Strategies to diminish colibacillosis in newly weaned piglets based on activation of immunity or prevention of colonization using antibodies or receptor analogues

E. Cox

Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University Belgium

Eric.Cox@ugent.be

The ban on growth promoters in feed of swine has led to an increase in infections with enteropathogens and as a result in increased use of antibiotics to treat these infections with increase in antibiotic resistance as a consequence. In the search for alternatives, ZnO was found to control the clinical signs of these infections, but its effect on antibiotic resistance and the environment has led to a ban on ZnO that will start in 2020. In the quest for alternatives, increasing resistance against enteropathogens is one of the important strategies.

The intestinal immune system is complexly regulated in order to cope with food antigens, commensal bacteria, pathogens and toxins. The dominant response mode of the intestine is immunological tolerance, but via pattern recognition receptors (PRRS) expressed by epithelial and cells of the immune system the mucosa can recognize danger signals which help to steer the immune system towards increased resistance. This resistance consists of rapid occurring innate immune responses which sometimes completely neutralize a pathogen. This innate immunity can be activated by adding microbe-associated molecular patterns (MMPs) to the feed, which bind to the PRRs. My lab elucidated some of the mechanism via which ß1,3-1,6 glucans activate the immune system of the pig and can lead to increased resistance against F4+ enterotoxigenic *Escherichia coli* infections ($F4^+$ETEC) (Baert et al., 2015. J. Controlled Rel.; Baert et al., 2015. Dev. Comp. Immunol.; Sonck et al., 2010. Vet. Imm. Immunopathol.; Stuyven et al., 2010. Vet. Imm. Immunopathol.). The glucans supplemented to the feed need to be sufficiently pure and consumed during at least 10 days in order to have this effect. As such colibacillosis occurring immediately post-weaning comes too early to be controlled using this strategy. The same is true for many other innate immune activators given via the feed.

Innate immune activation also forms the first step towards the development of the more pathogen-specific adaptive immunity. Indeed, the pro-inflammatory cytokines (IL-1, IL-6 and TNFα) secreted after interaction with the PRRs activate immune cells. Activated antigen-presenting cells can now sample the pathogen or its antigens and present them to T- and B-lymphocytes. This initiates the development of antibodies or cellular immunity which try to neutralize the pathogen. Special for the immune system of the gut is that the B-lymphocytes and T-lymphocytes have to be induced locally in order to obtain cells that home to the intestinal mucosa. This indicates that vaccination should be performed orally. Mainly IgA and some IgM can be secreted in the mucosal lumen where it is released as secretory IgA and IgM. These antibodies are more resistant towards degradation and can neutralize the pathogen and or its toxins. $F4^+$ and $F18^+$ *E. coli* use their fimbriae to adhere to receptors on small intestinal enterocytes. Antibodies directed against these fimbriae (or their adhesin) are sufficient to neutralize these strains. Some years ago, Prevtec registered a live oral vaccine against $F4^+$ *E. coli* based on an avirulent $F4^+$ E. coli strain. More recently this became supplemented with an avirulent $F18^+$ *E. coli* strain (Coliprotec F4/F18). They demonstrated that the vaccine can be administered to 17-day-old piglets weaned piglets with an onset of immunity 7 days later with a duration of immunity of 21 days. In Canada piglets are weaned at the age of 17 days. However, in Europe piglets should be at least 21 day old when weaned. Therefor one started to promote the use of the vaccine in suckling piglets, preferably seven days before weaning. However, on problems farms piglets have a robust lactogenic immunity against these pathogens and natural infections occur after weaning. It is not clear how the vaccine (should) work(s) in the presence of this robust immunity. It can be hypothesized that in cases where it is effective, there is a problem with the lactogenic immunity against colibacillosis or that the bacteria of the vaccine compete immediately after weaning with the pathogen for colonization of the mucosa.

Other strategies are the oral administration of antibodies. It is known for some time that egg yolk powder of chickens immunized with *E. coli* fimbriae, passively protect piglets against infection when given orally via the feed. More recently researcher of the VIB in collaboration with my group demonstrated that camelid variable single domain antibodies (VHH) specific for the adhesin of F4 fimbriae and fused to porcine IgA Fc can significantly reduce infection with the F4+ ETEC strain (Vikram et al., 2019. Nature Biotechnology). These antibodies can easily be produced in large quantities in soybean seeds or the yeast *Pichia pastoris*. This opens perspectives for large scale production of pathogen-neutralizing antibodies.

Also receptor analogues might be an interesting strategy. Our lab demonstrated that $F18^+$ *E. coli* bind with their adhesin to ABH type blood group antigens on glycosphingolipids in the membrane of enterocytes (Coddens et al., 2009. J. Biol. Chem.). Using these determinants we could decrease binding of the pathogen to the mucosa, diminish fecal excretion and prevent some clinical signs.

In conclusion, different strategies can be used of will become available to decrease colonization of F4+ and F18+ ETEC and/or shigatoxin producing *E. coli.* They are act via the innate or adaptive immune system or prevent colonization by diminishing binding of these pathogens to their receptor.