RESEARCH REPORT
SWINE HEALTH

Title: Development of an *Actinobacillus pleuropneumoniae* (APP) oral fluid antibody ELISA based on the detection of antibodies to APP ApxIV toxin in oral fluid specimens – **NPB #13-214**

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INDUSTRY SUMMARY

Actinobacillus pleuropneumoniae (APP) is a significant respiratory pathogen of swine, causing acute death in finishing pigs and adding significant treatment costs to the production of clinically-affected pigs. Several factors are involved in the pathogenesis of APP, including capsular polysaccharides, mural lipopolysaccharides, and proteinaceous exotoxins. There are four recognized APP toxins, but only ApxIV is specific to APP and expressed by all serotypes1. For this reason, the detection of anti-ApxIV serum antibodies has been used to identify APP infections.

Our research group has previously developed antibody ELISAs capable of detecting antibody against PRRSV, influenza A virus, and African swine fever virus in swine oral fluids. The efficiency of this approach has sparked commercial interest, i.e., 3 commercial diagnostic companies currently produce diagnostic tests for the detection of PRRSV antibody in oral fluids. Consistent with our experience with previous antibody assays, we found that we could develop an ELISA capable of detecting antibody against ApxIV in oral fluid specimens.

KEYWORDS

APP, ApxIV, oral fluids, ELISA, antibody, surveillance

SCIENTIFIC ABSTRACT

15 serotypes of APP are recognized (1-12, 15 (Biotype I); 13,14 (Biotype II)) and pathogenicity/ virulence vary among serotypes. In addition to lipopolysaccharides, toxins are the primary cause of the clinical disease. There are four recognized APP toxins, but only Apx IV is expressed by all serotypes of APP. (Note: Animals vaccinated with inactivated vaccines do not generate anti-Apx IV antibodies.) This "universal Apx IV expression" by all APP serotypes justifies the use of Apx IV antibody detection in APP screening tests. Currently, there are no USDA licensed assays for the detection of Apx IV toxin, but such assays are available on the global market. Optimizing a test for the detection of antibodies against Apx IV toxin in oral fluid would be a significant contribution to the swine industry.

INTRODUCTION

Actinobacillus pleuropneumonia (APP) is a significant respiratory pathogen of swine, causing acute death in finishing pigs and/or adding significant treatment costs to the production of clinically affected pigs. Outbreaks are often associated with a triggering factor – concomitant disease, animal management changes or other stressors - and APP is recognized as a component of the porcine respiratory disease complex (PRDC). In addition to clinical losses, APP causes fibrinous or fibrous pleural adhesions which may lead to condemnations at packing plants. "APP remains a significant cause of economic loss to the swine industry and there remains opportunity for improvement in control and eradication of this agent." (Gottschalk, 2012).

OBJECTIVES

The objective of this research was to develop an ELISA for the detection of APP carrier pigs based on the detection of ApxIV oral fluid antibody. The fact that the Apx IV toxin is universal to all APP serotypes will allow this assay to be used both as a diagnostic test and a surveillance tool for following APP infections in swine production systems.

MATERIALS AND METHODS

A bank of samples from pigs of precisely-known APP status was developed for initial test development. Three groups (6 pigs/group) of 14-week-old pigs were exposed to APP serotypes 1 (ATCC 27088), 5 (ATCC 33377), or 7 (ATCC WF83) intranasally (2 ml) and by direct application (3 ml) to tonsils using a challenge inoculum containing 1 x 106 CFU/ml. Animals were housed individually throughout the experiment to collect individual pig oral fluid and fecal samples. Pigs were monitored for clinical signs daily throughout the entire experiment. Oral fluids samples were collected daily, blood samples were collected weekly, and fecal samples were collected every 3 and 7 days for 56 days post inoculation (DPI).

Using the assay we developed, 124 paired samples (serum, oral fluid) from a commercial swine herd with clinical APP were tested for ApixIV antibody and the responses compared.

RESULTS

We successfully developed a test capable of detecting antibody against ApxIV in oral fluid specimens. Antibody responses in experimentally-inoculated animals were positively associated with the strength of the clinical response, i.e., both LPS and ApxIV antibodies were detected earlier (by DPI 7) and at higher levels in clinically-affected animals. In pigs inoculated with serotype 5, LPS serum ELISA responses were delayed (DPI 14-35), low, and sometimes transient, with some animals testing negative before the end of the observation period (56 DPI). Likewise, ApxIV ELISA responses were low or absent in this group. In particular, we believe that ApxIV is the result of chronic infection and detection of ApxIV antibody can be used to identify the presence of carrier animals in herds.

Testing of paired samples from a commercial swine herd with clinical APP showed good agreement between the tests (

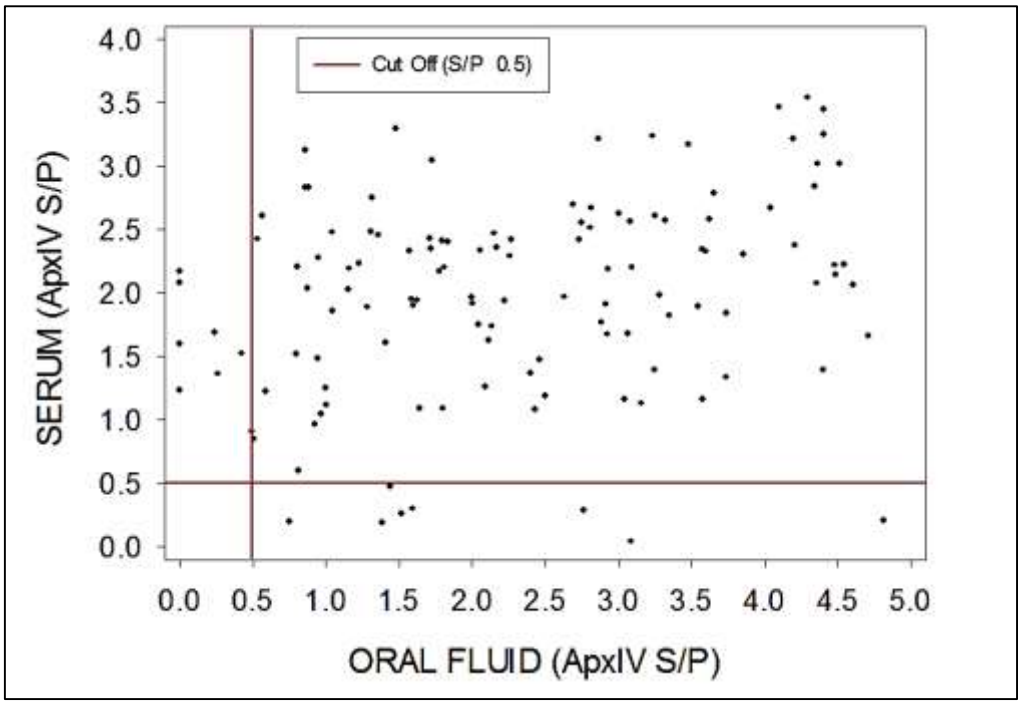

Figure 1. Quantitative (S/P) ApxIV antibody ELISA responses on 124 paired samples (serum, oral fluid) from a commercial swine herd with clinical APP

Table 1. Qualitative (agreement) ApxIV antibody ELISA responses on 124 paired samples (serum, oral fluid) from a commercial swine herd with clinical APP

		ORAL FLUID	
		Positive	Negative
SERUM	Positive	107	9
	Negative	8	0

DISCUSSION

Oral fluids are more practical for surveillance than bleeding and testing individual pigs. The pattern of APP infections in populations varies among production systems. Therefore, the benefit of oral fluid-based APP surveillance is in providing a practical method for collecting herd- and population-specific, timely, and accurate data. This approach gives producers the means to achieve targeted, efficient anti-APP interventions.