

## Vaccination against ETEC in pigs

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Enterotoxigenic *Escherichia coli* (ETEC) that bear F4 fimbriae on their surface (F4+ ETEC) are a major cause of postweaning diarrhoea (PWD) in pigs. The F4 fimbriae enable the bacteria to colonize the small intestine and subsequently, to produce enterotoxins causing diarrhoea. Consequently, an F4-specific secretory IgA response at the intestinal mucosa that neutralizes the fimbriae is desired for protection against postweaning diarrhoea.

Several strategies were tested in our lab to activate the intestinal mucosal immune system. In a first trial, we demonstrated that oral immunization of weaned F4-receptor positive (F4R<sup>+</sup>) pigs with soluble F4 fimbriae results in an antigen-specific secretory IgA response at the intestinal mucosa, preventing colonization of the bacteria and consequently, protecting the pigs against infection. However, to protect newly weaned pigs, the immune system should already be primed during the suckling period. During this period, the F4 fimbriae might be partially inactivated by maternal antibodies and other milk-factors. In order to overcome this, enteric-coated pellets containing the F4 fimbriae were developed. The pellets function by shielding the F4 during transport through the stomach and the duodenum and releasing the fimbriae at the beginning of the jejunum, close to the major intestinal immune induction sites, namely the jejunal Peyers patches. Oral vaccination of suckling pigs with these enteric-coated pellets resulted in a marginal but significant reduction in faecal F4+ ETEC excretion upon challenge infection. Nevertheless, in contrast to soluble F4 administration to weaned pigs, this could not prevent colonisation of the small intestine with F4+ ETEC. Subsequently, the F4 fimbriae were encapsulated in Gantrez nanoparticles. These nanoparticles were not expected to release the F4 in the jejunum, but only after transport through the intestinal epithelium. As compared to soluble F4, encapsulation of F4 in Gantrez nanoparticles didn't improve protection against F4+ ETEC, but did improve the serum IgG and IgA response in an oral immunisation experiment of weaned pigs. However, when suckling pigs were vaccinated, a positive effect of the Gantrez nanoparticles on the serum antibody response could neither be observed.

A second strategy we tested in order to activate the intestinal mucosal immune system was systemic vaccination using immunomodulating adjuvants. As successful results were already obtained in mice using  $1\alpha,25(\text{OH})_2\text{D}_3$ , the active form of vitamin D<sub>3</sub>, this adjuvant was tested in an intramuscular immunization experiment in pigs using the model antigen human serum albumin (HSA). Coadministration of  $1\alpha,25(\text{OH})_2\text{D}_3$  to HSA enhanced the serum IgA and IgM response and the IgA response in faeces, saliva and nasal secretions. In addition, it slightly increased the number of HSA-specific IgA antibody-secreting cells (ASC), suggesting that priming of the intestinal mucosal immune system occurred. To test this, pigs were immunized intramuscularly with soluble F4 in presence or absence of  $1\alpha,25(\text{OH})_2\text{D}_3$  and protection against F4+ ETEC was evaluated. In the  $1\alpha,25(\text{OH})_2\text{D}_3$  group, a secretory IgA response after infection and a reduced excretion of F4+ ETEC in faeces could be observed. Nevertheless, the duration of faecal F4+ ETEC excretion was not reduced, indicating that colonization could not be prevented. Currently, we are analyzing the

immunomodulating effect of retinoic acid, the metabolite of vitamin A on intramuscular immunization of pigs with HSA. Retinoic acid is produced by dendritic cells in the small intestinal lamina propria, Peyer's patches and mesenteric lymph nodes and is suggested to be involved in the imprinting of gut-homing specificity on lymphocytes. The results of this experiment are under evaluation.