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Novel *Haemophilus parasuis* ELISA test: Tracking systemic exposure and protective immunity

Nubia Macedo[1], Simone Oliveira[1], Eric van Esch[2], Paul Rush[2]
[1]Minnesota Veterinary Diagnostic Laboratory, USA; [2]Biochek, Netherlands

Introduction

Although *Haemophilus parasuis* continues to be one of the main causes of mortality in nursery pigs, we still don't have reliable information regarding the development and detection of protective immunity. Until now, there was no universal serological test to characterize decay of maternal immunity and development of active immunity following exposure or vaccination. In this study, we have developed a novel universal and species-specific ELISA test for detection of antibodies against *H parasuis*. This test is able to specifically detect antibodies produced following systemic contact with this pathogen. Our preliminary data suggests that these antibodies can be used as indicators of protective immunity.

Material and methods

A highly immunogenic and species-specific antigen was identified in *H parasuis* strains involved in a field outbreak by Western blot analysis. This antigen was sequenced and identified as a member of the *H parasuis* ATP-binding cassette (ABC) transport system (OppA). The OppA protein was present in *H parasuis* reference strains representing all serotypes and was absent in 10 bacterial pathogens commonly isolated from swine. OppA was cloned into *E coli*, expressed, purified and used as the coating antigen for the development of a novel ELISA test. Serum samples from convalescent pigs that have survived a *H parasuis* outbreak were used as positive controls for the development of the ELISA. Field samples collected from non-vaccinated pigs naturally colonized with *H parasuis* were also used to validate the new test.

Results

A tentative cut-off of 0.2 S/P was used to classify samples as positives for anti-OppA antibodies by ELISA. By utilizing this cut-off, all pigs that survived a *H parasuis* outbreak had high titers against the OppA antigen. (average S/P ratio of 0.6). Pigs (n = 30) from a non-vaccinated herd experiencing high mortality 7 to 10 days post weaning due to *H parasuis* were negative for anti-OppA antibodies at 3 weeks of age, whereas pigs from the same herd (n = 30) that survived the nursery outbreak were either suspects or positive for anti-OppA antibodies at 8 weeks of age (Figure 1).

Discussion and conclusions

Our preliminary results demonstrate that anti-OppA antibodies are present in pigs that have had systemic contact with *H parasuis*. Healthy and susceptible pigs colonized with *H parasuis* did not have antibodies against OppA. Characterization of serological profiles in a non-vaccinated swine herd that had increased mortality due to *H parasuis* at 7 to 10 days post-weaning demonstrated that all weaning age pigs lacked anti-OppA antibodies, whereas all pigs that survived the outbreak developed anti-OppA titers. We hypothesize that OppA can be used as a marker for detection of protective titers against *H parasuis* and we are currently generating data to prove this hypothesis.

Figure 1: Average S/P ratios for anti-OppA antibodies in a non-vaccinated population.