RESEARCH ABSTRACT

SWINE HEALTH

TITLE: Development of African swine fever diagnostic assays for oral fluids – NPB #13-048

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SCIENTIFIC ABSTRACT

Several ASFV structural and non-structural proteins have being identified as candidate antigens for serological tests (Gallardo et al., 2009; Cubillos et al., 2013). Among these, structural proteins p30, p54, and p72 have been identified as the principal serological immunodeterminants of ASFV (Kollnberger et al., 2002, Cubillos et al., 2013) and are the antigens most widely used in commercial ASFV serum antibody ELISAs.

We evaluated the serum antibody response against the three major ASFV proteins (p30, p72 and p54) using a multiplex fluorescent microbead-based immunoassay (FMIA). As shown in Figure 1, p30 provided the earliest response and best discrimination between known negative and known positive samples. Therefore, subsequent ELISA development was based on ASFV p30.

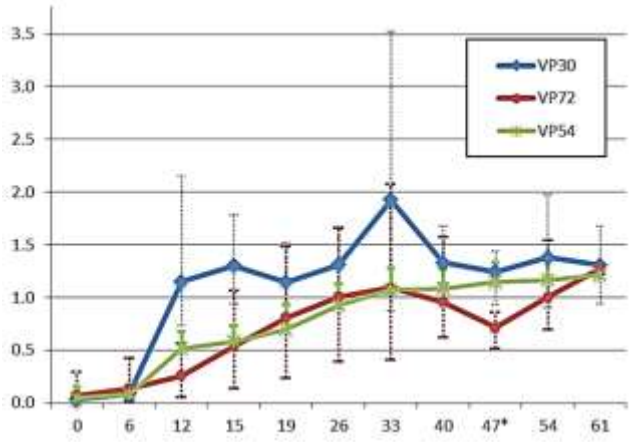

Fig 1. ASFV p30, p54, & p72 serum antibody responses (S/P ratio) over time as detected using a multiplex fluorescent microbead-based immunoassay (FMIA)

To create recombinant p30 for coating ELISA plates, a plasmid containing the p30 gene was over-expressed in *E. coli* and the recombinant His-tagged fusion p30 protein was purified from extracts of *E. coli* using nickel-affinity chromatography. ELISA conditions, e.g., rp30 coating/blocking conditions, sample/conjugate dilutions, buffers, and incubation times were optimized for antibody detection in serum and oral fluid specimens.

To evaluate the performance of the ASFV p30 ELISA, serum and oral fluid samples were collected from 9 pigs following inoculation with an attenuated ASFV isolate (NHV) that produces chronic infection. Oral fluid and serum samples were collected over days post inoculation (DPI 0, 6, 12, 15, 19, 26, 33, 40, 47, 54, and 61). ELISA specificity was evaluated using samples (200 oral fluid and 200 serum samples submitted to the Iowa State University Veterinary Diagnostic Laboratory for routine diagnostic testing from swine herds in the U.S., i.e., a known ASFV-negative pig population.

The mean ASFV rp30 antibody ELISA ODs for serum and oral fluid are shown in Figure 2. IgG antibody was detected by DPI 12 in both serum and oral fluid specimens. The evaluation of known ASFV negative field samples showed specificities of 99.5% and 100% for serum and oral fluid samples, respectively.

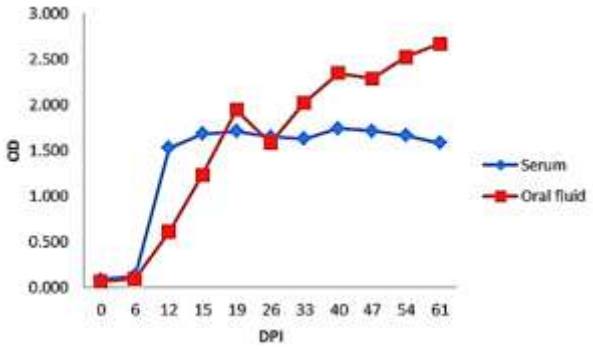

Fig 2. Mean optical density for serum (♦) and oral fluid (■) specimens collected over time from pigs inoculated with ASFV isolate NHV