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AFFINITY COMPARISON OF FOUR NEW SPECIFIC ANTIBODIES TOWARDS PORCINE SERUM AMYLOID A

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OBJECTIVES. The main goal of this study was the development of specific antibodies towards porcine SAA and the comparison of their performance in different immunological detection systems.

METHODS. A recombinant porcine SAA protein developed in our laboratory was employed as immunogen for the production of rabbit and chicken polyclonal and rat monoclonal antibodies. Different species were employed due to the difficulty in raising high-affinity antibodies against the highly-conserved SAA protein. The affinity of the antibodies was tested in two ways. Firstly, a competitive ELISA was performed by coating polystyrene plates with each antibody followed by a mix of a serial dilution of acute phase porcine serum and a fix amount of biotinylated competitor (recombinant porcine SAA). After adding a streptavidin-HRP conjugate and an ABTS substrate, the response of each antibody was checked by fitting a non linear 4-parameter regression curve and comparing these curve parameters. Secondly, recombinant porcine SAA and acute phase porcine serum were run in a SDS-PAGE gel and subsequently separated proteins were transferred onto PVDF membranes. SAA was detected in those membranes by western blot analysis with each of the antibodies. The performance of each antibody in immunoblotting was contrasted.

RESULTS. The monoclonal antibody and one of the rabbit polyclonal antibodies gave good and very similar response to the competitive ELISA assay, with results showing a very good fitting with the regression model.

All the antibodies recognized recombinant and native porcine SAA in western blot, being the monoclonal antibody the most sensitive, since it able to neatly recognize both SAA monomers (15 kDa) and different-order multimers.

CONCLUSIONS. The affinity results showed by a rat monoclonal antibody towards porcine SAA was much higher than any polyclonal antibody, demonstrated by its good performance in different immunological methods and corroborating that rats are perfect for anti-SAA antibody production.