ApicoWplexa virtual Meeting, November 18, 2021:

Vector-borne apicomplexan parasites

Keynote 1:

Babesia: from the lab to the wild

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Babesiosis is considered an emerging zoonoses caused by intraerythrocytic protozoa that are transmitted by Ixodid ticks, blood transfusion, organ transplantation and perinatally. More than 100 *Babesia* species infect a wide spectrum of wild and domestic animals worldwide. Parasite infection of natural hosts such as in cattle causes bovine babesiosis, resulting in high economic losses.

Six species of *Babesia* have been identified as human pathogens. *Babesia microti* is the predominant species that infects humans, is found throughout the world, and causes endemic disease in the United States and China. *Babesia venatorum* and *Babesia crassa*-like agent also cause endemic disease in China. *Babesia divergens* is the predominant species in Europe causing "redwater" fever in cattle and sporadically but fulminant babesiosis cases in humans.

Beyond the epidemiological status of *B. divergens*, this parasite is considering an excellent *in vitro* model for studying basic biological aspects of the apicomplexan parasites. Taking this advantage, we have recently explored, by time-lapse video microscopy *in vivo* and soft-X-ray tomography, the whole *B. divergens* asexual life cycle. The obtained results have allowed to better understanding of the role that *B. divergens* performs outside and inside the human red blood cell (RBC).

We also are studying different biological aspects of the parasite by the integration of different omics, including genomic, transcriptomic and metabolomic. This integrative approach are revealing several genes, proteins, metabolites and metabolic pathways that are active at specific points during the *B. divergens* life cycle, thus ensuring the propagation and survival of the parasite.

These all studies together are providing a vision of the infection of the RBCs by *B. divergens* and molecular and serological tools that we are currently using for studying *Babesia* and babesiosis in humans and in wild.

Keynote 2:

Identification of anti-theilerials and host-parasite interactions in *Theileria annulata*

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Tropical theileriosis or Mediterranean Coast fever is a tick-borne haemoprotozoan disease caused by the intracellular parasite *Theileria annulata*. Tropical theileriosis is geographically distributed in India, China, Central Asia, Southern Europe, North Africa, and Middle East. *T. annulata* is transmitted by *Hyalomma anatolicum* and infects *Bos taurus*, *Bubalus bubalis* and other ruminants. Death may occur after 3-4 weeks of infection. The economic impact of theileriosis is approximately US$ 300 million worldwide. And 250 million cattle were estimated to be at risk of tropical theileriosis. Buparvaquone, a hydroxynaphthoquinone, is effective in the treatment of bovine tropical theileriosis. Point mutations in *T. annulata* cytochrome b and alanine-to-proline mutation at position 53 of TaPIN1 lead to a gain of resistance towards buparvaquone. The macroschizonts subvert various cell signaling pathways and transform the host leucocytes, finally leading to the lymphoproliferation of infected leukocytes. Host-parasite interactions play a vital role in subverting the cell signaling pathways. We are interested in studying the repurposing abilities of the known anti-cancer or anti-parasitic chemical compounds against tropical theileriosis. Also, we are inquisitive to study the host-parasite interactions which may involve parasite-derived host transformation.

Short presentation 1:

Studying *Theileria* parasite-host interactions to gain insights into cell transformation

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Intracellular Apicomplexa parasites are masters of manipulating their hosts, targeting transcriptional machineries and inducing epigenomic remodeling events which lead to veterinary and human diseases. *Theileria* spp. are bovine-specific pathogens that cause a lymphoproliferative disease (similar to some human leukemias) with significant economic impact due to animal mortality and decreased productivity. *Theileria*-infected lymphocytes and macrophages are transformed and immortalized; they display uncontrolled proliferation *in vitro*, independent of exogenous growth factors, and increased ability to migrate and form invasive metastases. Buparvaquone is the only known treatment able to reverse these phenotypes and kill infected cells, but there are many reports of emerging drug-resistance in the field.
We study the genetic and epigenetic events involved in *Theileria*-host interactions and parasite-induced transformation. We identified a Peptidyl-prolyl isomerase (TaPin1) secreted into the host cell [1]. We showed that TaPin1 induces proliferation via the host transcription factor c-Jun and leads to metabolic rewiring of host glycolytic enzymes via the HIF-1α factor [1,2]. Furthermore, the parasite gene encoding TaPin1 is mutated in Buparvaquone drug-resistant parasites [1,3]. We established a microscopy-based drug screen to identify new theilericidal inhibitors. We identified hits from the "Pathogen box" (Medicines for Malaria Venture) and showed that one of them is as effective as Buparvaquone in disrupting host cell transformation, but it does not appear to target the TaPin1 protein. The identified compound decreases the *T. annulata* and *T. parva* parasite load in infected B cells and macrophages. We also show effects on temperature-induced merogony, host cell cycle or host gene expression. Identifying the targets and mode of action of this new compound will provide insights into the unique host-parasite interactions underlying *Theileria*-induced transformation and offer an alternative to target Buparvaquone-resistant parasites.

References

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Short presentation 2:

Discovery of a new locus of repetitive effector proteins in *Theileria* and their role in malignant transformation of the host cell

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Theileria spp. are a group of apicomplexan parasites which are transmitted by ticks and are closely related to *Plasmodium* spp. The two most virulent species, *Theileria annulata* and *Theileria parva*, transform bovine leukocytes into a malignant, hyperproliferative state, reminiscent of cancer cells. We hypothesized that a multitude of parasite effector proteins must be responsible for the observed alteration of host signaling pathways leading to continuous proliferation and immortality. Very few exported parasite proteins have been described so far, and our knowledge of their contribution to host cell malignancy is very limited. To identify uncharacterized *Theileria* effector proteins translocating to the host cell nucleus, we utilized the TurboID proximity labelling technique. Promiscuous biotin ligase BirA* was targeted to the host cell nucleus and parasite surface of *T. annulata* schizont-infected macrophages. Subcellular fractionation followed by affinity purification and liquid chromatography-tandem mass spectrometry (LC-MS/MS) was done in triplicate and coupled with bioinformatic analysis. By this approach we identified a novel repetitive protein locus comprising four proteins which are absent in the non-transformative species *T. orientalis*. Antibodies raised against these newly discovered proteins confirmed their localization to the host nucleus and suggests that one of these protein shuttles between the parasite surface and the nucleus. Further investigations are now being undertaken to analyze the contribution of these proteins to host cell transformation.

Short presentation 3:

Identification of *Theileria orientalis* genotypes in asymptomatic grazing cows in São Miguel, Azores – silent commuters or neglected opponents?

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Azores archipelago is one of the most suited regions for dairy cattle production in Portugal and represents 30% of national dairy production. It is mainly characterized by a pasture-based feeding system where cows maintain longer productive lifespans.
A molecular survey targeting tick-borne Apicomplexa parasites (*Babesia* spp. and *Theileria* spp.) was conducted in 2019, on 10 dairy farms in São Miguel Island. At each farm, 10 cows wererandomly selected and sampled. Initial screenings were performed by PCR targeting a 680 base pairs region of the 18S rRNA gene. The amplicons showed 100% identity to *Theileria orientalis* complex and a second PCR, targeting the Major Piroplasm Surface Protein (MPSP) gene (656 bp), was designed to allow genotype identification. In total, 45 samples were positive for *T. orientalis* (45/100, 45%). Only one farm had no positive samples. At the other farms, the number of positives varied from one (1/10, 10%) to all the samples tested (10/10, 100%). Sequencing allowed to characterize 38 of these samples so far, with 26 samples compatible with buffeli genotype (type 3), 10 samples compatible with Chitose genotype (type 1) and 2 cows revealing to be co-infected by both genotypes. At the farm level, both genotypes were detected in three out of the nine positive farms.
This is the first report on the presence of *Theileria orientalis* complex in Azores archipelago and the first report of Chitose genotype circulating within the country. Although buffeli genotype is characterized by silent infections, Chitose is a more virulent genotype associated to production losses, especially on the first wave of infection. Even though all the surveyed cows were apparently asymptomatic, the repercussions of *Theileria orientalis* infections are probably being undervalued or confounded by other health problems.

Short presentation 4:

Transcriptional analyses of bovine macrophages infected with *Besnoitia besnoiti*

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Bovine besnoitiosis is caused by the intracellular cyst-forming apicomplexan parasite *Besnoitia besnoiti*. This parasitic disease impairs reproductive parameters since infected bulls may develop orchitis during the acute infection that may end up with sterility during both acute and chronic besnoitiosis. During the acute stage of the disease, the fast-replicating tachyzoites infect target cells such as endothelial cells and macrophages. However, the role of macrophages in the immune response raised against *B. besnoiti* remains to be elucidated. Therefore, the aim of this study was to explore the interaction of *B. besnoiti* tachyzoites with isolated primary bovine monocyte-derived macrophages *in vitro.*

First, the *B. besnoiti* tachyzoite lytic cycle was characterized. Next, host-parasite interactions were investigated by RNA-Seq at 4h post-infection (pi) (during initial parasite-host cell interaction) and at 8h pi (early parasite invasion) in macrophages infected with live tachyzoites (MO-Bb) and macrophages inoculated with heat-killed tachyzoites (MO-hkBb). Non-infected macrophages (MO) were used as control.
Besnoitia besnoiti was able to invade and proliferate in macrophages. A higher number of invasion events were observed in infected macrophages at 12h pi (37.35%) compared to other bovine primary cell cultures. Other lytic cycle parameters did not change significantly (eg. doubling time: 15.1h; proliferation from 24hpi onwards; egress at 72h pi). Upon infection, MO-Bb were smaller, round and lacked filopodial structures compared to MO-hkBb, that might be associated to a hypermigratory phenotype demonstrated in other apicomplexan parasites.
Differences in macrophage activation were also evidenced between MO-Bb and MO-hkBb at the transcriptional level and the number of differentially expressed genes (DEGs) increased substantially during the course of infection: 545 DEGs at 4h pi and 1739 at 8h pi in MO-Bb *vs* MO, 514 DEGs at 4h pi and 2813 DEGs at 8h pi in MO-hkBb *vs* MO, and 148 DEGs at 4h pi and 231 DEGs at 8h pi in MO-Bb *vs* MO-hkBb. “Apoptosis” (*BCL2, CASP6, ATF4, CHOP*) and “MAPK” (*MAPK13, MAPK14, MAP3K6, MKNK2,*) pathways were enriched in MO-Bb compared to MO-hkBb at 4hpi. The frequency of TUNEL positive apoptotic cells increased significantly in MO-Bb at 8h pi, showing that *B. besnoiti* infection induces macrophage apoptosis. “Herpes simplex 1 virus infection” pathway (*CCL2, IRAK1, OAS, CASP3*) was found to be significantly enriched in MO-Bb compared to MO-hkBb at 8hpi. Interestingly, relevant DEGs of the “Herpes simplex 1 virus

infection" (*IFN- α, CCL2*) and the "Apoptosis" (*ATF4, CHOP-2*) pathways were significantly regulated in the testicular parenchyma of naturally infected bulls.

The present work has set the basis for further studies that should clarify whether these relevant transcriptional changes might favor parasite survival and proliferation.

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