

# Mycoplasma hyopneumoniae detection and genotyping in wild boar and domestic pigs and investigations on the possible reservoir role of free-ranging wild boar

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## Key words

Enzootic pneumonia (EP), epidemiology, Multilocus sequence typing (MLST), transmission

## Aim of the study

Investigate the prevalence and presence of *M. hyopneumoniae* in wild boar, compare strains from wild boar and pig, assess the role of wild boar by EP outbreak investigations

## Material and methods

Culture and real-time PCR for detection of *M. hyopneumoniae*, genotyping using MLST and p146, cluster analysis of genotypes, genome sequencing and proteomics

## Results and significance

In project part A a prevalence of *M. hyopneumoniae* in wild boar of about 50% was determined by testing bronchial swabs that proved more sensitive than nasal swab analysis. Based on these semi-quantitative real-time PCR results wild boar generally harbor much less *M. hyopneumoniae* than diseased pigs.

- It was for the first time possible to isolate *M. hyopneumoniae* from wild boar. Genome sequencing of this isolate indicated that it does not significantly differ from the known genome sequences of porcine isolates. This was also supported by proteome analyses using 1D- and 2D-PAGE in combination with MALDI-TOF-MS/MS and LC-MS/MS. These comparative studies have been carried out in addition to the project description.

- Genotyping of wild boar and pig samples was possible and proved very useful for epidemiological investigations. The same picture as in pig was seen in wild boar with high strain variability but still limited clonality, whereby strains from geographically clustered animals had identical genotypes. Four scenarios were observed during outbreak investigations: i) the same genotype was found in recurrent EP outbreaks in the same farm, ii) the same genotype was found in geographically or operationally linked outbreak farms, iii) strains from outbreak farms differed from those in wild boar from the vicinity, iv) identical genotypes were found in outbreak farms and wild boar in the neighbourhood but only after and so far never before an EP outbreak. We conclude that *M. hyopneumoniae* in wild boar are very similar and can even be identical to strains in pig. A transmission between the two is therefore possible. Because wild boar harbour much lower loads of *M. hyopneumoniae* than affected pigs and since identical strains in wild boar were so far only found after an outbreak in a nearby pig farm, wild boar does not play a major role in EP outbreaks. Our results rather indicate that *M. hyopneumoniae* is transmitted from outbreak farms to wild boar and that animal transport and lack of proper hygiene measures remain the main reasons for the few outbreaks seen in Switzerland.

- A reliable routine typing method for epidemiological EP outbreak investigations was established including an extensive genotyping database. This allows future outbreak clarifications by routine analysis of samples from EP cases.

## Publications, posters and presentations

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Batista Linhares, M.; Belloy, L.; Origgi, FC.; Lechner, I.; Segner, H.; Ryser-Degiorgis, M.-P. (2014) Investigating The Role Of Free-Ranging Wild Boar (*Sus Scrofa*) In The Re-Emergence Of Enzootic Pneumonia In Domestic Pig Herds: A Pathological, Prevalence And Risk-Factor Study. Submitted To Plos One, Re-Submitted On October 20<sup>th</sup> (Manuscript Under External Revision).

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