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Absence of *Actinobacillus pleuropneumoniae* in semen from serologically positive tested AI-boars

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Introduction
Transmission of *Actinobacillus pleuropneumoniae* (Ap) between animals occurs mainly due to direct contact between pigs, due to nose-nose contact or uptake of nasal/oral fluids. To assess the risk of transmission of Ap by semen, first it is needed to know whether Ap can be detected in semen. The aim of this study was to evaluate the performance of a qPCR for the *ApxIVA* gene, on semen and test semen of seropositive but healthy boars for the presence of Ap.

Materials and Methods
To enable detection of the *ApxIVA* gene by qPCR in semen, the validated protocol for tonsil brush samples was adjusted, according to a validated protocol for detection of *Brucellosis* in semen. In short, DNA isolation was performed using the DNEasy kit. To assess the qPCR efficiency and minimal detection limit for Ap in fresh and undiluted semen a pooled semen sample was spiked with a 10-fold serial dilution of Ap in triplicate. Thereafter DNA isolation was performed and the qPCR was performed as described earlier (Tobias, 2012). Finally, 19 fresh and undiluted semen samples of serologically positive boars (by *ApxIV* ELISA and/or LC-LPS ELISA) were processed and tested by *ApxIVA* qPCR.

Results
The minimal detection limit of the *ApxIVA* qPCR in semen was >34 - ≤340 Ap DNA copies per reaction, when testing spiked semen. None of the semen samples of serologically positive tested boars (0/19) returned a positive qPCR result.

Conclusion
This study shows that *ApxIVA* qPCR testing of semen for Ap is feasible, but that detection limit increased from 5 copies / reaction in tonsil brush material to between 34 and 340 Ap DNA copies /reaction, which equals ~10^3 – 10^4 CFU /mL semen. As seminal transmission is already considered unlikely and in addition targeted screening of semen samples of serologically positive boars resulted in negative results, the risk of Ap seminal transmission by serological positive boars seems low.

Keywords: semen, artificial insemina, *Actinobacillus pleuropneumoniae*, *ApxIVA* qPCR, transmission