Title:

ELISPOT ON ENRICHED IgA$^+$ B-LYMPHOCYTES CAN BE USED TO DETECT THE IgA RESPONSE FOLLOWING ORAL IMMUNIZATION WITH F4 FIMBRIAE OF PIGS WITH COLOSTRAL F4-SPECIFIC SERUM ANTIBODIES

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Diarrhoea and mortality in piglets are mainly caused by F4 enterotoxigenic *Escherichia coli* (ETEC) and lead to serious economical losses. Oral immunization of F4 receptor positive F4-seronegative pigs with F4 fimbriae can induce a protective intestinal immune response evidenced by an F4 specific serum and intestinal mucosal IgA response. However, on farms most pigs obtain maternal F4-specific colostral IgG at birth and F4-specific IgA from milk during the suckling period resulting in the presence of F4-specific serum antibodies. The aim of the present study was to determine if piglets with F4-specific passive immunity could be orally immunized with F4. Whereas the F4-specific immune response of orally immunized F4-seronegative pigs can be easily measured in serum, maternal antibodies will obscure the response in orally immunized pigs with colostral F4-specific serum antibodies. In the current experiment, a protocol was optimized to detect the F4-specific IgA response following oral immunization of 3- to 4-week-old pigs with passive immunity. As controls, pigs that were intramuscularly immunized and pigs that are not immunized were included. To determine the humoral F4-specific immune response, blood was taken weekly until 3 weeks post immunization and ELISA's were performed to determine the F4-specific serum IgA antibody titres as well as ELIspot assays to enumerate the induced F4-specific antibody secreting cells. The ELIspot assays were determined on peripheral blood mononuclear cells (PBMCs) following density gradient centrifugation and on the IgA$^+$ B cell population enriched from the PBMCs by magnetic-activated cell sorting (MACs). Whereas the intramuscularly induced IgA response could be measured with both ELISA and ELIspot assays, active immune responses in the orally immunized pigs could only be detected by the ELIspot assay performed 21 days post immunization on the enriched IgA$^+$ B-cell populations. These results demonstrate that piglets with maternal antibodies against F4 still can be immunized orally with F4 fimbriae, most likely protecting these piglets against infection with F4 ETEC. Furthermore, our ELIspot assay on IgA$^+$ B-cells has the potential to be used as a rapid procedure to optimize immunization protocols in pigs with passive immunity in comparison with the much more labour intense challenge assays.