Development and evaluation of a mixed long-chain lipopolysaccharide based ELISA for serological surveillance of infection with Actinobacillus pleuropneumoniae serotypes 2, 6 and 12 in pig herds

The objective was to develop an enzyme-linked immunosorbent assay (ELISA) for simultaneous detection of antibodies against Actinobacillus pleuropneumoniae (Ap) serotypes 2, 6 and 12. The assay was designated MIX-ELISA. Lipopolysaccharide (LPS) from Ap serotypes 2, 6 and 12 was purified using hot phenol-water extraction followed by fractionation by size-exclusion chromatography. A mixture of fractions containing molecules with molecular weight above 50 kDa from all three serotypes was used as antigen. The MIX-ELISA was evaluated with sera from pigs experimentally infected with the serotypes 1, 2, 5b, 6, 7, 8, 10 and 12 of Ap biotype 1. In addition to reaction with sera from pigs inoculated with Ap serotypes 2, 6 and 12, reaction was observed with sera from pigs inoculated with serotype 8. Furthermore, the sensitivity and specificity of the test on a herd level were evaluated with sera from herds naturally infected with serotypes 2, 6 or 12 and with sera from herds free of infection with any Ap serotype of biotype 1. The ELISA showed a high herd sensitivity (0.98; 95% confidence interval: 0.89-1.00) and specificity (0.95; 0.88-0.99). The high diagnostic sensitivity and specificity of the assay indicate that screening of herds for Ap infection can be performed using this ELISA. Efficient serological surveillance can be achieved by using such mixed antigen ELISAs coated with size-selected LPS-antigens from the most prevalent serotypes.

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