Seed produced anti ETEC antibodies for oral passive immunisation of piglets against post weaning diarrhoea

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Enterotoxigenic *Escherichia coli* (ETEC) related diarrhoea is a common pig disease upon weaning. Passive immunisation of weaned piglets with anti ETEC antibodies was shown to reduce the disease occurrence. However, this therapy needs large amounts of protective antibodies produced by immunized animals, which makes it an unrealistic treatment, particularly for immunising large herds of pigs. A low cost alternative could be to produce the ETEC-specific antibodies in a seed crop and administer them via the starter feed. Therefore, we first investigated the production of anti ETEC antibodies in Arabidopsis seeds, to later transfer the technology to a major grain crop.

The success of such a treatment of course depends on the seed ability to produce abundant amounts of antibodies. Moreover the antibodies need to survive the stomach and gut transit, and the antibodies need to be bivalent in order to agglutinate the ETEC bacteria and to prevent them from binding the gut villi. Therefore, a novel antibody strategy was applied.

First, antigen-binding domains of camel antibodies (nanobodies) were isolated against FaeG, the major subunit protein of F4 fimbriae of ETEC bacteria causing post weaning diarrhoea. Then, the functional ELISA positive nanobodies were grafted onto the Fc-fragment of a pig IgG3 immunoglobulin, because IgG3 was described as being most proteolytically stable. These fusion camel-like antibodies were expressed in Arabidopsis seeds by using the regulatory sequences of the seed storage protein genes arceline 5-I and -phaseolin of a *Phaseolus* species. Each antibody expressed to its own particular accumulation level, the highest being 3% of seed weight. The fusion immunoglobulins formed bivalent complexes via disulphide bridges across the cysteine residues in the hinge region. Importantly, the seed produced antibodies did agglutinate the ETEC bacteria *in vitro* and also showed inhibition of bacterial attachment to villi isolated from the gut of piglets.

We conclude that production of these antibodies in a major feed crop holds great promise to protect piglets from ETEC during the weaning period.