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Development of an enzyme-linked immunosorbent assay for the diagnosis of porcine proliferative enteropathy

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Introduction

For monitoring porcine proliferative enteropathy (PPE) distribution and its kinetics in the herd, serological testing is preferred because it is less expensive than fecal PCR testing and allows processing of larger numbers of samples. Currently, the available serology tests for diagnosis of PPE in pigs are the indirect fluorescent antibody test (IFAT) (5) and the immunoperoxidase monolayer assay (IPMA)(2). Previously, enzyme-linked immuno-sorbent assay (ELISA) techniques have been developed for detecting immunoglobulin IgG (4, 1) Those ELISA tests used unpurified and purified *Lawsonia intracellularis* (LI) coated as antigen. The objective of this study was to develop an indirect ELISA test using LI whole cell as antigen.

Materials and Methods

The purified LI strain PHE/MN1-00 was ultrasonicated on ice, the sonicated antigen was centrifuged and the supernatant was used as antigen.

The sensitivity (DSn) and the specificity (DSp) of the ELISA test was determined using 355 negative reference sera obtained from negative controls and 332 positive reference sera from challenge studies. The mean and standard deviation of the OD were calculated and the Receiver Operating Characteristic (ROC) was performed to determine the accuracy of the ELISA. For the ELISA, the sonicated antigen was diluted in carbonate buffer with a pH of 9.6 to a final protein concentration of 250ng/well and incubated 2 hours at 37°C. The coated plates were washed and blocked with 200 µl of blocking buffer (5% skim milk in PBST) for one hour. The tested sera were diluted 1:120 in blocking buffer and 100µl of each transferred to the coated plates. After a 1-hour incubation, the sera were discarded and washed 3 times. Goat anti-swine HRP conjugate(100µl) at 1:4,000 in blocking buffer was added and incubated for 1 hour. After 4 washing steps, 3,3',5,5'-tetra-methylbenzidine was added and incubated for 15 minutes and then the reaction was stopped with 1M phosphoric acid. Cross reactivity of the sonicated LI whole cell ELISA was tested with serum from pigs experimentally exposed to *B. pilosicoli, B. hyodysenteriae, C. mucosalis, C. jejuni,* and *C. coli.*

Results and Discussion

The ROC curve had an area under the curve of 0.991 (95% CI of area 0.986-0.997, P=0.0001). This indicates an excellent differentiation of positive and

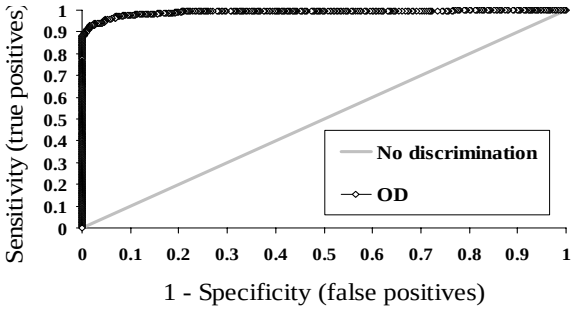

Figure 1. The ROC curve

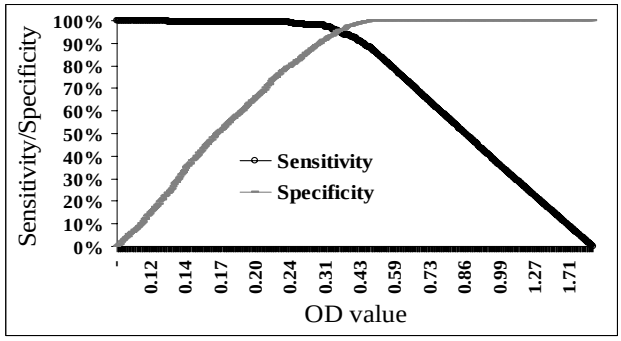

Figure 2. Sensitivity and specificity of the LI whole cell ELISA test based on test samples

negative controls. We determined the cutoff for the whole cell LI ELISA to be at 0.45 OD with 89.8% DSn and 99.4% DSp based on a combination of good sensitivity with high specificity. The DSn and DSp of the whole cell ELISA is similar to the IFAT and the IPMA (2, 3). No cross reactivity was found in sera from pigs exposed to *B. pilosicoli, B. hyodysenteriae, C. mucosalis, C. jejuni*, or *C. coli.* These results indicate that the whole cell ELISA should be further evaluated as an alternative serological test for the diagnosis of PE. For further investigation, we will perform a comparative analysis of agreement between the IPMA and the LI whole cell ELISA.

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