RESEARCH ABSTRACT

SWINE HEALTH

Title: Testing of a live enterotoxigenic E. coli vaccine candidate for its potential as a competitive exclusion probiotic for preventing colibacillosis in weaned pigs –
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Scientific Abstract

Enterotoxigenic *Escherichia coli* (ETEC) is an important cause of diarrhea in both humans and livestock. Virulence of ETEC is associated with fimbrial adhesins that enable attachment to the mucosa of the small intestine and enterotoxins such as heat-labile (LT) and/or heat-stable (ST) enterotoxins that stimulate host diarrhea. Our recent *in vitro* work using a cell culture model system suggests that exclusion of ETEC from attachment to epithelial cells requires expression of both an adhesin such as K88 fimbriae, and the LT enterotoxin. In addition, we have observed that LT contributes to ETEC adherence independent of secretory activity. To further test the ability of non-pathogenic *E. coli* (probiotic) strains to colonize the intestine and competitively exclude ETEC at the level required to produce clinical disease, we utilized a piglet ETEC challenge model. Thirty-nine 5-day-old piglets were inoculated with either a placebo (negative control group) or with any of the three $K88^+$ *E. coli* strains isogenic with regard to modified LT expression. The isogenic strains were 8017 (pBR322 control), the non-toxigenic mutant 8221 (LT(R192G) in pBR322), or 8488 (LT(R192G) gene fused to the gene for STb in pBR322). Piglets were challenged with virulent ETEC strain 3030-2 ($K88^+$/LT/STb) 24h after piglets were inoculated with the isogenic strains. All K88ac receptor-positive piglets in the control group (given no isogenic strain) developed diarrhea and became dehydrated after 12 hours post-challenge. Piglets inoculated with strains 8221 or 8488 did not exhibit any clinical signs of ETEC disease while piglets inoculated with ETEC strain 8017 showed mild diarrhea to no diarrhea post-challenge. There was significant weight loss in the control pigs compared to the piglets inoculated with the isogenic strains and blood total protein was significantly higher in the control pigs compared to the pigs inoculated with strains 8221 or 8488. Quantitative culture of the challenge strain in washed ileum and jejunum indicated a significant higher number of pathogenic *E. coli* in the control group compared to groups inoculated with strains 8017 or 8488 for both segments of the small intestine. There was a significantly higher number of pathogenic *E. coli* in the ileum of pigs inoculated with strain 8017 compared to the 8488 group. In further studies to assess whether the protection was highly specific to K88 ETEC, we inoculated the piglets with the strains competitive exclusion strains as indicated above, but challenged with an unrelated ETEC strain (B44; O9:K99:F41: H-; STa). The vaccine strains were not protective against this unrelated ETEC, suggesting that protection is highly specific. In further studies, we substituted the vaccine strains with strains K12 strains possessing K88 fimbriae and the modified LT toxin. K12 strains grow poorly if at all in mammalian intestines. Thus, these bacteria are living, but non-proliferative. When pigs were treated with these strains, then challenged with K88+ ETEC, they were not protected. This observation would suggest that protection may require sufficient bacterial proliferation for substantial colonization to occur. Thus, it is unlikely that the mechanism of protection is primarily an up-regulation of innate immunity precipitated by epithelial contact with a few ETEC-like bacteria. Rather it is likely a competitive exclusion event made possible by a large population of bacteria similar the ETEC pathogen, but unable to cause diarrheal disease.