Topics for summer internships 2024 at ENVT

1- VIROLOGY (VIRéMIE team)

Our team of virology is part of the joint research unit of the National Veterinary School of Toulouse (ENVT) and the National Research Institute for Agriculture, Food and Environment (INRAe) Interactions Host-Pathogens. This year we are 8 permanent staff members (including scientists and research technicians), 6 PhD students and 1 master student. 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* an *in vivo* approaches.

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A- Molecular epidemiology of avian 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. We have strong collaborations with Northern (Morocco), Western (Benin, Togo, Côte d'Ivoire, Mali), 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 embryonnated 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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B- Detection and characterization of influenza D virus

Recent studies in the USA, in China, and our preliminary work in France have identified a new genus of the *Orthomyxoviridae* family, named Influenza D virus (IDV). This novel virus was shown to infect farm animals including swine and cattle, and to efficiently replicate and transmit in ferrets, the animal model of choice for transmission of influenza A virus (IAV), a well-known other Orthomyxovirus, to humans. IAV is well known to infect poultry and wild birds, with huge economic consequences for the food industry. In addition, IAV is also a major concern for human health; once this virus crosses the species barrier it might even adapt to its new host, becoming highly infectious and gaining the ability to spread from man to man and cause pandemics.
In contrast to IAV, there is only very scarce information on Influenza D and its risks for animal and human health. The virus seems to be circulating at a global level in farm animals, as antibodies against IDV has been detected in cattle in America (USA, Canada, Mexico, and Argentina), Asia (China and Japan), Africa (Benin, Togo, Morocco and Kenya), but also in France, Italy, Ireland, Luxembourg and Turkey. In addition, it has been shown that IDV can be

isolated from sick cattle, while it might be associated in a complex of pathogens, which might hamper early detection.
The main scope of the internship is to generate highly needed knowledge for better risk assessment of IDV. We will work towards the evaluation of diagnostic tools and assessing the virus genetic and antigenic properties. The project is part of a European Union funded project with partners from Sweden, Belgium, Italy, Luxembourg and Ireland.

The internship will be co-supervised by Prof. Gilles Meyer.

Keywords: influenza D virus, molecular biology, serology, evolution, phylogeny, diagnostics.
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C- AVIAN INFLUENZA LOW PATHOGENICITY H9N2 VIRUS SURVEILLANCE IN AFRICA

Avian influenza viruses (AIVs), characterized by a broad host spectrum and evolutionary capacity, *can* have a strong impact for animal and human health. Knowing which viruses are circulating in animals is therefore important for characterizing them and estimating the risk they represent for animal and public health. The H9N2 virus, because of its zoonotic nature and its presence in Africa, is the subject of surveillance measures. 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).

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D- 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.
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https://interactionshotesagentspathogenes.weebly.com/

2- VIRAL - UMR ENVT-INRAe IHAP Team

Development of a serological diagnostic tool for *Carp Edema Virus*

Carp Edema Virus (CEV, *Poxviridae* family), is an emerging pathogen associated with a disease that causes high mortality in common and koi carp (*Cyprinus carpio*). CEV has been detected in many countries, sometimes associated with trading context. In France, most carp mortalities are now associated with CEV.

Our laboratory, in collaboration with other partners, aims to evaluate the distribution of CEV in France and to enhance the knowledge of its circulation in order to improve its control.

Currently, the surveillance of CEV is only carried out by direct detection by PCR. However, a serological tool would allow a better understanding of the distribution of CEV even in the absence of clinical signs or mortality.

To develop this serological diagnostic tool, and since CEV cannot be isolated *in vitro*, recombinant viral proteins will be produced. The target proteins will be selected by analogy with what is known for other members of the *Poxviridae* family. They will be expressed in prokaryote and/or baculovirus models, depending on their properties. Once the proteins are produced, they will be purified and the presence of antibodies against them in carp sera will be tested by Western blot.

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3- MICROBIOLOGY (Myco team)

Our team is part of the joint research unit of the National Veterinary School of Toulouse (ENVT) and the National Research Institute for Agriculture, Food, and Environment (INRAE), named IHAP (Interactions Host-Pathogens). Today, our Myco team integrates nine permanent staff members (including scientists and research technicians), 2 PhD students, one postdoc, and one resident veterinarian. DVM and undergraduate students frequently join the team for their theses or short internships. Our group investigates the mechanisms of host-microbiota dialogue, using both *in vitro* and in vivo approaches, focusing on *Mycoplasma* spp.

Evaluating the forecasting power of mucin-microbiome interplay in livestock

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Respiratory infectious diseases are one of the most significant challenges for the livestock industry, impacting animal health and welfare, the environment, and public health. One of the first lines for respiratory pathogen defense combines the mucus layer, a highly viscous material primarily formed by mucins and the thriving multi-kingdom microbial ecosystems. The mucin-microbiome interplay can be considered a mighty two-edged sword, as its usual function is to protect from unwanted pathogens and substances. At the same time, its dysfunction may be a clue for microbial infection and disease onset. However, this information still needs to be explored for animals. To fill this gap, we aim to unearth the complex mucin-microbiome crosstalk in ruminants and then underline which mucin-microbiota interactions are pivotal for holobiont health and resilience against infectious diseases. Using *in vivo* and *in vitro* experiments, we will decipher these main conceptual questions: (i) What are the types of mucins, their abundance, and their distribution in the respiratory and digestive tract? (ii) How does the mucin glycan profile impact microbiota composition in the mucus and the host immune response? (iii) How do biotic (pathogens: viruses + *Mycoplasma* spp) and abiotic (antibiotic administration) stressors modify the microbiota-mucin interplay? and (iv) What molecular mechanisms enable the microbiota-mucins system to respond to different pathogens and antibiotics? Collectively, this project will lay the framework for characterizing the protective and therapeutic nature of mucins and microbiota against infections and animal diseases in livestock and how they modulate the behavior and pathogenicity of different microorganisms.

The main scope of the internship is to help optimize the protocols needed to discover the types of mucins, their abundance, and their distribution in the respiratory tract of calves. Then, the student will participate in an *in vivo* research experiment at the ENVT to characterize the health and welfare of individuals following antibiotic administration and experimental pathogen coinfections. Altogether, the candidate can work from the farm to the lab, from wet to dry. The project has the financial support of French national institutions and the European Union, involving a multidisciplinary consortium and partners from France, the Netherlands, Germany, Poland, Sweden, Italy, England, Switzerland, and Spain.

Keywords: bovine respiratory pathogens, mucins, microbiota, animal health and welfare

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4- PHARMACOLOGY (INTHERES TEAM)

IN VITRO EVALUATION OF THE EFFICACY OF DIFFERENT ANTIBIOTICS AGAINST RHODOCOCCOCUS EQUI

Background

Rhodococcosis is a bacterial respiratory disease of foals associated with mortality of up to 80% if left untreated. No randomized controlled trials are comparing different treatments in foals, and the recommendation to combine rifampicin with a macrolide since the 1980s has been based mainly on the results of retrospective clinical studies and in vitro studies that are not representative of the clinical situation.

The combination of rifampicin and macrolides is based on evidence of in vitro synergy. However, all the tests demonstrating synergy were performed by exposing bacteria to antibiotic concentrations that were not representative of the antibiotic concentrations obtained in vivo. Indeed, in foals, concentrations vary over time due to absorption and elimination mechanisms. The data from these in vitro studies are therefore not very predictive of the effects that may be obtained during treatment in foals. In addition, rifampicin is a known enzyme inducer and has recently been shown to significantly decrease macrolide concentrations reaching the lungs, which may call into question the value of this combination. The evaluation of this combination for the treatment of foals should be based on the benefit (synergy for antibacterial activity) / risk (under-exposure to antibiotics) ratio.

Since the ability of Rhodococcus equi to survive in macrophages is correlated to the virulence of the bacteria in the foal, the activity of antibiotics on R. equi in macrophages must be systematically studied in in vitro models more representative of the concentrations obtained in the foal and capable of better predicting the effects of the different treatments in terms of bacterial eradication and selection of resistant bacteria

Project

The objective of the training period is to test in vitro the effects of antibiotics that could be used, alone or in combination, in the treatment of rhodococcosis in foals.

Efficacy will be tested by exposing extracellular and intracellular bacteria (after phagocytosis) to the maximum concentrations obtained in the foal (either free plasma concentrations or pulmonary epithelial lining fluid concentrations (PELF) as a single agent or in combination). Antibiotics tested will be rifampicin plus clarithromycin or azithromycin, marbofloxacin alone or in combination with macrolides and possibly gamithromycin. The concentrations tested will be taken from available publications for these antibiotics administered to foals.

To test the efficacy on intracellular bacteria, two cell lines capable of phagocytosing R. equi will be tested.

The efficacy of the antibiotics will be evaluated by counting the bacteria over time and by looking for resistance selection. For antibiotics effective at maximum concentrations, lower concentrations will be tested to evaluate the impact of altered absorption in some foals (inter-individual variability). In parallel, we will try to maintain extracellular and intracellular bacteria under identical experimental conditions and we will try to obtain non-adherent phagocytic cells to be able to use the dynamic Hollow Fiber system later on.

Ultimately, the results will allow us to better understand the effects of rifampicin in combination with clarithromycin or azithromycin (rate of effect, comparison of extra- and intracellular activity, the advantage of the combination, ...) and to determine if therapeutic failures (death or prolongation of treatment) can be attributed to under-exposure of certain foals to antibiotics. Finally, new data will also be generated for other treatments and may guide future research.

Methods used:

- Cell culture

- Bacterial culture (plating, enumeration, ...)

Required skills:

There are no specific skills required. All the methods can be learnt during the training period.

The internship will be supervised by Aude Ferran, DVM. PhD. HDR. Dip ECVPT

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