

## Experience in a foreign country: Switzerland

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Research conducted in the Institute of Veterinary Bacteriology, Bern, Switzerland

I spent the six weeks period of my fellowship in the Institute of Veterinary Bacteriology and Swine Clinic of the Vetsuisse Faculty in Bern, Switzerland. I took this decision to have the opportunity to work as a Vet in a relatively new environment for me: a research laboratory of veterinary bacteriology. During this period, I had the opportunity to work on my own and with very experienced people that taught me how to work in a scientific manner. My research project was about the genotypic characterization of 60 isolates of *Mycoplasma hyorhinis*, by employing Multilocus Sequence Typing (MLST) based on six housekeeping genes, as described by Toqueville et al. in 2014. These 60 isolates belong to more than twenty-five Swiss and German fattening units, previously included in a transnational cross study.

*Mycoplasma hyorhinis* is a common inhabitant of the upper respiratory tract and tonsils of pigs. Its role as a potential pathogen remains controversial. In fact, most infections due to this organism are subclinical, even though pneumoniae, arthritis, polyserositis, conjunctivitis and otitis are clinical disorders that may be associated with the infection. Varieties in the virulence of different *M. hyorhinis* strains have been discussed but no virulence factors have been identified to distinguish between pathogenic and non-pathogenic strains. Therefore, information on the genome structure might help to get a clearer picture on specific strains involved in clinical disorders.

For this purpose, each isolate was submitted to several passages before the genome's analysis was feasible. Firstly, it was cultured from stocks kept at -80° C, lysated at 96° C for fifteen minutes and submitted to the PCR. The products were then verified on agarose gel prior to purification. After the PCR product purification, every sample underwent MLST, to target six housekeeping genes, *adK* (adenylate kinase), *dnaA* (chromosomal replication initiation protein), *gltX* (glutamyl-tRNA synthetase), *gmK* (guanylate kinase), *gyrB* (DNA gyrase subunit  $\beta$ ) and *rpoB* (RNA polymerase  $\beta$  subunit). After the sequence process, an arbitrary allele number was conferred at each given locus and the alleles were then assigned to a specific sequence type (ST). The STs were analyzed with the program Bio Numerics<sup>®</sup> and compared to the PubMLST database ones ([pubmlst.org](http://pubmlst.org)).

Data obtained shows a high variability of *Mycoplasma hyorhinis* strains. In fact, all the STs except one resulted new, when compared to the strain analyzed in the study of Toqueville et al. The farms where we analyzed more than a single isolate revealed the same strain being present, with some exceptions. The first exception appeared in three Swiss farms in which we found the same *Mycoplasma* strain. The second exception was in three German herds, where different isolates were simultaneously present in the same farm and even within single animals.

In conclusion, the high diversity of both Swiss and German strains shows that recombination of the *M. hyorhinis* genome appears, even with a certain and limited clonality, similar to what is observed for *M. hyopneumoniae*. Some interesting results of intra-herd and intra-pig concomitant infections with different isolates were detected in German herds, where the *M. hyopneumoniae* prevalence is high, if compared to Switzerland, which has been considered free from enzootic pneumoniae since 2003. We are then going to implement further investigations based on the comparison of MLST data and herd health status information, to define possible virulent strains related to different clinical presentations.