

# Are wild boars in Switzerland a reservoir for enzootic pneumonia and what is the current serological status of the Swiss domestic pigs?

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## Key words

*Mycoplasma hyopneumoniae*, diagnostics, experimental infection, wild boar, serology, monitoring

## Aim of the study

The main objectives of this project were to **(i)** Evaluate the relevance of atypical *M. hyopneumoniae* (*Mhyo*) strains isolated from Swiss wild boars with respect to their epizootic potential for Swiss pig farming **(ii)** Generate and characterize urgently-needed diagnostic reference specimens for the National Reference Laboratory at ZOBA, **(iii)** Implement an aerosol-based infection model at IVI using an infection chamber, which better mimics the natural route of infection and fosters animal welfare, **(iv)** Investigate the serological status of domestic pigs in Switzerland comprehensively, and finally, **(v)** Get insight into the phylogenetic position of atypical *Mhyo* strains.

## Material and methods

**Aims i, ii and iii:** Aliquoted and at -80°C-stored *Mhyo* cultures grown in Mycoplasma experience medium were titred out. Aliquots were used for subsequent *in vivo* challenge studies. In a first trial, 16 pigs (2 x n=8) were infected with a nebulized Swiss enzootic pneumonia (EP) outbreak strain using either **(a)** a PARI LC Sprint nebulizer in combination with a PARI BOY® Classic compressor or **(b)** a MAD nebulizer. 6 pigs were used as mock controls. Half of the pigs of each group were euthanized 2 weeks post infection and the other half at 4 weeks post infection. In a second trial, 16 pigs (2 x n=8) were infected repeatedly using either **(a)** a German EP outbreak strain or **(b)** a Swiss wild boar isolate. All pigs were euthanized 4 weeks post infection. In the two experiments, clinical and haematological parameters as well as *Mhyo* shedding were followed daily. All pigs were submitted to post-mortem analyses. **Aim iv:** The serological status of slaughtered sows (5,021 sera from 968 farms collected in 2018 at eight different slaughterhouses) in Switzerland was investigated using the IDEXX *Mhyo* Ab Test. Data on domestic pig populations and wild boars were plotted graphically with previous EP cases in Switzerland. **Aim v:** Genomic DNA of *Mhyo* strains from wild boars and domestic pigs was isolated, PacBio-sequenced and genomes were compared using a suite of bioinformatic tools.

## Results and significance

We were not able to fully address **aim i**. The reason for this is that we were not able to set up an aerosol-based infection model as envisaged. The nebulization reduced the number of viable cells to approximately one log and therefore we infected with 10<sup>7</sup> colony-forming-units (CFU) per animal. The infection with a Swiss wild boar strain and a Swiss outbreak strain did not result in clinical disease nor typical pathomorphological lesions. The infection with a German outbreak strain resulted in pathomorphological lesions in one out of six animals. We refined a model to expose pigs to aerosols. In summary, we have not been able to infect SPF pigs with *Mhyo* using an infectious dose of 10<sup>7</sup> CFU. More work is needed to establish the challenge model for *Mhyo*. **Aim ii:** we were able to collect infected tissue and serum from one pig infected with the German outbreak strain. **Aim iii:** We were able to establish an aerosol-based stress-free challenge model, but this model requires optimization. **Aim iv:** The overall seroprevalence of EP was very low with 0.98% of sows tested positive and these seropositive animals could be allocated to 3.92% of farms tested. Most seropositive farms presented weakly positive singleton reactors and only one farm showed several strongly seropositive animals. The serological status mirrors the successful progressive control of *Mhyo* in the Swiss domestic pig population over the years. The current study underlines the added value of serological testing in the surveillance of EP in Switzerland and confirms the sustained benefit of strategic control programmes.

**Aim v:** A genome comparison of atypical Swiss *Mhyo* strains with four Swiss EP outbreak strains, the virulent strains F7.2C & 232 as well as the avirulent strain J did not reveal big differences. Differences with respect to the copy number of the gene encoding the P97-adhesion point towards a lower virulence of atypical strains compared to outbreak strains. We were not able to compare the virulence directly between atypical and outbreak strains since we did not manage to set up a robust aerosol-based challenge model.

Additionally, most recently we modified the original Friis medium recipe and included supplements enhancing and prolonging *Mhyo* and *M. hyorhinis* growth under laboratory conditions, which extended the viability of the cultures so they reached titers of  $10^9$  cells/mL and grow faster than in standard Friis. Moreover, we confirmed the presence of two sets of the MIB/MIP system in *Mhyo* and the ability of *Mhyo* to cleave immunoglobulins, which presents a candidate virulence factor. Furthermore, we characterized the proteome of wild boar and domestic pig derived *Mhyo* strains, which will enable us to identify membrane-localised proteins in atypical and outbreak *Mhyo* strains that are likely to interact with the host cells.

### **Publications, posters and presentations**

- Nadia Scalisi, Gudrun Overesch, Nicolas Ruggli, Peter Kuhnert, Joerg Jores, Title: Presence of *Mycoplasma hyopneumoniae*-specific antibodies in Swiss domestic pigs, Annual Congress of the Swiss Society of Microbiology 2021, Full virtual event, September 2-3, 2021, poster presentation
- Nadia Scalisi, Peter Kuhnert, Maria Elena Vargas Amado, Gudrun Overesch, Katharina D.C. Stärk, Nicolas Ruggli and Joerg Jores (2022) Seroprevalence of *Mycoplasma hyopneumoniae* in sows fifteen years after implementation of a control programme for enzootic pneumonia in Switzerland, Veterinary Microbiology, 270: 109455
- Nadia Scalisi (2022) Seroprevalence of *Mycoplasma hyopneumoniae* in sows fifteen years after implementation of a control programme for enzootic pneumonia in Switzerland, Doctoral Dissertation, Vetsuisse faculty, University of Bern
- Jörg Jores, Nicolas Ruggli, Nadia Scalisi, Jaeyoun Jang, Sergi Torres-Puig, A stress-free and easy-to-use system to expose pigs to aerosols, submitted and in review in the journal Vaccine: X (Elsevier)

### **Project 1.21.03**

**Project duration** April 2021 – June 2023