# ApicoWplexa virtual Meeting, September 16, 2021:

# Models for studying apicomplexan parasites

## Keynote 1:

## *In vitro* modelling of coccidia – the *Cystoisospora suis* story

**Anja Joachim**, Anna Feix, Bärbel Ruttkowski, Teresa Cruz-Bustos
*Institute of Parasitology, University of Veterinary Medicine Vienna, Austria*
Anja.Joachim@vetmeduni.ac.at

Apicomplexan parasites are notoriously difficult to cultivate throughout their life cycle, which presents a serious obstacle for research on both basic and applied aspects of parasite biology, disease and control. *Cystoisospora suis* is a monoxenous coccidian species and an important cause of enteric disease of suckling piglets worldwide. Despite its taxonomic position in the genus *Cystoisospora*, it is transmitted directly via oocyst contamination in the piglets' environment, and extraintestinal stages or paratenic hosts have not been described. Cystoisosporosis is an economically important disease, as affected piglets suffer from poor weight gain and secondary enteric infections can cause considerable mortality. However, the only available animal model is the neonatal piglet which is genetically poorly defined, not regularly available and difficult to maintain. In the 1980ies when piglet production was intensified cystoisosporosis was a rising star in the firmament of veterinary parasitology, and also first *in vitro* cultivation experiments were undertaken in swine testicular (ST) cells and in chicken embryos. However, neither system sustained the parasite it its full life cycle. After several years of research on *C. suis* in animals we started to develop a cell culture model that would sustain at least asexual replication of merozoites. After initial experiments with ST cells we changed to intestinal porcine epithelial cells (IPEC) as an obviously more suitable host cell, and managed to optimize culture conditions to sustain the whole life cycle of our laboratory strain, Wien-I from sporozoites (derived from sporulated oocysts recovered *ex vivo* from experimentally infected piglets) to unsporulated oocysts. Sporulation rates *in vitro* are still very limited, and during the cultivation the stages first increase but then decline again in numbers. However, the cultivation of merozoites can be used for testing bioactive compounds potentially effective against merozoites *in vitro* in a quantitative assay. Applying the principles of serendipity to experimental research, we recently found out that

merozoites harvested from cell culture supernatant and kept in cell-free medium develop further to gamonts, gametes and oocysts. In follow-up experiments, the whole life cycle of C. suis could be established in an IPEC-1 culture followed by harvest of merozoites and continuing development in host-cell-free culture. This simple cultivation system enables the harvest of pure stages, especially gamonts, macro- and microgametes, for further analysis by -omics, detailed morphological examination by electron and confocal laser scanning microscopy and evaluation of proposed intervention strategies. Further research will include adaptation of the culture system to the optimized production of specific stages and focus on the gametes and the process of gamete fusion and zygote formation as a key event in the coccidian life cycle that could be targeted by novel intervention strategies.

## Keynote 2:

## Modeling *Toxoplasma* sexual development in mice & microphysiological devices

Bruno Martorelli De Genova, Nicole Davis, Mouhita Humayun, David Beebe, **Laura Knoll**
*Department of Medical Microbiology, University of Wisconsin-Madison, 1550 Linden Drive, Room 3303, Madison WI 53706, USA*
*ljknoll@wisc.edu*

Our research centers are studying the host/pathogen interactions of protozoan parasites that are common in food and water. We use mice as well as intestinal organoids from many different species to model infection. We have recently focused on modeling the sexual cycle of *Toxoplasma gondii*, which was previously limited to the cat intestine. We determined cell culture conditions for *T. gondii* sexual development by supplementing cat intestinal organoids with linoleic acid. Cats are the only warm-blooded animals that do not readily metabolize linoleic acid because they do not express delta-6-desaturase in their intestines. To generate a mouse model of *T. gondii* sexual development, we inhibited delta-6-desaturase activity and supplemented their diet with linoleic acid. This protocol allowed *T. gondii* sexual development in mice, including the production of sporulation-competent oocysts in their feces. We are currently modeling this pathway in tissue culture microphysiological devices for maximum oocyst yield. Our goal is to make *T. gondii* sexual development easily accessible for all research labs to permit a molecular analysis of the sexual stages and allow labs to perform classical genetic crosses on a routine basis.

## Keynote 3:

### In-cell structural investigation of the apicomplexan rhoptry secretion systems by cryo-electron tomography

**Yi-Wei Chang**[1]*, Shrawan Kumar Mageswaran[1], Amandine Guérin[2], Liam M. Theveny[1], William David Chen[1], Matthew Martinez[1], Maryse Lebrun[3], Boris Striepen[2]
[1]*Department of Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania, USA*
[2]*Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, USA*
[3]*LPHI, UMR 5235 CNRS, Université de Montpellier, France*
*ywc@pennmedicine.upenn.edu*

Parasites of the phylum Apicomplexa cause important diseases including malaria, cryptosporidiosis, and toxoplasmosis. These intracellular pathogens inject the contents of an essential organelle, the rhoptry, into host cells to facilitate invasion and infection. However, the structure and mechanism of this eukaryotic secretion system remain elusive. In this talk, I will present our recent work using cryo-electron tomography to image the supramolecular architecture of this secretion system directly inside intact, frozen-hydrated *Cryptosporidium parvum* sporozoites and *Toxoplasma gondii* tachyzoites at a resolution of few nanometers in three dimensions. In both species, we identify helical filaments which appear to shape and compartmentalize the rhoptries, and an apical vesicle (AV) which facilitates docking of the rhoptry tip at the parasite's apical region with the help of a remarkable multi-component molecular machine named the rhoptry secretory apparatus. Moreover, *T. gondii* contains a line of AV-like vesicles which interact with a pair of microtubules and accumulate towards the AV, leading to a working model for AV-reloading and discharging of multiple rhoptries. Together, our analyses provide a structural framework to understand how these important parasites regulate and conduct rhoptry's discharge.
Reference: Mageswaran S, Guérin A*, Theveny L*, Chen W*, Martinez M, Lebrun M, Striepen B, Chang YW. 'In situ ultrastructure of two evolutionarily distant apicomplexan rhoptry secretion systems' *Nature Communications* 12: 4983 (2021)

## Short presentation 1:

### Development of placental ex vivo models for the study of host-parasite interactions in toxoplasmosis and neosporosis

**Bárbara Pérez-Arroyo**[1], Pilar Horcajo[1], Esther Collantes-Fernández[1], Julio Benavides[2], Roberto Sánchez-Sánchez[1], Luis Miguel Ortega-Mora[1], Iván Pastor-Fernández[1*].
[1] *SALUVET, Animal Health Department, Faculty of Veterinary Sciences, Universidad Complutense, Madrid, Spain.*

[2] *Mountain Livestock Institute (CSIC-ULE), León, Spain.*
barbpe03@ucm.es; ipastor@ucm.es

Worldwide, the productivity of domestic ruminant livestock is compromised by the existence of multiple pathogens that cause reproductive failure, including the apicomplexan parasites *Toxoplasma gondii* and *Neospora caninum*. The mechanisms underlying this failure are not fully understood, but are related to the interaction of the replicating parasites with the placenta, a complex and temporary organ that guarantees the survival and development of the foetus. Current availability of models able to reproduce the complex structure of the ruminant placenta is limited. Hence, the use of in vivo models is the only alternative available to study placental host-pathogen interactions. In order to reduce the dependence on animal models and circumvent the limitations of established in vitro tools, we propose the use of ex vivo approaches based on ruminant placental explants. Considering their short lifespan, this project aims to develop suitable protocols for explant cryopreservation and create tissue bio-banks that could be used on a demand basis, minimizing the number of animals required. The use of bio-banked explants will be first validated through viability, functionality, structural and immunological assays, and subsequently exploited to perform experimental infections with *T. gondii* and *N. caninum* to dissect the interactions of both parasites with the placenta. This work has the potential to expand the knowledge on the pathogenesis of toxoplasmosis and neosporosis, but also offers a powerful tool for the study of other transmissible reproductive diseases affecting ruminants.

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## Short presentation 2:

## *In vitro* methods to assess sporozoite invasion and endogenous development of *Eimeria tenella* and its application for the evaluation of anticoccidial compounds

**Virginia Marugan-Hernandez**
*The Royal Veterinary College, University of London, Hawkshead Lane, Hertfordshire, AL9 7TA, UK*
vhernandez@rvc.ac.uk

The development of *in vitro* models can support important advances in biomedical sciences and significantly reduce the use of experimental animals. The transference of these models for a wider uptake is paramount to make results comparable between different research groups and to support a global reduction of animal use in research. At the Royal Veterinary College (University of London), we have recently developed an *in vitro* model for the evaluation of anti-parasitic properties of novel compounds intended to be used for control of chicken coccidiosis.

This model has also supported other relevant studies in basic research of these parasites, supporting experiments and observations that cannot be done in animals. We have used cell culture to assess localisation of proteins during the endogenous development, studies in organelles morphology and morphometrics or supporting systems biology studies by producing high quality samples for transcriptomes, proteomics and phosphor-proteomics of both parasite and host. Chicken coccidiosis is a costly disease for the poultry industry (>£10 billion annually) caused by protozoa parasites of the genus *Eimeria*. The control of this disease is mostly achieved by chemoprophylaxis with drugs; nonetheless, there is an overall agreement in the need to improve current methods of control due to the reported resistances, new regulations on their use and public concerns. Research in novel compounds with anti-parasitic properties is necessary to control coccidiosis and allow the expansion of the poultry industry to keep feeding the global population. At the moment, the anti-parasitic activity of new compounds are usually tested in live animals by expensive experiments that involve large numbers of chickens. With the aim of getting a wider uptake of this *in vitro* model, we have recently obtaining funding from the NC3R (National Centre for the Replacement, Refinement and Reduction of Animals in Research) for the optimisation and transfer of the model to SALUVET-innova, a R&D company in Spain that will implement the model and test compound from animal nutrition companies *in vitro* before they performing final experiments in animals prior the commercialisation of the product.

## Keynote 4:

## *Neospora caninum*: an immunotherapeutic protozoan against cancer

**Louis Lantier**[1], Agathe Poupée-Beaugé[1], Anne di Tommaso[1], Céline Ducournau[1], Mathieu Epardaud[2], Zineb Lakhrif[1], Stéphanie Germon[1], Françoise Debierre-Grockiego[1], Marie-Noëlle Mévélec[2], Arthur Battistoni[1], Loïs Coënon[1], Nora Deluce-Kakwata-Nkor[3], Florence Velge-Roussel[3], Céline Beauvillain[4], Thomas Baranek[5], Gordon Scott Lee[6], Thibault Kervarrec[1,7], Antoine Touzé[1], Nathalie Moiré[2], Isabelle Dimier-Poisson[1]
[1] *Université de Tours, INRAE, ISP, F-37000, Tours, France*
[2] *INRAE, Université de Tours, ISP, F-37380, Nouzilly, France*
[3] *GICC EA 7501, Université de Tours, UFR de Médecine, 10 Boulevard Tonnellé, F-37032 Tours, France.*
[4] *Inserm U1232, Faculté des Sciences, CRCINA, CHU d'Angers, Université Angers, Angers, France*
[5] *INSERM, Centre d'Etude des Pathologies Respiratoires (CEPR), UMR, 1100, Université de Tours, Tours, France*
[6] *Kymeris Santé, SA, F-37000, Tours, France*
[7] *Department of Pathology, Université de Tours, CHU de Tours, Tours, France*
*llantier@kymerisrx.com*

Immunotherapy induces, provides, and/or reactivates anti-tumor immune responses. Some microorganisms can also initiate response that lyzes infected tumor and/or stimulates systemic immunity. Attenuated viruses or bacteria are well studied as oncotherapeutics, but no protozoa

except *Toxoplasma gondii*. We assessed the effect on tumors of other protozoa that were naturally non-pathogenic to humans. Thus, we discovered the ability to use *Neospora caninum (Nc)* in a manner and form that demonstrated a synergistic array of pertinent immunotherapeutic characteristics against solid cancers.

We demonstrated that the treatment of thymoma EG7 by *Nc* strongly inhibited tumor development. Analysis of immune responses and interactions between *Nc* and tumor cells showed that *Nc* had the ability to lyze infected cancer cells, reactivated immune competence within the Tumor Microenvironment (TME), and activated the systemic immune system by promoting the recruitment of immune cells to the site of tumor. We also established in a NOD/SCID mouse model that *Nc* was able to induce a strong regression of human MCC. Recently, to further enhance oncotherapeutic effect, we engineered an *Nc* strain to secrete human IL-15 (cross reactive with mouse cells), associated with alpha subunit of IL-15 receptor, increasing its stability. This strain induced proliferation of human PBMCs and their secretion of IFN-γ. In the EG7 model, human IL-15 secreting Nc showed greater protection against tumor development, confirming enhancement of immunotherapy by engineering *Nc* to deliver/secrete IL-15.

These results highlight *Neospora caninum* as a potentially extremely efficient, and non-toxic anti-cancer agent, capable of being engineered to express at its surface or to secrete bio-drugs, like human IL-15 cytokine. Our work has identified the broad clinical possibilities of using *N. caninum* as an oncolytic protozoan in human medicine capable of vectoring molecular therapy, overcoming TME defenses.

Reference:

Lantier L, Poupée- Beaugé A, di Tommaso A, et al. Neospora caninum: a new class of biopharmaceuticals in the therapeutic arsenal against cancer. Journal for ImmunoTherapy of Cancer 2020;**8**:e001242. doi:10.1136/ jitc-2020-001242