ApicoWplexa virtual Meeting, April 22, 2021:

Host-parasite interactions
Abstracts

Keynote:

Metabolic needs and capabilities of *Toxoplasma gondii* during acute and chronic infection

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In the intermediate hosts*, Toxoplasma gondii* alternates between two life cycle stages: the fast-replicating tachyzoites that cause potentially life-threatening acute infection and the slowly replicating bradyzoites, which persist in the brain and muscle tissues, establishing a chronic infection. Survival of *T. gondii* relies on the uptake of essential nutrients from the host, as well as on the *de novo* synthesis of metabolites, which cannot be sufficiently salvaged. Understanding the needs and capabilities of the acute and chronic stages is therefore vital for the development of eradicating drugs. To investigate these complex changes following developmental transitions, we have generated a well curated, genome-scale metabolic model (iTgo), harmonized with experimentally observed phenotypes for the fast-replicating tachyzoite stage. To validate the parasite's needs and capabilities, we have generated mutants, performed in-depth i*n vitro* and *in vivo* phenotyping including targeted metabolomics. This led to unexpected insights into the remarkable flexibility of the parasite, addressing the dependency on biosynthesis or salvage of fatty acids (FAs), purine nucleotides (AMP, GMP), vitamins (pyridoxal-5P, pantothenate) and a cofactor (heme), in the acute and latent stages of infection. Taken together, the metabolic network combined with experimental validation leads to a deeper understanding of the parasite's biology, and new insights for development of successful therapeutic intervention.

Short presentations:

Short presentation 1:

Characterization of the potential *Neospora caninum* virulence factors NcGRA7, NcROP40 and NcROP in a pregnant mouse model

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Previous studies have extensively characterized the wide biological variability between different *Neospora caninum* isolates in terms of virulence. In particular, transcriptomic and proteomic comparisons among high- and low- virulence isolates have evidenced a higher expression/abundance of specific genes/proteins in high virulence isolates, and proposed the dense granule protein NcGRA7 and two rhoptry proteins (NcROP40 and a protein of the ROP20 subfamily, NCLIV_068850) as potential virulence factors. Thus, the objective of this study was to evaluate the role of these proteins in the virulence of *N. caninum* through experimental infections in a well-established pregnant BALB/c mouse model using CRISPR/Cas9 knock-out parasites for the three genes. In general, deletion of Nc*GRA7*, Nc*ROP40* and *NCLIV_068850* was associated to a partial loss of virulence. Specifically, dams infected with the *NCLIV_068850* defective mutant displayed significant milder clinical signs and lower mortality rates compared to those infected with the wild-type parasite (Nc-Spain7), and this was associated to a significant lower parasite burden in their brains. On the other hand, median survival times of the offspring from dams infected with all three knock-out parasites were significantly improved. A reduction of neonatal mortality rates was recorded in all groups, but significant differences were only found in that infected with the *NCLIV_0688500* mutant. Overall, virulence impairment was remarkable upon *NCLIV_068850* disruption, while the impact of Nc*GRA7* or Nc*ROP40* deletion was more discrete. These results prove the relevance of NcGRA7, NcROP40 and NCLIV_0688500 in the virulence of *N. caninum* in mice and lay down new areas of research in the field of neosporosis.
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Short presentation 2:

Overexpression of rhoptry kinase alters the length of *Eimeria tenella* life cycle

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Eimeria tenella is a deadly and contagious apicomplexan protozoan parasite, which is responsible for avian coccidiosis. This pathology induces major economic losses for poultry industry worldwide. *E. tenella* invades the digestive epithelial cells, causing intestinal lesions that can lead to death. Coccidiostatic drugs and vaccination are necessary to control coccidiosis. However, with the apparition of resistance against anticoccidian molecules in parasite field strains and the high cost of vaccines, it appears necessary to improve the means of control of this parasite.
Our research is focused on the understanding of *E. tenella* rhoptry protein kinase (ROPK) functions. It is well known that invasion of apicomplexan parasite is orchestrated by protein secretion. Among the proteins secreted, ROPKs are well described in *Toxoplasma gondii* as key virulence factors. ROPKs are involved in the modulation of numerous cellular functions and pathways allowing parasite development.
The knowledge about the functions of *E. tenella* ROPK is limited. *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. In this context, our research is focused on understanding the mode of action of *E. tenella* ROPK. We previously established that the first kinase, EtROP1, is active and phosphorylates the cellular p53, inhibiting the apoptosis of parasited cells and thus promoting the parasite development. The second kinase, EtROP2, is an active kinase localized in the rhoptry compartment and is early expressed during the parasite life cycle. Interestingly, we show that the overexpression of EtROP2 could speed up the parasitic life cycle, favoring the excretion of oocysts 1-2 days earlier than a wild-type strain.
Understanding the role of EtROP2 in the host-parasite interaction may reveal this kinase as a good candidate in the race of improvement of the means of control for coccidiosis.

Short presentation 3:

Glycosylphosphatidylinositols of apicomplexan parasites: a structure-activity relationship analysis

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Glycosylphosphatidylinositols (GPIs) are widespread among eukaryotes, and the expression of GPI-anchored proteins and free GPIs is particularly abundant among the parasitic protozoa. The basic structure of GPIs consists of ethanolamine, mannose, non-acetylated glucosamine, inositol and a lipid-moiety, in part linked by diphosphate bridges. In addition, the evolutionary conserved core structure undergoes species-specific structural variations. The synthesis of GPI anchors takes place in the endoplasmic reticulum and requires many different steps.
Protocols used for the identification and characterization of GPIs depend mainly upon sufficient amounts of material or metabolic labelling techniques using radioactive GPI precursor molecules, organic solvent extraction procedures, the use of specific enzymes an of thin-layer chromatography analysis.
Responses of host cells to GPIs of apicomplexan parasites (*Babesia divergens*, *Plasmodium falciparum*, *Neospora caninum, Toxoplasma gondii*) have been studied and a structure-activity relationship can be outlined on cytokine production (**1-3**), Toll-like receptor activation (**2-4**) and apoptosis (**5-6**).

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Short presentation 4:

Characterization of the intestinal mononuclear phagocytic cells in the intestine of the lamb to better understand the immune response against *Cryptosporidium parvum*

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Cryptosporidiosis is a poorly controlled zoonosis caused by the intestinal infection by a protozoan parasite, *Cryptosporidium parvum* (*Cp*), presenting a high prevalence in ruminant farms (cattle, sheep, goats). Young animals with an immature intestinal immune system are particularly susceptible to this infection. In a neonatal mouse model, we previously demonstrated the key role of innate immunity and in particular of CD11c+CD103+CD11b- dendritic cell (DC) subset in controlling the acute phase of *Cp* infection [1]. During infection, in response to chemokine production by infected epithelial cells, newly recruited CD11c+CD103+CD11b- DC produce IL12 and IFNγ contributing to the elimination process of the parasite. According to the current common classification of DC in different species (human, mouse, pig, sheep, and chicken), this subpopulation can be identified as the cDC1 subset of conventional DC.
The aim of this project was to better characterize intestinal DC subpopulations in lamb and to determine their role during *Cp* infection. As in the mouse model, the parasite invades and multiplies mainly in the ileum of young lambs. However, a peculiarity of young ruminants is the presence of a large ileal Peyer's patch (lymphoid tissue) that extends all along the ileum. We characterized in various compartments of the lamb small intestine (lymphoid and non-lymphoid)

the various mononuclear phagocyte populations by performing a phenotypic and functional analysis by flow cytometry and transcriptomic methods respectively. We followed the evolution of the cell subsets according to the age of the animals and during Cp infection. This work represents the first fine description of mononuclear phagocyte cell subsets in the small intestine of young lambs at homeostasis and during the course of *C. parvum* infection.

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