Development and validation of an ELISA tests to detect antibodies against *Haemophilus parasuis*

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**Introduction**
*Haemophilus parasuis* is one of the main bacterial pathogens involved in mortality of pigs between 7 and 9 weeks of age. *Haemophilus parasuis* is a commensal of the upper respiratory tract and it can cause systemic infection during stressful events or due to decay of maternal immunity. Affected pigs usually develop severe fibrinous polyserositis, arthritis, and meningitis with sudden death occurring as early as 24 hours post systemic infection. One of the main challenges in *H. parasuis* control is the lack of a reliable serological test to characterize the development of antibodies in sows and piglets. This information is critical to understand herd immunity and to identify the best timing for vaccination. In this study, we have utilized a highly immunogenic and species-specific antigen recently discovered in *H. parasuis* to develop and validate a *H. parasuis*-specific ELISA test.

**Material and Methods**
Serum samples obtained from 10 pigs that survived a *H. parasuis* outbreak were used to screen outbreak strains for immunogenic *H. parasuis* proteins. Whole cell proteins obtained from 6 outbreak strains were screened for the presence of immunodominant proteins by western blot analysis. An immunodominant protein present in all outbreak strains was identified and sequenced. The specificity of the identified protein was evaluated by screening *H. parasuis* reference strains representing 14 different serotypes and 10 bacterial species frequently isolated from swine using convalescent sera. The GenBank sequence for the complete gene coding for the identified species-specific immunogenic protein was utilized to synthesize a recombinant antigen in *E. coli*. The size and identity of the purified antigen were confirmed by western blot analysis and sequencing. The purified antigen was used to develop an indirect ELISA. Concentrations for coating antigen, primary, and secondary antibodies were optimized to produce a specific and cost-effective ELISA test. Once the conditions of the ELISA were defined, serum samples obtained from 10 convalescent pigs involved in a *H. parasuis* outbreak and 80 healthy 3-week old pigs colonized with *H. parasuis* were tested.

**Results**
A species-specific and highly immunogenic 52 kDa protein was identified in all *H. parasuis* strains by western blot analysis. This protein was absent in all non-*H. parasuis* swine bacterial pathogens tested. The 52 kDa protein was sequenced and identified as *H. parasuis* oligopeptidase permease A (OppA). This protein is located on the cytoplasmic membrane of *H. parasuis* and it constitutes the main component of *H. parasuis* ATP-binding cassette (ABC) transport system. The OppA gene of *H. parasuis* was successfully cloned and expressed in *E. coli* and the highly purified recombinant protein was used as the coating antigen at the OppA ELISA. All pigs that survived the *H. parasuis* outbreak were positive by OppA ELISA with an average S/P ratio of 0.6. The average S/P ratio for the 80 healthy 3-week old piglets colonized with *H. parasuis* was 0.046.

**Conclusion**
We have successfully developed an OppA-based ELISA test to detect clinically relevant anti-*H. parasuis* antibodies. Pigs colonized with *H. parasuis* are negative for anti-OppA antibodies, whereas pigs that have survived an outbreak have high titers against this antigen. We are currently testing serum samples from vaccinated pigs to characterize the use of the OppA ELISA to track protective immunity.