Diagnostic investigations on mycobacterial abortion in a sow herd

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Introduction
Swine abortion associated with a mycobacterial infection is an uncommon finding (Ellsworth et al.). Since intradermal testing is time-consuming and therefore cost-intensive, alternative test-systems are needed to get an economically justifiable overview of the situation in an affected sow herd. In March 2009, three neonatal porcine fetuses were submitted for diagnostic investigations. The sow was necropsied one month later.

Materials & Methods
Histopathology: Samples were fixed in 10% buffered formalin. 4-µm-thick sections were cut and stained with haematoxylin and eosin, as well as Ziehl-Neelsen’s stain.

Microbiology & PCR: Tissue samples were decontaminated using NALC-NaOH, inoculated onto two solid media (Löwenstein-Jensen, Stonebrink) and one liquid medium (Middlebrook 7H9) and incubated for 8 weeks. Simultaneously, DNA was extracted from fetal and maternal tissue with the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. Species of the mycobacterial isolates and presence of mycobacteria of the Mycobacterium-tuberculosis-complex and Mycobacterium-avium-complex were examined using the primers according to Rodriguez et al., Guerrero et al., Kunze et al. and Moser et al., respectively.

Immunological tests: Blood samples were collected from 30 sows and 10 suckling, growing and fattening pigs, respectively. They were stimulated with phosphate-buffered saline (PBS), Avian PPD (Prionics) and poke-weed mitogen (Sigma), respectively. Thereafter plasma was analyzed for IFN-γ with Quantikine® Porcine IFN-γ Immunoassay (R&D Systems, Wiesbaden; Cut-off 40 pg/ml). Serum was tested for antibodies with two prototype ELISA ID3Screen® Paratuberculosis Indirect Multispecies (IDVet-MAP) and Mycobacterium avium hominisuis (IDVet-MAHS) ELISA.

Results
Necropsy & histopathology results
The fetuses showed numerous, small (2-6 mm), white foci in the liver. Sow: Several white, small, firm nodules projected from the surface of the endometrium. Several lymph nodes were enlarged. Multifocal, small (2-4 mm), white, firm nodules were seen on the surface of the lungs and in the kidneys. Fetal livers and the affected organs of the sow showed a mild to severe, multifocal, granulomatous inflammation, multinucleated giant cells with single to numerous, intracytoplasmatic, acid-fast and rod-shaped bacteria.

Figure 1: Necropsy & histopathology results
A: Fetal livers B: Uterus of the sow C: Lung of the sow D: Histology: First column fetal liver, second column uterus of the sow; HE and Ziehl-Neelsen’s stain, respectively

Microbiology & PCR
M. avium ssp. hominisuis was isolated from fetal liver and organs of the sow (including endometrium, lung, kidney, liver, tonsil and several lymph nodes). Mycobacteria of the Mycobacterium-tuberculosis-complex were not detected.

Immunological tests
Eleven of 27 sows responded to Avian PPD (3x >1000; 4x 10031000 and 4x 40-100 pg/ml). Only one suckling piglet was positive for IFN-γ (104 pg/ml). Three sows had detectable antibodies (one positive in both ELISAs had to be excluded from IFN-γ-test; one positive in IDVet-MAHS® and IFN-γ-test; one positive in IDVet-MAP and negative in IFN-γ-test). None of the other animals showed a serological reaction.

Discussion
Tuberculous lymphadenitis caused by M. avium ssp. hominisuis is common in swine but genital and/or systemic infections seem to be rare. Detection of subclinically infected animals is essential, as they could be a source of infection for others. Frequent infection of sows was demonstrated by IFN-γ-test (41%). In contrast, antibodies were detectable only in single cases. Preliminary data are promising, but further investigations are necessary to validate this new test system.

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References

* Called now ID Screen © Mycobacterium avium Indirect ELISA