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427 - ÁREA: VETERINÁRIA

INDIRECT ELISA USING A RECOMBINANT NUCLEOPROTEIN FOR INFLUENZA A VIRUS ANTIBODY DETECTION IN SWINE

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Resumo

Influenza A virus (IAV) causes an important respiratory disease in pigs leading to significant economic losses in the swine industry. The major subtypes that circulate endemically in the swine population worldwide are H1N1, H1N2 and H3N2. Although surveillance of IAVs in Brazilian swine population is not systematically conducted, it is relevant due to public health concerns. Nucleoprotein (NP) is highly conserved between IAV subtypes and broadly used for diagnostics. Considering the absence of a cost competitive serologic assay available in Brazil for IAV screening, an indirect ELISA based on the NP was developed for use in serologic surveys. The NP gene was amplified from an IAV (A/swine/Brazil/12A/2010) strain, cloned into pET23d plasmid and transformed into E. coli. The recombinant protein (rNP) was expressed and purified by Ni-based affinity chromatography followed by anion exchange chromatography. The protein mass (55 kDa) and identity were confirmed by SDS-PAGE and western blot using anti-histidine antibody and anti-NP monoclonal antibody. In order to produce antisera as a positive control, two 3-week-old piglets were immunized with 200 µg rNP. The rNP-ELISA plates were coated with 200 ng/well of rNP along with negative and positive controls. Plates were blocked using 3% non-fat dry milk. Swine serum samples were diluted (1:100) in 3% non-fat dry milk containing E. coli extract (1:10). Secondary antibody (anti-swine IgG peroxidase-conjugated antibody) was diluted to 1:5000. After color development using OPD in citrate phosphate solution, the enzymatic reaction was stopped with 12% H2SO4 solution and the optical density (OD) was read. The test sensitivity (SE) and specificity (SP) of rNP-ELISA was estimated by evaluating 151 pig sera (92 positive and 59 negative), previously tested by a commercial ELISA (AI Multi-Screen Ab test®, IDEXX). The ROC curve analysis resulted in a cut-off (0.542) derived from the maximum SE (84.8%) and SP (89.8%) values. Test SE and SP estimated by a Bayesian analysis were 84.7% (95% CI: 76-91%) and 93.4% (95% CI: 93-98%), using the same cut-off (0.542). Based on the test performance analysis, the rNP-ELISA was considered suitable for serologic detection of IAV in pigs. The test developed here is also a cost-effective, safe, and a rapid tool to detect influenza-specific antibodies in swine herds.

Palavras-chave: Diagnosis, ELISA, Influenza A virus, Nucleoprotein, Swine