RESEARCHREPORT

PORK SAFETY

Title: Effects of Competitive Exclusion on Post-Weaning *Escherichia coli* Infection - **NPB #00-137**

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I. Abstract:

The objective of the proposed research was to evaluate the effects of a porcine-derived competitive exclusion culture (RPCF) on an experimental enterotoxigenic *E. coli* (ETEC) infection in weaned pigs. The litters of eighteen sows, split into 3 studies, were used. Piglets in the RPCF group received the RPCF culture within 12 hours of birth by oral administration. Control piglets received a placebo. Upon weaning, piglets in the RPCF and infected control groups were administered 10^8, 10^9, or 10^{10} colony-forming units (CFUs) of an enterotoxigenic *Escherichia coli* (studies 1, 2, 3, respectively) that also expressed the F 18 fimbrial antigen and produced the STx2e toxin associated with edema disease in weaned pigs. In studies 1 and 2 where a lower challenge dose was used, reductions in the tissue positive and the CFU determinations for EC F18 STx2e were observed in the RPCF group. No mortality was associated with the two lower challenge doses and no differences in weights were recorded. However, in the high challenge dose studies (Study 3), no reductions in gut colonization by EC F18 STx2e were observed. The infected control group in Study 3 experienced 14.3% mortality, while no mortalities were recorded in the RPCF-treated pigs. Again, no differences in weight gain were observed. These studies demonstrate that the RPCF competitive exclusion culture may be an alternative or possibly adjunct prophylactic measure for the control of ETEC in weaned pigs.

II. Introduction

Escherichia coli has been described as the most important cause of neonatal and post-weaning diarrhea in pigs (1). In weaned pigs, multiple changes occur which could predispose these pigs to an increased incidence of disease caused by *E. coli*. These changes include decreased levels of maternal antibody, changes in diet, changes in their environment, including temperature and exposure to pathogens, and stress associated with such changes. In addition, information regarding the immune competence of pigs at weaning has pointed to a lack of

immune responsiveness in both the cell- and antibody- mediated arms of the immune system (2,3).

Treatment and prevention of *E. coli* infections in weaned pigs have, thus far, relied upon the use of both subtherapeutic and therapeutic levels of antibiotics. However, recent public and scientific concerns over the use of antibiotics in food animals and the possible development of multiple antibiotic resistant bacterial strains have caused researchers to look for alternative methods to control and prevent disease caused by pathogenic bacteria. One such alternative is the use of competitive exclusion cultures. The theory of competitive exclusion cultures follows from the idea that when an animal is born, its digestive tract is a sterile environment devoid of the normal bacterial flora found in adults of that species. Due to the lack of a normal bacterial flora in the gut, it is believed that the neonatal animal is uniquely susceptible to colonization and infection by enteropathogens. In addition, recent studies have suggested that it is the establishment of a normal flora early in life that may affect the production of an effective immune response throughout an animal's life. A competitive exclusion culture provides the neonatal animal with the normal flora of an adult which may prevent the colonization and subsequent infection of the host by enteric pathogens. The exact mechanism of action of the competitive exclusion of bacterial pathogens has not been established, but may include the binding/blocking of receptor sites in the gut which are used by pathogenic bacteria to attach to and/or invade the host, the production of conditions within the gut that are unsuitable for the pathogen's growth, or the production of specific antimicrobial substances that negatively effect the pathogen's survival (4-6).

Our laboratory has recently developed a swine-derived, defined competitive exclusion culture (PCF-1) that has been found to reduce the incidence of *Salmonella choleraesuis* shedding and cecal colonization in young pigs (7). A derivative of this culture, designated RPCF, has been found to protect both nursery-raised and suckling neonatal pigs against enterotoxigenic *E. coli* infections (8,9).

III. Objective: The objective of the proposed research was to evaluate the effects of a porcine- derived competitive exclusion culture (RPCF) on an experimental enterotoxigenic *E. coli* (ETEC) infection in weaned pigs.

IV. Procedures:

Challenge *Escherichia coli* strain:

The *E. coli* strain used in these studies was isolated from a case of clinical porcine colibacillosis at the North Carolina Department of Agriculture and Consumer Services, Rollins Animal Disease Diagnostic Laboratory, Raleigh, North Carolina and generously provided to our laboratory by Dr. Karen W. Post DVM, MS, D-ACVM. The *E. coli* strain, designated *EC F18/STx2e*, exhibits the following virulence determinants: F18, STx2e, STa, STb.

Animals:

Delivery of pregnant sows to our facilities will occur one week prior to farrowing. Sow health and vaccination histories were obtained from the supplier. Upon farrowing, piglets in all groups were ear tagged and treated as described below. Upon farrowing, litters of pigs in the RPCF treatment groups were administered,

by oral gavage, 5 ml of RPCF (~ 10^9 to 10^{10} colony forming units/ ml of mixed bacterial species) within 12 hours of birth. Pigs from the challenged control groups received 5 ml of VL broth by oral gavage within 12 hours of birth. Piglets designated as untreated, unchallenged controls did not receive any oral treatment. Piglets remained on the sows for a period of 14 days post- farrow.

Study Groups:

1. **Control group** – did not receive any treatment upon birth and were not challenged with EC F18/STx2e upon weaning. Upon birth, piglets in this group were given an intramuscular injection of 0.5 ml of iron dextran i.m. as per standard industry practices.
2. **Infected Control Group** – were administered 5 ml of sterile VL broth by oral gavage within 12 hours of birth. Upon birth, piglets in this group were given an intramuscular injection 0.5 ml of iron dextran i.m. as per standard industry practices. Upon weaning, piglets in this group were challenged with ~ 10^8, 10^9, or 10^{10} CFU EC F18/STx2e by oral gavage.
3. **RPCF Group** – were administered 5 ml of the RPCF CE culture by oral gavage within 12 hours of birth. Piglets in this group received 0.5 ml i.m. iron dextran injection upon birth. Upon weaning, piglets in this group were challenged with ~ 10^8, 10^9, or 10^{10} CFU EC F18/STx2e by oral gavage.

Experimental Groups

Groups	Treatment @ birth	Tx 48 hr after birth
1) Control	none	none
2) Infected control	*per os* administration VL broth (5 ml)	*per os* administration 10^8, 10^9, or 10^{10} CFUs EC F18
3) RPCF culture	*per os* administration RPCF culture (5 ml) (~ 10^9 CFUs/ml bacteria)	*per os* administration 10^8, 10^9, or 10^{10} CFUs EC F18

On day 14, piglets in all groups were weighed. Piglets were placed into floor pens with *ad libitum* access to water and an unmedicated piglet starter diet. Piglets acclimated to the diet for a period of 3 days post-wean. On day 18 post farrow (day 4 post-wean), piglets in the RPCF and Infected control groups were then challenged with 10^8, 10^9, or 10^{10} cfu *EC F18/STx2e* by oral gavage (see Results). Daily rectal swabs were taken from all infected pigs and at two weeks post-wean, piglets in all groups were again weighed. Mortalities were cultured for the presence of *EC F18/STx2e* using standard isolation methods. At two weeks post-wean, piglets were euthanized and tissue samples were cultured for the presence of *EC F18/STx2e*. Tissues cultured included the ileum, ileocolic lymph nodes, ileocecal junction, cecum, and colon and CFU determinations from ileal and cecal contents. Comparisons of mortality levels, rectal swab data, CFU's, and organ culture data were then made between RPCF- treated and

infected controls and weight gain over the study period was compared for all three groups.

V. Results: Three separate sets of litters from six sows per set were used in these studies. Piglets from the first set received 10^8 CFUs ECF18/STx2e; second set received 10^9 CFUs ECF18/STx2e; third set received 10^{10} CFUs ECF18/STx2e. The different doses of *E. coli* were administered to allow for the measurement of CFUs of ECF18/STx2e in the gut. In laboratory experiments, higher doses of *E. coli* have been previously found to be isolated from the gut equally between controls and treated piglets.

As can be seen in Study 1, Table I, there were no differences between control and treated piglets in the isolation of *E. coli* from the gut and associated lymph nodes except in the ileum where there was a 41% reduction in the number of pigs positive for ECF18/STx2e. Rectal swabs also showed no difference between controls and treated pigs, with all pigs shedding ECF18/STx2e throughout the study period.

In Study 2, weight gain was recorded from birth to six weeks post-weaning (Table I). There were no significant differences in weights recorded for the infected controls and the RPCF-treated. The untreated, uninfected controls gained an average of 5 lbs more than both the infected controls and the RPCF-treated. Tissues cultured at necropsy for the presence of ECF18/STx2e showed no difference between the control group and the treated group (Table II). Determinations of CFUs of EC F18/STx2e in the ileum and the cecum were done for the infected controls and the treated pigs. There was a significant reduction ($P< 0.05$) in the $\log_{10}$ CFUs in the ileum of the RPCF pigs when compared to those of the infected controls (Table III). Rectal swabs were found to be positive for all pigs throughout the duration of the study.

In Study 3, piglets were challenged with 10^{10} CFUs of EC F18 STx2e. No differences were noted in either the ileal dilutions or the cecal diltions between infected controls and RPCF-treated (Table I). Samples cultured for positive and negative growth of EC F18 STx2e are presented in Table I. No differences between groups were detected. **Mortality in the control group was 14.30% (2/14 pigs), while there was no mortality in the RPCF-treated**. Weight gain measured in Study 3 also showed no significant difference between the infected controls and the RPCF-treated pigs (Table II). The unifected, untreated controls gained an average of 12.34 pounds compared to 7.22 and 7.10 gained by the infected controls and the RPCF-treated, respectively.

STUDY 3: Challenge dose – 10^{10}cfus ETEC

Table 1.
Culture of tissues for the recovery of ETEC

Group	ICLN	JEJ	IL	IJ	COL	CEC
Infected control	3/12 (25%)	9/12 (75%) (IL CFUs = 2.67 $\log_{10}$)	12/12 (100%)	11/12 (91.6%)	11/12 (91.6%) (CEC CFUs = 4.25 $\log_{10}$)	10/12 (83.3%)

RPCF	2/7 (28.5%)	6/7 (85.7%) (IL CFUs = 2.78 $\log_{10}$)	7/7 (100%)	6/7 (85.1%)	7/7 (100%)	5/7 (71.4%) (CEC CFUs = 4.10 $\log_{10}$)

(ICLN-ileocecal lymph nodes; JEJ-jejunum; IL-ileum; IJ-ileocecal junction; COL-colon; CEC-cecum)
Rectal swabs for all pigs in all groups were positive for ETEC throughout the study.

Table II. Weight Gain (lbs.)

Group	Average gain from wean to two weeks post-wean
Control	12.34
Infected control	7.22
RPCF	7.10

STUDY 2: Challenge dose - 10^9cfus ETEC

Table 1. Weight Gain (lbs.)

Group	Average gain from birth to 6 weeks post-wean
Control	35.69
Infected control	30.84
RPCF	29.67

Table II. Culture of tissues for the recovery of ETEC

Group	ICLN	JEJ	IL	IJ	COL	CEC
Infected control	2/15 (13.3%)	10/15 (66.6%)	14/15 (93.3%)	15/15 (100%)	15/15 (100%)	13/15 (86.6%)
RPCF	1/13 (7.6%)	10/13 (76.9%)	12/13 (92.3%)	13/13 (100%)	12/13 (92.3%)	10/13 (76.9%)

(ICLN-ileocecal lymph nodes; JEJ-jejunum; IL-ileum; IJ-ileocecal junction; COL-colon; CEC-cecum)

Table III. Ileal and cecal colony forming unit counts (log_{10}) of ETEC

Group	Ileum	Cecum
Infected control	1.81	4.64
RPCF	0.56**	4.63

** indicates a significant difference between RPCF and Infected control groups ($P < 0.05$).

Rectal swabs for all pigs in study 2 were positive for ETEC for the duration of the study (38 days post-weaning).

STUDY 1: Challenge dose – 10^8 cfus ETEC

Table I. Culture of tissues for the recovery of ETEC

Group	ICLN	JEJ	IL	IJ	COL	CEC
Infected control	1/7 (14.2%)	2/7 (28.5%)	7/7 (100%)	7/7 (100%)	7/7 (100%)	6/7 (85.7%)
RPCF	0/17 (0%)	7/17 (41.1%)	10/17 (58.8%)	16/17 (94.1%)	17/17 (100%)	14/17 (82.3%)

(ICLN-ileocecal lymph nodes; JEJ-jejunum; IL-ileum; IJ-ileocecal junction; COL-colon; CEC-cecum)

Rectal swabs for all pigs in study 1 were positive for ETEC for the duration of the study (21 days post-weaning).
Ileal and cecal dilutions were not performed on pigs in study 1.
Weights were also not recorded for study 1.

VI. Conclusions

The present studies were conducted as an extension of the porcine CE project in our laboratories. Initial studies involving neonatal pigs and ETEC infections have shown the RPCF culture to be effective in the prevention of mortality and gut colonization associated with ETEC in neonatal pigs (8,9). While the results of the current weaned-pig studies are encouraging, the studies were not as definitive as the neonatal studies. The CFU and tissue determinations for EC F18/STx2e in Study 1 and 2 seem to indicate that the RPCF- treated pigs had reduced numbers of EC F18 STx2e in the gut. However, the next set of experiments did not show any reductions in the number of CFUs of EC F18STx2e in the gut. Weight gains for both infected controls and the RPCF-treated pigs were similar, and rectal swab isolation from all pigs showed fecal shedding throughout the three studies. Studies 1 and 2 had no mortality in either group, while Study 3 showed a 14.30 % mortality in the infected control group.

Perhaps the protection observed against *E. coli* in neonatal pigs provided by the RPCF culture does not continue to be a significant benefit during weaning. The virulence determinants of the neonatal *E. coli* used in previous studies also differ from those of the *E. coli* isolate used in these studies. In addition, mortality data was only generated with a high challenge dose of EC F18/STx2e which would indicate that various other

factors may be involved with the incidence of mortality due to strains of E. coli. The results of these studies indicate that the RPCF CE culture did reduce mortality due to an E. coli infection and that there may be some reducing effect on the colonization of the porcine gut. Further studies on a larger scale need to be conducted before definitive conclusions can be drawn about the RPCF CE culture.

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