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Detection of African Swine Fever Virus Antibodies in Serum and Oral Fluid Specimens Using a Recombinant Protein 30 (p30) Dual Matrix Indirect ELISA

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Abstract

In the absence of effective vaccine(s), control of African swine fever caused by African swine fever virus (ASFV) must be based on early, efficient, cost-effective detection and strict control and elimination strategies. For this purpose, we developed an indirect ELISA capable of detecting ASFV antibodies in either serum or oral fluid specimens. The recombinant protein used in the ELISA was selected by comparing the early serum antibody response of ASFV-infected pigs (NHV-p68 isolate) to three major recombinant polypeptides (p30, p54, p72) using a multiplex fluorescent microbead-based immunoassay (FMIA). Non-hazardous (non-infectious) antibody-positive serum for use as plate positive controls and for the calculation of sample-to-positive (S:P) ratios was produced by inoculating pigs with a replicon particle (RP) vaccine expressing the ASFV p30 gene. The optimized ELISA detected anti-p30 antibodies in serum and/or oral fluid samples from pigs inoculated with ASFV under experimental conditions beginning 8 to 12 days post inoculation. Tests on serum (n = 200) and oral fluid (n = 200) field samples from an ASFV-free population demonstrated that the assay was highly diagnostically specific. The convenience and diagnostic utility of oral fluid sampling combined with the flexibility to test either serum or oral fluid on the same platform suggests that this assay will be highly useful under the conditions for which OIE recommends ASFV antibody surveillance, i.e., in ASFV-endemic areas and for the detection of infections with ASFV isolates of low virulence.