Internships ENVT – Summer 2018

1- Regulation of the innate immune response to Influenza virus in ducks

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While rapid death is the usual outcome of highly pathogenic avian influenza (HPAI) virus infection in gallinaceous poultry, such as chicken, HPAI infected ducks usually present only mild clinical signs and recover from infection. Evidence suggests that the innate immune response contributes to the efficient control of HPAI replication in ducks. Using, samples from in vivo experiments, as well as in cell culture experiments, the goal of this project is to analyse how the innate immune response to Influenza virus infection is regulated in ducks.

2- Influenza "D" virus: which host range for this novel pathogen?

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Cross-species transmission of pathogens from the animal reservoir to domestic species and ultimately to humans constitutes a major risk for animal or human health. Recent studies in the USA and our preliminary work in France have identified a new Genus tentatively named Influenzavirus D within the *Orthomyxoviridae* family. The novel virus was shown to infect swine and cattle and to efficiently replicate and transmit in ferrets, the animal model of choice for the study of influenza in human, suggesting that humans could be infected. Our project aims at assessing the emergence threat associated with influenza D viruses' circulation. We will screen samples from different animal species (wild and domestic) for the presence of influenza D virus and antibodies against the novel virus to assess the host range of the pathogen. Our field samples originate from Europe and from Africa.
The internship will be co-supervised by Pr. Gilles Meyer.

Keywords: influenza D virus, host range, serology, molecular biology

References:

Hause, Ducatez et al, Isolation of a novel swine influenza virus distantly related to influenza, PloS Pathogen 2013
Ducatez, Pelletier and Meyer, Influenza D Virus in Cattle, France, 2011–2014, Emerging Infectious Diseases 2015
Salem et al, Serologic Evidence for Influenza C and D Virus among Ruminants and Camelids, Africa, 1991-2015, Emerging Infectious Diseases 201

3- **Molecular epidemiology of avian viruses in Morocco**

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Within the framework of our collaboration with the Institut Agronomique et Vétérinaire Hassan II in Rabat, Morocco, we study the circulation and evolution of avian coronaviruses in Morocco. In Africa, very little is known on avian viruses, their circulation, evolution and spread. The aim of the internship is to understand putative virus exchanges between wild and domestic birds and between geographic areas. We will work on Moroccan field samples, screen them for avian viruses by RT-PCR, isolate virus in embryonnated eggs, and carry out a molecular characterization of the strains (partial or full genome sequencing, phylogenetic analyses). Our findings will be compared with available data available from Europe and North Africa.

Keywords: avian respiratory virus, avian coronavirus, Morocco, molecular epidemiology

References:

El Houadfi M, et al, First outbreaks and phylogenetic analyses of avian influenza H9N2 viruses isolated from poultry flocks in Morocco. Virology Journal 2016

Fellahi et al, Phylogenetic Analysis of Avian Infectious Bronchitis Virus S1 Glycoprotein Reveals Emergence of a New Genotype in Moroccan Broiler Flocks, Virology Journal 2015

Fellahi et al, Prevalence and molecular characterization of avian infectious bronchitis virus in poultry flocks in Morocco from 2010 to 2014 and first detection of Italy 02 in Africa, Avian Pathology 2015

4- **Myxoma virus evolution from the 1960s to now**

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Myxomatosis in Europe is the result of the release of a South America strain of myxoma virus in 1952. Several attenuated strains with origins in South America or California have since been used as vaccines in the rabbit industry. Using our archive virus collection (with samples from 1960s to now), we will aim at understanding the evolution and molecular clock of European myxoma viruses. Techniques to be used here: DNA extraction, PCR reactions for targeted genome regions, Sanger sequencing, phylogeny and molecular clock analyses.

The internship will be co-supervised by Pr. Stéphane Bertagnoli.

Keywords: myxoma virus, molecular biology, evolution, phylogeny, molecular clock

References:

Camus-Bouclainville et al, Genome sequence of SG33 strain and recombination between wild-type and vaccine myxoma viruses. Emerging Infectious Diseases 2011

Bertagnoli and Marchandeau. Myxomatosis. Rev Sci Tech. 2015

5- Potential roles of stable fly *Stomoxys calcictrans* and *Aedes albopictus* as vectors of the Lumpy skin disease (LSD)

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Lumpy skin disease is a poxviral disease affecting cattle. It is of primary economic importance and notifiable for the world organization for animal health (OIE). LSD is widely distributed in many African countries where it is considered as endemic. Since 1989, LSD spread on an unusually large scale affecting Middle Eastern countries, Turkey, Kazakhstan, Azerbaijan, Russia, but also European countries. Indeed, since 2015 where the first outbreak was reported in Greece, LSD expanded uninterruptedly to new territories with ongoing outbreaks still arising in Greece, Macedonia, Albania, Bulgaria, Montenegro and Serbia (2016, OIE, WAHID).

Previous studies demonstrated a potential role of certain hematophagous arthropod vectors (stable flies, mosquitoes, and certain tick species) in the transmission of the virus. The stable fly *Stomoxys calcitrans* is indeed considered as the most likely candidate to play a role in the epidemiology of the LSD. However a formal demonstration of its role in the transmission of LSDV has not been made yet. The mosquito *Aedes aegypti* has been reported once to transmit LSDV in cattle under experimental conditions (Chihota et al., 2001). However no data are available on the potential vectorial role of *Aedes albopictus* now widely settled in Europe. In this context, the Parasitology and Virology teams of the National Veterinary School of Toulouse, France will focus on the cosmopolitan stable fly *Stomoxys calcictrans* and on the invasive mosquito species *Aedes albopictus* to ascertain their potential roles as vectors of the LSDV. These investigations are conceivable as these two species of insects are maintained under laboratory colonies in the National Veterinary School of Toulouse.

These investigations will have the folllowing objectives: i/ to confirm the transmission of LSDV by *S. calcitrans* using an in vitro model and to investigate its possible transmission by *Aedes albopictus*, ii/ to assess the virus titer threshold still enabling a transmission in vitro by these two vectors, iii/ to determine the virus lifespan in its vectors, iv/ to ascertain the maximal delayed transmission after a contaminated blood- meal.

During his internship the student will actively take part to the experiments: he will participate to the experimental infection of the insects with the LSD virus, and will learn how to dissect the insects, to isolate the mouthparts, and abdominal parts to further analyze them using molecular tools, to look for the presence of the virus.

6- **Investigations on how OMVs produced by hlyF+ E. coli alter the autophagic and inflammatory processes in vitro and ex vivo**

Laure David - Pathogenesis and commensalism of enterobacteria" au sein du Digestive Health Research Institute (IRSD), inserm UMR1220, INRA UMR1416, ENVT, Université Toulouse Paul Sabatier

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The crosstalk between gut bacteria and intestinal cells emerges as pivotal in intestinal homeostasis and autoimmune diseases or infections. Among other types of communication between eukaryotic and prokaryotic cells, Gram negative bacteria are able to produce Outer Membrane Vesicles (OMVs). OMVs are spherical nanoparticules that are shed by the outer membrane of these bacteria. OMVs mediate diverse functions depending on their content, including promoting pathogenesis and regulating microbial interactions within bacterial communities or with their host. In line with this idea, we have shown in the team that some pathogenic *Escherichia coli* (*E. coli*) carry a virulence gene, *hlyF* gene, that promotes the production of Outer Membrane Vesicles (OMV) with a very specific biological activity: the capacity to block autophagy in eukaryotic host cells. Autophagy is a catalytic process that have a major role in inflammation regulation. Thus, autophagy blockade contributes to an excess of inflammation in the gut, participating in infection or the progression of autoimmune diseases.

Thus, this project aims at investigating how OMVs produced by hlyF+ *E. coli* alter the autophagic and inflammatory processes *in vitro* and *ex vivo*. The student will perform cellular biology (study of autophagy and inflammation) and bacteriology (bacteria culture and OMVs production) techniques.