tissue as determined by qPCR. qPCR will be compared to histopathology and results will be discussed. These results demonstrate that *T. gondii* is an important disease of domestic turkeys, especially birds that are raised outdoors in organic or backyard propagation facilities.

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Effect of meat processing on viability of Toxoplasma gondii: Towards replacement of mouse bioassay by in vitro testing

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Felines are the definitive hosts of *T. gondii* and primary infection results in fecal shedding of infectious oocysts. Infected intermediate hosts will develop tissue cysts, which are infective to both cats and intermediate hosts. Meat containing viable tissue cysts is considered one of the main sources of human infection. In contrast to fresh meat, raw meat products usually undergo processing, including salting and mixing in additives such as acetate and lactate, which affects the viability of *T. gondii*. However, the experiments currently described in literature, are not always performed in line with the processing methods applied in industry. Therefore we aimed to study the effect of salting and additives according to the recipes used by commercial producers. Mouse or cat bioassay is the gold standard to demonstrate the presence of viable *T. gondii*. However, it is costly, time consuming and for ethical reasons not preferred for large-scale studies. Therefore, our second aim was to develop an alternative for mouse bioassay that can be used to determine the effect of processing on the viability of *T. gondii* tissue cysts. We focused on a tissue culture method to determine the parasite's ability to multiply, and a PMA-based assay to selectively detect DNA from live cells. Results with the PMA-based method were inconsistent and did not sufficiently discriminate between live and dead parasites. The tissue culture method showed promising results, but further optimization is needed before it can replace or reduce the number of mouse bioassays needed. Small scale experiments with minced meat incubated for 20h with low concentrations of salt, lactate and acetate showed a large but incomplete reduction of the number of infected mice. In future, in vitro methods are needed to allow more extensive testing of product-specific

processing methods, thereby providing a better indication of the risk of *T. gondii* infection for consumers.

P65

Bumped kinase inhibitor BKI-1748: Studies on in vitro efficacy, safety and in vivo effects in pregnant mice infected with the highly virulent N. caninum isolate Nc-Spain7

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Neospora caninum is one of the major causes of abortion in cattle and thus exerts considerable economic impact. Bumped kinase inhibitors (BKIs), which target calcium dependent protein kinase 1 (CDPK1), represent a promising class of compounds that are active against numerous apicomplexans species. *In vitro*, BKI-1748 added concomitantly to infection of human foreskin fibroblast (HFF) monolayers with *N. caninum* tachyzoites impaired parasite proliferation with an IC50 of 68 nM. When BKI-1748 (2.5 µM) was added to already infected HFF monolayers, treatment efficacy was much lower, but resulted in the formation of large multinucleated schizont-like structures that contain newly formed zoites, which were unable to separate and form tachyzoites. These multinucleated complexes remained largely viable for several weeks. As *N. caninum* tachyzoites are vertically transmitted, it is essential that BKI-treatment does not interfere with embryonic development and pregnancy outcome. BKI-1748 was therefore assessed for potential interference in embryo development using a Zebrafish (*Danio rerio*) model. Analysis for lethality/malformations demonstrated no embryonic growth interference within a time frame of 96 hours with BKI-1748 treatments at concentrations up to 10 µM, while embryo development impairment was evident at 20 µM and above. In pregnant mice treated by oral gavage with either 50, 20 or 5 mg/kg BKI-1748 emulsified in corn oil during days 9-13 of pregnancy, the highest concentration resulted in severe loss of offspring, while 20 and 5 mg/kg did not impair neither fertility nor pup survival. Based on these results, we are currently performing a BKI-1748 treatment study in pregnant mice that are infected with 10^5 *N. caninum* Nc-Spain7 tachyzoites at day 7 of pregnancy, and are then treated with 20 or 5 mg/kg/day for 5 days, starting 2 days post infection. We will present data on clinical signs, fertility, litter size, neonatal and postnatal mortality.

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Treatment with bumped kinase inhibitor 1294 alters Neospora caninum protein expression, which lead to a diversified humoral immune response in N. caninum infected mice

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Bumped kinase inhibitors such as BKI-1294 target apicomplexan calcium-dependent protein kinase 1 (CDPK1), a protein kinase that is involved in host cell invasion and egress. CDPK1 is encoded by apicoplast DNA, and thus an interesting drug target. In vitro, the drug inhibits host cell invasion, and induces the formation of multinucleated complexes (MNCs) by interfering in the process of completing cytokinesis. However, the drug does not act parasiticidal. MNCs are composed of several parasite nuclei and newly formed zoites with an inner membrane complex but lacking a SAG1-positive outer membrane. They form a schizont-like organism that remains trapped within the host cell, but intact tachyzoites with an outer SAG1-positive membrane are formed readily and resume proliferation once the drug is removed. In spite of the lack of parasiticidal activity, BKI-1294 dramatically reduced cerebral infection and vertical transmission in mice. IFA and RT-PCR showed that these MNCs exhibit a dysregulated antigen expression pattern compared to non-treated tachyzoites. Thus, we hypothesize that BKI-1294 treatment induces the expression of other antigenic parasite proteins that could potentiate the immune response. We developed a procedure for enrichment of MNCs and performed comparative

proteomics of MNCs and tachyzoites. Several proteins, expressed at distinct levels, were identified. In addition, MNC and tachyzoite extracts were subjected to SDS-PAGE and Western blotting using mouse sera from *N. caninum*infected mice, either treated or not with BKI-1294. 6 protein bands that could be detected only or much stronger in MNC extracts were excised and analyzed by LC-MS.Further investigations are now focusing on proteins specifically recognized in MNC extracts in order to elucidate their identity and their role in the host-parasite interaction. This could lead to the identification of novel immune-relevant antigens and potential candidates for further vaccination studies.

P67
Preliminary validation of IgM-capture ELISA for early detection of Besnoiti besnoiti in cattle

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Besnoitia besnoiti (*Bb*), an apicomplexan parasite closely related to *Toxoplasma gondii* and *Neospora caninum*, is the cause of bovine besnoitiosis, mainly transmitted by blood sucking insects. Besnoitiosis was recently classified as an emerging disease in Europe. It causes economic loss (reduced milk production, weight loss, infertility) and increased mortality.
Serological tools such as ELISA, western blot or IFAT play an essential role for diagnosis. In ELISAs, IgG Ab are detected 5 to 6 weeks post-infection. To date, there is no Bb tests with good diagnostic performance during the febrile/acute phase of infection: Bb detection by qPCR on whole blood in the early stages of infection is unconstant and therefore unreliable, making differential diagnosis difficult.
Could IgM antibodies be a good marker of acute Bb infection ? This study will present the first data of different Bb IgM ELISA prototypes (indirect or IMAC) using samples from experimental infection as well field samples.
2 bovine (A and B) were inoculated with *B. besnoiti* at 1x104 and 2x107 tachyzoites per mL, respectively, by intradermic and intravenous ways, and were bled at different days post inoculation (dpi). Only one IgM ELISA allowed IgM Ab detection from 11 to 42 dpi (A) and from 7 to 42 dpi (B), whereas IgG Ab were detected as of day 32 (A) to 21 (B).
59 cattle samples from a high prevalence infected herd were tested in parallel by qPCR, IgM and IgG ELISAs. 8/59 samples, qPCR positive, were IgM positive whereas they were IgG-negative.
As a conclusion, experimental results confirm that IgM antibodies are present in Bb infection If their persistence could be quite short (around 4 weeks), they seem to appear in the early stages of infection and could be detected 4 weeks before IgG. Preliminary data obtained on field samples indicate that Bb IgM Ab ELISA could potentially be used as an early detection tool for Besnoitiosis.

P68
Biological tools to study Toxoplasma gondii in its definitive host

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Toxoplasmosis is a zoonotic disease caused by the protozoan *Toxoplasma gondii*. Up to a third of the global human population is estimated to carry a *T. gondii* infection, which can result in severe complications in immunocompromised individuals and pregnant women [1]. *T. gondii* can infect humans but also any warm-blooded animal and its definitive host is the *Felidae* family (mostly cats) where it undergoes its sexual reproductive cycle with the release of infectious oocysts.
Most of the studies on *T. gondii* rely on infectious tissue cysts or tachyzoites which can be obtained either by the use of laboratory intermediate hosts or by cell culture, respectively. However, working on oocysts is trickier as it involves the experimental infection of cats. Therefore, there are still unrevealed parts of the sexual cycle of the parasite, due to the difficulties to obtain such oocysts.
In the last couple of years, with the approval of the local ethic committee (Anses/EnvA/UPEC), representing French Ministry of Research and Innovation, we performed vaccination and challenge trials on cats with a genetically modified vaccine candidate against *T. gondii*. Despite an immunogenic and safe vaccine candidate, it did not protect animals from oocysts shedding after the challenge with a wild-type strain of *T. gondii* [2]. This challenge mimicking a first natural infection, the antibodies produced after it, protect the cats from a second oocysts shedding. Therefore, at the end of the experimental trials, the animals have been

included in an adoption program, within the larger frame of a general agreement between the National Veterinary School of Alfort and animal-care associations for the retreat and welfare of laboratory animals. These trials allowed us to develop an expertise in obtaining oocysts from experimentally infected cats, which the scientific community can benefit from.

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A base-exchange type phosphatidylserine synthase is essential for the lytic cycle of *Toxoplasma gondii*

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Phosphatidylserine (PtdSer) is a universal lipid, which is involved in a variety of functions such as, apoptosis, membrane potential, protein sorting and secretion in mammalian cells. Among these multiple functions, some depend on the acidic nature and negative charge of PtdSer, which allows it to interact with calcium and cationic domains of several proteins. Additionally, to these characteristics, its biophysical characteristics help maintain the membrane potential across organelle membranes. Herein, we have identified the parasite enzyme underlying PtdSer synthesis in tachyzoites of T. gondii. The protein localizes in the membranes of the endoplasmic reticulum. Our efforts to delete the PSS gene concluded to its inability to successfully knockout it, something that suggests its essential nature during the lytic cycle, which is also consistent with the phenotypic score (-4.8) in ToxoDB. Conditional regulation of PSS by tetracycline or Shield1 significantly reduced the synthesis and amount of PtdSer in tachyzoites, corroborating its role in PtdSer biogenesis. Surprisingly, however, both mutants survived almost complete depletion of PSS concurrent with a reduction in PtdSer, likely due to compensation by other lipids including phosphatidylthreonine and posttranslational control of PSS activity. In future work, the PSS gene will be tagged with an auxin-inducible degradation (AID) domain, which we believe has a chance to deliver a strain with a severe phenotype that will be useful for further characterization. Additionally, we are now implementing a Cre-LoxP-based gene excision method to demonstrate the essentiality of the PSS gene. Our work also will expand towards making a double mutant of PSS and PtdThr synthase to resolve the functional relationship between the two related lipids.

Keywords
Toxoplasma gondii, phospholipid synthesis, phosphatidylserine synthase

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Molecular epidemiology of cryptosporidiosis in Zambia

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Cryptosporidium spp. infections are major cause of severe diarrheal diseases in young children and immune-compromised individuals especially in sub-Saharan African countries. Nevertheless, it is largely neglected and molecular epidemiological study have not been performed in Zambia. In this study, to understand the epidemiology of Cryptosporidium infection in urban setting of Zambia, 48 microscopically confirmed cryptosporidiosis stool samples from the central hospital in Lusaka were used for molecular analysis. Two loci encoding a small subunit (SSU) rRNA and a 60kDa glycoprotein (GP60) were used for species identification and subtyping. The sequence analysis of the 18SrRNA gene identified *Cryptosporidium hominis* (54%), *C. parvum* (42%), *C. felis* (2%) and *C. meleagridis* (2%). The sequence analysis of the *gp60* gene identified 4 subtype families (Ia, Ib, Id, Ie) from *C. hominis* and 2 subtype families from *C. parvum* (IIc, IIe). The major subtypes were Ie (35%) and IIc (24%) in *C. hominis* and *C. parvum*, respectively. Since *C. hominis* and *C. parvum* IIc and IIe are considered to be anthroponotic, our result indicated that Cryptosporidiosis in the urban setting Zambia can be mainly transmitted from human to human. The identification of *C. felis* and *C. meleagridis* suggest an atypical zoonotic transmission cycle also exist in Zambia. Further investigation including peri-urban, rural setting will be required to understand epidemiology, transmission cycle to better control of the disease.

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Molecular survey for cyst-forming coccidia (Toxoplasma gondii, Neospora caninum, Sarcocystis spp.) in Mediterranean periurban micromammals

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Rodents and other micromammals are important reservoirs of infectious diseases; their role in the life cycle of cyst-forming coccidia as *Toxoplasma gondii*, *Neospora caninum* and *Sarcocystis* spp. still needs clarification. We analyzed, by PCR and Sanger sequencing methods, the presence of specific parasite DNA within brain and heart tissues of 313 individuals of five synanthropic small mammal species (*Apodemus sylvaticus*, *Mus spretus*, *Mus musculus*, *Rattus rattus* and *Crocidura russula*) collected in Barcelona metropolitan area (NE Spain). Specific DNA of *T. gondii*, *N. caninum* and *Sarcocystis* spp., was detected in 0.32% (*n* = 1), 1.27% (*n* = 4) and 3.83% (*n* = 12) of the animals, respectively. No mixed infections were observed. The brain can be highlighted as the tissue with the highest detection rate, and *Crocidura russula* as the main *Sarcocystis* spp. host. *T. gondii* DNA detected in an individual house rat was genetically characterized by RFLP-PCR, presenting type II and III alleles (*SAG1* [II], *SAG3* [III], *GRA6* [II], *c22-8* [III], *Apico* [III]). *N. caninum Nc5* specific sequences showed up to 97% homology with those deposited in GenBank (EU0735991, KU253799), and microsatellite typing analysis is still in progress. Likewise, *Sarcocystis* spp. identity was studied by *cox1* sequence alignment of 14 positive samples resulting in an unknown organism closely similar (95.14%) to *S. canis* (KX721495) and *Sarcocystis canis*-like (KX721496); no *cox1* sequences belonging to the expected *S. (Frenkelia) glareoli* or *S. (Frenkelia) microti* are yet deposited in public databases. Prevalence figures found in this first survey carried out in Spain are in agreement with other international studies also focused on periurban areas. Further surveys should be conducted in farms and surroundings in order to unravel the role of wild micromammals in the epidemiology of such protozoan parasites.

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An in vitro system to study heteroresistance and metabolic host interaction on mature tissue cysts of *Toxoplasma gondii*

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Persisting stages of *T. gondii* are a key source of transmission and acute toxoplasmosis but cannot be targeted with currently available treatments.
Because access to mature cysts is limited to in vivo models the mechanistic basis of this resistance is hard to study and identification of compounds that target tissue cysts efficiently remains challenging. To address both problems we optimized the generation of mature *T. gondii* cysts in vitro. To this end we infected terminally differentiated human myotubes as natural host cells with a wide range of parasite strains. Indeed, these cells support long-term culture of cysts, including type I RH parasites, without interfering growth of tachyzoites.
Confirming their phenotypic similarity to in vivo cysts, our cysts lack expression of the tachyzoite antigen SAG1, develop pepsin resistance after three weeks and survive one week-long treatments with high doses of Pyrimethamine, Sulfadiazine, the quinolone HDQ and bumped-kinase inhibitors against CDPK1. Our EM-based ultrastructural analyses indicate broad distribution of polysaccharide stores, parasite packaging densities and proliferation within the same cyst, suggesting a heteroresistance mechanism against these compounds. Interestingly, host cell mitochondria associate with the vacuoles of type 2 parasites in these cells. This contrasts observations in fibroblasts and we are currently investigating implications on lipid uptake using mass spectrometry-based metabolomics.
Summarized, we identified a human host cell line that can be used to raise *T. gondii* tissue cysts that are functionally similar to in vivo cysts. Our method will facilitate future studies on bradyzoite biology and enable the identification of bradyzoidal compounds.

Keywords: bradyzoites, phenotypic heterogeneity, metabolism, host-parasite interaction

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The mode of action of *T. gondii* tissue cyst inhibitors

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The intracellular, apicomplexan parasite *Toxoplasma gondii* infects up to 30% of the global human population and causes life-threatening diseases in immuno-compromised patients. Chronically persisting bradyzoites form cysts in brain and muscle tissues and are responsible for transmission and remission of this disease. However, currently available medical treatment options are only effective against the virulent tachyzoites but fail to target the chronic stages of *T. gondii*.
To address this shortcoming, we are screening MMV and Myxobacterial antimicrobial compounds against both stages of the parasite in a plate based assay by observing a growth-dependent fluorescence. We identified effective substances against both stages. To identify their molecular target in *T. gondii*, we pursue an untargeted metabolomics approach using HILIC-UHPLC-MS. We compare the global metabolic response of the parasite to candidate drugs to a library of metabolic fingerprints of established inhibitors with known modes of action.