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Abstracts

Keynote

Scaling up parasite genomics

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Over the past two decades we have developed an excellent understanding of the genomes of key parasite species. However, many taxa still have little or no genomic resources to help drive their study. But large projects, with the bold aim of assembling high-quality genome sequences for all species of eukaryote are now underway. At the Sanger Institute we are involved in the Darwin Tree of Life project to sequence all ~60,000 named eukaryotic species in the UK in the next 10 years. As part of this, we aim to vastly improve genomic resources for parasites. These sequences and associated data will improve our understanding of parasite diversity and evolution, and lead to new strategies for their control. I will discuss our recent work on genome assemblies of the Apicomplexans *Hepatocystis* and *Eimeria* as examples of the quality achievable with current sequencing technologies and the insights that can be made. Finally, I will describe the software we are developing to better understand chromosome structure and evolution in parasites, and across the whole tree of life.

Short presentation 1:

Evaluating Potential Peridomestic Wildlife Sentinels of *Toxoplasma gondii*

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Toxoplasma gondii prevalence of peridomestic wildlife was examined on a dairy farm in eastern Tennessee. Blood samples were obtained from small mammals and mesopredators trapped on the farm in 2016 and 2017. Serological testing by the modified agglutination test (MAT; cutoff 1:50) found 3.7% (1/27) of house mice (*Mus musculus*), 52.9% (9/17) of raccoons (*Procyon lotor*), and 50% (1/2) of domestic cats (*Felis catus*) were seropositive for *T. gondii* antibodies. No antibodies were found in five white-footed mice/deer mice (*Peromyscus* spp), one Norway rat (*Rattus norvegicus*), six cotton rats (*Sigmodon hispidus*), one Eastern mole (*Scalopus aquaticus*), 16 opossums (*Didelphis virginiana*), and two skunks (*Mephitis mephitis*). Twenty-nine tissue samples from small animals were also collected and subjected to PCR-RFLP detection; four (13.7%) were positive for *T. gondii*, however, *T. gondii* DNA was not consistently detected. Seroprevalence in rodents was low, but higher in mesopredators such as raccoons and cats. Spatial partition of rodent species was evident, which may lead to partitioning of *T. gondii* genotypes through different transmission cycles on farms and in natural environments. Our study showed that mesopredators, especially the raccoon and cat, would be a suitable indicator species to monitor *T. gondii* environmental infection. The study of *T. gondii* infection in animals is a critical parameter in assessing environmental contamination, especially in areas where low contamination is indicated. Mesopredator surveillance is imperative in future research to better understand susceptibility of these animals to *T. gondii* infection, but also environmental contamination in their habitats.

Short presentation 2:

Contrasting population genetics of co-endemic cattle- and buffalo- derived *Theileria annulata*

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A study was designed to improve understanding of the genetics of *Theileria annulata* populations in sympatric cattle and water buffalo. The study was undertaken in the Punjab province of Pakistan, where the prevalence of tropical theileriosis is high. Parasite materials were collected from infected animals in defined regions, where cattle and water buffalo are kept together. Six satellite DNA markers and a mitochondrial cytochrome b marker were used to explore the multiplicity of *T. annulata* infection and patterns of emergence and spread of different parasite genotypes. The results show differences in the numbers of unique satellite locus alleles, suggesting that *T. annulata* is genetically more diverse in cattle- than in water buffalo-derived populations. Heterozygosity (H_e) indices based on satellite and cytochrome b loci data show high levels of genetic diversity among the cattle- and water buffalo-derived *T. annulata* populations. When considered in the context of high parasite transmission rates and frequent animal movements between different regions, the predominance of multiple *T. annulata* genotypes and multiple introductions of infection may have practical implications for the spread of parasite genetic adaptations; such as those conferring vaccine cross-protection against different strains affecting cattle and buffalo, or resistance to antiprotozoal drugs.

Key Words: *Theileria annulata*; tropical theileriosis; vaccine cross-protection; multiplicity of infection; antiprotozoal drugs.

Short presentation 3:

Detection of *Neospora caninum* in the blood and tissues of one-humped camels (*Camelus dromedarius*)

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Neospora caninum is a cyst-forming apicomplexan protozoan parasite that infects canids as definitive and a wide range of mammals as intermediate hosts. The parasite is mostly known by its role in the abortion in cattle with a significant economic loss however, serological evidences suggest the infection of the one-humped camel (*Camelus dromedarius*). The aim of this study was to employ molecular and histopathological methods to examine *N. caninum* infection in blood and tissues from 100 camels in Iran.

Genomic DNA was extracted from blood, brain, liver and portal lymph node specimens of the camels, and nested-PCRs targeting ITS-1 region of *N. caninum* (NN1, NN2, NP1, NP2 primers) were performed. In addition, brain tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections (5 µm) were cut, stained with haematoxyline and eosin (H&E), and examined microscopically.

Results revealed the DNA of *N. caninum* in the blood, brain and portal lymph nodes of two (2%) camels. Histopathology also revealed cysts resembling *N. caninum* in brain samples of PCR-positive camels. This study provides the first insight into detection of *N. caninum* in *Camelus dromedarius*. Further molecular studies specifically on the fetal tissues are required for better understanding of the epidemiology and transmission dynamics of *N. caninum* in camels.

Short presentation 4:

Toxoplasma gondii in Spanish farm animals: opening new avenues from genotype to phenotype.

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Toxoplasma gondii possess a significant genetic and biological diversity that was proposed as responsible for the variation in clinical presentations during infections. Therefore, it is crucial to characterize *T. gondii* strains infecting food animals at all possible levels and explore their variability. Two decades of scientific effort on isolation, and molecular and phenotypic characterization of the parasite have revealed a population structure more complex than the traditional three clonal lineages composition, and a virulence degree classification under discussion.

Previous studies allowed us to identify several polymorphic strains and a predominant type II PRU variant genotype (ToxoDB#3) infecting domestic sheep in Spain, coexisting with other much less frequent clonal genotypes (ToxoDB#1 and ToxoDB#2). Furthermore, new investigations showed similar results in free-ranging Iberian pigs. In addition, *in vitro* and *in vivo* phenotypical characterization assays have enabled us to describe intra-genotype differences between Spanish isolates, in terms of traits such as proliferation *in vitro* in ovine trophoblast target cells, or mortality and morbidity rates, parasite burden and tissue lesions in a murine model. Besides, recent results from our group reopen the debate of *Toxoplasma* strains virulence sorting with a clonal type III (ToxoDB#2) isolate from Iberian pig that presents close to 90% mortality and 100% morbidity rates and notable consequences for tissues in a standardized murine model. Complementing these studies, Multilocus Microsatellite Typing and Whole-Genome Sequencing analyses are, respectively, bringing us the opportunity to

explore the variability of the parasite population in Spain, and hopefully find new *T. gondii* virulence effectors associated with phenotypic findings.

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Short presentation 5:

Spatial distance between sites of sampling associated with genetic variation among *Neospora caninum* in aborted bovine foetuses from northern Italy

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Neospora caninum, a coccidian protozoan, represents an important cause of bovine abortion. Available *N. caninum* strains show considerable variation *in vitro* and *in vivo*, including different virulence in cattle.

A total of 198 aborted bovine foetuses were collected from 165 Italian Holstein Friesian intensive dairy farms located in Lombardy between 2015 and early 2019. *N. caninum* samples were subjected to multilocus-microsatellite genotyping (MLMG) using ten previously established microsatellite markers. In addition to own data, those from a recent study [1] providing data on five markers from other northern Italian regions were included and analyzed.

Of the 55 samples finally subjected to MLMG, 35 were typed at all or 9 out of 10 loci. Linear regression revealed a statistically significant association between the spatial distance of the sampling sites with the genetic distance of *N. caninum* MLMGs ($P < 0.001$). Including data from a previous North Italian study (eBURST analysis) revealed that part of *N. caninum* MLMGs from northern Italy separate into four groups; most of the samples from Lombardy clustered in one of these groups. Principle component analysis revealed similar clusters and confirmed MLMG groups identified by eBURST. Variations observed between MLMGs were not equally distributed over all loci, but predominantly observed in MS7, MS6A, or MS10.

Our findings confirm the concept of local *N. caninum* subpopulations. More comprehensive studies on microsatellites in *N. caninum* should be undertaken, not only to improve genotyping capabilities, but also to understand possible functions of these regions in the genomes of these parasites.

[1] Regidor-Cerrillo J, Horcajo P, Ceglie L, Schiavon E, Ortega-Mora LM, Natale A. Parasitol Res. 2020, 119, 1353–62.

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