

## Internship subjects –Summer 2020

### **I- MENTOR : Mariette Ducatez, PhD, director of research UMR 1225 IHAP INRAE-ENVT**

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Our team of virology is part of the joint research unit of the National Veterinary School of Toulouse (ENVT) and the National Institute for Agronomy Research (INRA) Interactions Host Pathogens. This year we are 14 permanent staff members (including scientists and research technicians), 7 PhD students and three post-doctoral fellows. DVM and undergraduate students also join the team for their theses or short internships. Our group investigates the mechanisms of host-virus dialogue, using both *in vitro* and *in vivo* approaches. More details can be found on our website: <http://www.envt.fr/virology-ihvv-research-group?destination=node/341>.

Three internship topics are available for the summer of 2020:

#### **1. Molecular epidemiology of animal influenza viruses**

Only two avian viruses are able by themselves to cause 100% mortality in chicken flocks: highly pathogenic influenza virus and velogenic Newcastle disease virus (NDV). Several other avian viruses cause severe losses in poultry flocks when associated with co-infecting pathogens. In Africa, very little is known on avian viruses, their circulation, evolution and spread. In cattle and small ruminants, influenza D viruses have been shown to circulate throughout the globe, including in Africa (Salem et al, EID 2017). We have strong collaborations with Northern (Morocco, Tunisia, and Egypt), Western (Benin, Togo, Côte d'Ivoire), and Eastern (Uganda) Africa and are conducting molecular epidemiology studies mainly on respiratory viruses on the continent to be able to understand putative virus exchanges between wild and domestic birds and between geographic areas. We will work on African field samples, screen them for avian viruses by (RT-)PCR, take care of the virus isolation in embryonated eggs, and carry out a molecular characterization of the strains (partial or full genome sequencing, phylogenetic analyses).

**Keywords:** avian and bovine respiratory viruses, Africa, molecular epidemiology

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### **2. Co-infections with influenza D virus and other respiratory pathogens in the bovine host**

The frequency of respiratory diseases and their economic impact represents a major obstacle in the breeding of young cattle. The most often, these conditions are multifactorial and involve pathogens on the one hand (viruses and bacteria) and, on the other hand, factors related to the animal, the environment and farming systems. Generally, the role of a single pathogen or virus/bacteria association in the development of respiratory disease is being studied. Few studies describe the effects of an association between two viruses, which nevertheless share the same tissue and cellular tropism. In accordance with the work by Mitra et al. in 2016, we found by PCR and NGS (next generation sequencing) a high frequency of viral co-infections in calves with severe respiratory signs (manuscript in preparation, Salem et al). In particular, we found associations between influenza D virus (IDV), bovine coronavirus (BCoV), bovine respiratory syncytial virus (BRSV), and *Mycoplasma bovis*. We assume that these co-infections contribute to the development of respiratory disease in relation to the conditions of mono-infections. In our team, we are interested in the role of IDV, which we have identified for the first time in Europe (Ducatez et al, 2015), in association with BCoV, BRSV, or *Mycoplasma bovis* on the occurrence of respiratory disease in cattle. In particular, we are interested in the role of the innate immune response, the first line of defense for infected and surrounding cells, in the monoinfection framework versus viral co-infections in cattle.

The objective of the internship will be to study kinetics of replication of pathogens in mono- and co-infections and to characterize the innate immune response.

The internship will be co-supervised by Dr. Adrien Lyon and Pr. Gilles Meyer.

**Keywords:** influenza D virus, co-infections, pathogenesis, immune response

#### **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 2017

Mitra et al, Metagenomic characterization of the virome associated with bovine respiratory disease in feedlot cattle identified novel viruses and suggests an etiologic role for influenza D virus, Journal of General Virology 2016

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### 3. Myxoma virus evolution from the 1960s to now

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

**II- MENTOR : Aude Ferrand, DMV, PhD, Dip ECVPT – Laboratory of physiology - ENVT**

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### Staphylococcus spp. is one of the leading causes of infections associated with biofilms.

Biofilms are characterized by groups of bacteria adhering to a surface and covered by an extracellular polysaccharide matrix. The structure of the biofilms and the phenotype of the bacteria they harbor limit the activity of the immune system and antibiotics. The eradication of biofilms is extremely difficult and currently they are recognized as a major source of recurrent or chronic infections such as dermatitis caused by *Staphylococcus pseudintermedius* in dogs and cats and chronic mastitis caused by *Staphylococcus aureus* in cattle.

The veterinary armamentarium is very limited as most of authorized antibiotics have no efficacy on biofilms even when they are used at high doses for long duration. The aim of this study is to evaluate the bactericidal activity of innovative combinations of different antimicrobial and non-antimicrobial drugs on *Staphylococcus* biofilms. The experiments will be performed *in vitro* in static conditions (exposing bacteria to constant concentrations of drugs) and in dynamic conditions (exposing bacteria to fluctuating concentrations similar to those obtained in treated animals).

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### **III- MENTOR: Romain Volmer DVM, PhD – Laboratory of virology – Ecole nationale vétérinaire de Toulouse - France**

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#### **Emergence of highly pathogenic avian influenza**

This project will use state-of-the art molecular virology to assess how the nucleotide sequence of the hemagglutinin gene segment modulates the genetic evolution from low pathogenic avian influenza to highly pathogenic avian influenza viruses.

<https://interactionsnotesagentspathogenes.weebly.com/>

### **IV – MENTOR: Claire Guinat - DVM, MSc, RCVS, PhD, Postdoctoral research associate, UMR 1225 IHAP**

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#### **Structure of the poultry rendering network and H5N8 outbreaks in France, 2016-2017**

In 2016-17, Europe was hit hard by an unprecedented wave of Highly Pathogenic Avian Influenza (HPAI) A(H5N8) outbreaks, having a severe socio-economic impact on poultry production, global trade and people's livelihoods (Napp et al. 2018). The highest number of outbreaks was reported in France, with ducks being the most affected poultry species (Alarcon et al. 2018; Guinat et al. 2018). Duck movement networks have been recently identified as underlying factors for the spatial distribution of HPAI outbreaks (Guinat et al. 2019), suggesting that these factors should be taken into account to appropriately describe the epidemic spread.

The general objective of this study is to investigate the association between the structure of the poultry rendering network and the H5N8 epizootic in France, 2016-17. Accurate data on the date and location of infected poultry holdings suspicion and poultry rendering network have been collected in France. The specific objectives are (1) to clean and organise the database of the poultry rendering network provided by the ENVT, (2) to calculate general descriptive statistics of the poultry rendering network and (3) to compare the descriptive statistics of the poultry rendering network before/during/after the outbreak. The internship will be co-supervised by Claire Guinat, Billy Bauzile and Debapryio Chakraborty.

**Keywords:** Highly Pathogenic Avian Influenza, poultry rendering network, H5N8 epizootic, France

**Date of the internship:** May-June 2020

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### References

Alarcon, Pablo, Adam Brouwer, Divya Venkatesh, Daisy Duncan, Chrysostomos I. Dovas, George Georgiades, Isabella Monne, et al. 2018. “Comparison of 2016–17 and Previous Epizootics of Highly Pathogenic Avian Influenza H5 Guangdong Lineage in Europe.” *Emerging Infectious Diseases* 24 (12): 2270–83. <https://doi.org/10.3201/eid2412.171860>.

Guinat, C, J Artois, A Bronner, JL Guérin, M Gilbert, and MC Paul. 2019. “Duck Production Systems and Highly Pathogenic Avian Influenza H5N8 in France, 2016–2017.” *Scientific Reports* 9 (1): 6177.

Guinat, C., G. Nicolas, T. Vergne, A. Bronner, B. Durand, A. Courcoul, M. Gilbert, J.L. Guérin, and M.C. Paul. 2018. “Spatio-Temporal Patterns of Highly Pathogenic Avian Influenza Virus Subtype H5N8 Spread, France, 2016 to 2017.” *Eurosurveillance* 23 (26). <https://doi.org/10.2807/1560-7917.ES.2018.23.26.1700791>.

Napp, S., N. Majó, R. Sánchez-González, and J. Vergara-Alert. 2018. “Emergence and Spread of Highly Pathogenic Avian Influenza A(H5N8) in Europe in 2016-2017.” *Transboundary and Emerging Diseases* 65 (5): 1217–26. <https://doi.org/10.1111/tbed.12861>.

**IV – MENTOR: Agnes Waret - DVM, MSc, PhD, swine lecturer and clinician, epidemiologist**

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### Assessing gilts and sow welfare on farms

Welfare is today of utmost importance in livestock animals. To answer the societal demand in Europe a project named ‘Welfare quality’ established a protocol to assess pig welfare on farms and slaughterhouses. However, if the method is very much detailed for growing pigs an overall assessment is not possible to our knowledge today for gilts and sows. The assessment can only be summarized per individual criteria. The objective of the externship will be to investigate and propose a mean to fulfil this gap.