DEVELOPMENT AND EVALUATION OF A NOVEL ORALLY ADMINISTERED SUBUNIT VACCINE TO CONTROL FOODBORNE PATHOGENS

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Introduction

Development of vaccines for effective control of foodborne pathogens and infection represents an important development in reducing public health risk. Advancements in the area of biotechnology have increased innovative potential and allow new technologies to be used as a promising control strategy for alternatives to antibiotics. We have been working to create a novel vaccine platform that incorporates a subunit/epitope sequence, common for all \textit{E. coli} strains (broad spectrum), into an inactivated orally administered vaccine platform that protects against infection and disease by inducing mucosal immunity.

The mucous membranes constitute the major portal of entry for infectious agents and include membranes of the nasal, respiratory, gastrointestinal, and genitourinary tract; as well as the ocular conjunctiva, the inner ear and the ducts of all exocrine glands. Collectively they cover more than 400m\textsuperscript{2} in humans, compared to only 2m\textsuperscript{2} of skin, and serve as the first line of defense against infection at the entry points for a variety of pathogens (Ogra et al., 2001). The gastrointestinal system is the largest lymphoid organ in the body containing an estimated 70\% to 80\% of the body’s immunoglobulin–producing cells (Kaul 1999). 80\% of all the activated B cells in the body are located at the mucosal tissues (Brandtzaeg et al., 1989) In fact the only way to contract an infection other than the mucosal portal of entry is through blood-borne vectors or damage to epithelial surfaces.

Despite its important role, currently only a handful of vaccines specifically target this area of the immune system despite strong evidence that a robust mucosal response can effectively prevent systemic infections (Ogra et al., 2001). Increasing evidence has indicated that mucosal vaccination can induce both systemic and local mucosal immunity, while systemic immunization generally fails to elicit strong mucosal immunity (Valosky et al., 2005). Also, the concept of a common mucosal immune system predicts that induction of immunity at one mucosal surface, such as the gut, can provide immunity at another mucosal surface, such as the lung (Cerkinsky et al., 1995) providing a necessary link for immunity transfer throughout mucosal surfaces. Mucosal immunity may prove to be the link in fighting a complex infection in which systemic and local immunity are necessary in preventing the spread and transmission of infectious disease and foodborne pathogens.

Pathogenic \textit{E. coli} infections, or colibacillosis, is one of the most prevalent diseases affecting the global swine industry (Fairbrother J. et al., 2005). Enterotoxigenic \textit{Escherichia coli} (ETEC) is a major cause of illness and death in neonatal and recently weaned pigs in some cases young pigs can lose up to 40\% of their body weight and in severe cases mortality can reach 100\% (USDA 2002). Colibacillosis not only has a direct economic impact on producers it also represents a potential human transmission route of foodborne illness. Common treatment options often include incorporation of antibiotics to control and limit spread of the disease; however, the disease is becoming
increasingly more difficult to treat due to acquired antibiotic resistance. Moreover, consumer pressure and changing government regulations may limit or omit the use of antibiotics necessitating the need for alternative intervention strategies.

Our vaccine development, in which a single vaccine, can simultaneously and effectively control all or the majority of serotypes/strains that make up the 150-200 serotypes that represent the *E. coli* family of pathogens (broad spectrum) and provide protection in multiple species (swine, poultry, bovine, fish, humans) potentially represents practical biotechnological progress as an alternative intervention strategy in controlling diseases and foodborne pathogens.

**Materials and methods**

**Development of subunit orally administered inactive vaccine against *E. coli* spp. (Biotech Vac E. Coli)**

Briefly vaccine construction was as follows: a synthetic, antigenic, epitope, genetic sequence common for *E. coli* spp. was inserted by direct ligation into a *Bacillus subtilis* expression plasmid. The genetic sequence was put under the control of an IPTG inducible promoter present on the expression plasmid and inserted into the multiple cloning site of the plasmid for Open Reading Frame expression. The modified expression plasmid was then transfected into *E. coli* TOPO 1 cells for confirmation of gene insert and multiplication of the plasmid. The multiplied confirmed plasmid was then isolated, concentrated and transfected into the vector *Bacillus subtilis* VBTSLL11™, a proprietary *Bacillus* strain selected specifically for use in the Biotech Vac platform. Once plasmid insertion was confirmed by colony PCR, DNA sequencing was performed to confirm correct genetic sequence and protein expression was quantified by SDS-PAGE western blotting. This newly constructed and verified *Bacillus* strain was used to manufacture the antigenic *E. coli* subunit. The bacteria were grown under normal Bacillus culture conditions in Tryptic Soy Broth (TSB) at 37°C, after 4 hours of growth the culture was induced with 1mM of Isopropyl β-D-1-thiogalactopyranoside (IPTG) followed by an additional 5 hours of growth. Once fermentation was complete the culture was inactivated and added to an encapsulation media for incorporation of the epitopes into micro-particles for oral delivery. Subunit concentration for each 2.0ml dose of vaccine is approximately 500ng.

**Challenge with *Escherichia coli***

Two wild-type field isolates (VBTEcoli1-2) of Enterotoxigenic *E. coli* (ETEC) originally isolated from swine farms in Argentina were grown individually to log phase, combined, serially diluted, and enumerated by spectrophotometric density and comparison to a previously generated standard curve.

These strains were diluted to approximately 10^8 cfu/ml for challenge by oral gavage at a dose of 2.0ml/pig for 2 consecutive days.

**Vaccination study 1**

In the first challenge trial, two groups of 3 day old piglets at a commercial production farm were assigned to one of two experimental groups, the piglet and sows in these two groups were isolated from the rest of the commercial farm. A non-treated
control group (n=13) that received 2.0ml of saline by oral gavage on days 3 and 14 of life or a treated group (n=12) that received 2.0ml of Biotech Vac E. coli vaccine by oral gavage on days 3 and 14 of life. All piglets in both groups were challenge with 2.0ml of ETEC E. coli by oral gavage (challenge preparation described above) on days 17 and 18 of life. Piglets were observed for 1 week following challenge and presence or absence of diarrhea was recorded daily. Additionally, individual weight gain was calculated. During the course of the experiment, neither the piglets nor the lactating sows received antibiotic treatment and antibiotics were not present in the commercial feed.

Vaccination study 2

In the second challenge trial, thirty 28 day old pigs from a commercial production farm were randomly assigned to either a non-treated control group or a Biotech Vac E. coli vaccine treated group (n=15/group), transferred to adjacent weaning boxes isolated from the rest of the commercial farm and allowed to acclimate for 5 days. Following the acclimation period (day 33 of life), pigs were either given by oral gavage 2.0ml saline (control group) or 2.0ml Biotech Vac E.coli. and subsequently administered the same treatment 10 days (day 43 of life) following the first administration. Three and four days following the second treatment administration (day 44 & 47 of life), all pigs in both groups were challenged with 2.0ml of ETEC E. coli by oral gavage (challenge preparation described above). Pigs in both groups were observed for 10 days following challenge and presence or absence of diarrhea was recorded daily. Pigs were fed standard commercial diets containing no antibiotics.

Results

Results from the 1st challenge trial done in newborn piglets under standard commercial production conditions showed that on day 20 48 hours after trial 100% (13/13) of the piglets in the control non-treated group had developed clinical signs of diarrhea consistent with Colibacillosis with the diarrhea continuing for 72 hours. While the piglets that were vaccinated with Biotech Vac E. Coli, exhibited no clinical signs and did not develop diarrheas throughout the 1 week observation period. Additionally, piglets in the Biotech Vac E. coli treated group had a slightly increased total weight gain and daily body weight gain when compared to the non-treated controls; 46.4kg vs 45.6kg respectively (total weight gain) and 1.93kg vs 1.90kg respectively (daily body weight gain). No mortality was observed in either experimental group.

In the 2nd challenge trial, conducted in weanling pigs, results demonstrated significant reductions in the percentage, severity and duration of ETEC associated diarrheas in the group vaccinated with Biotech Vac E. coli when compared to the non-treated control group under standard commercial production conditions. In the group treated with the vaccine 4 days after challenge 20% (3/15) of the pigs exhibited diarrheas lasting 1 day and 6.7% (1/15) of the pigs had mild diarrhea that lasted 3 days. Also, in the group receiving the vaccine, 1 pig exhibited severe diarrhea (perineal congestion) and ultimately was diagnosed with pneumonia. While in the non-treated control group:

- Day 3 post-challenge: 20% (2/15) of the pigs began with diarrhea.
- Day 4 post-challenge: 73% (11/15) of the pigs were present with diarrhea ranging in severity and consistency.
- Day 5 post-challenge: 73% (11/15) of the pigs were still present with diarrhea.
Day 6 post-challenge: 20% (2/15) of the pigs continued to exhibit mild diarrhea. 
No mortality was observed in either experimental group.

Conclusions

Results of 2 separate ETEC E. coli challenge trials in commercial newborn piglets and weanling pigs that received two doses of Biotech Vac E. coli demonstrated: significant reductions of clinical symptoms associated with E. coli, significant reductions in the severity of E. coli associated diarrheas and a reduction in time in which the clinical signs and diarrheas persisted. These preliminary results suggest that our inactivated orally administered subunit vaccine platform offers a promising alternative for the control of infections and pathogens associated with foodborne diseases.

References


