Competitive exclusion treatment reduces the mortality and fecal shedding associated with enterotoxigenic *Escherichia coli* infection in nursery-raised neonatal pigs

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Abstract

We have previously reported that the administration of a competitive exclusion culture (PCF-1), derived from the cecal microflora of a young, healthy pig and maintained in a continuous flow fermentation system to neonatal pigs resulted in a decrease in the incidence of fecal shedding and cecal colonization by *Salmonella choleraesuis* in pigs at weaning. In the present experiment, we describe the effects of the administration of a derivative of the PCF-1 culture, RPCF, against an enterotoxigenic *E. coli* infection in neonatal pigs raised off-sow. The administration of RPCF at 12 and 24 hours after birth resulted in significant ($P < 0.05$) reductions in mortality, incidence of fecal shedding, and in gut colonization by *E. coli* when compared to control values. The RPCF reduced mortality from 17.5%, observed in untreated pigs, to 4.4% in RPCF-treated pigs. Fecal shedding of *E. coli* was reduced significantly ($P < 0.05$) in RPCF-treated pigs between Days 1 and 3 post-challenge. These results indicate that the RPCF culture is effective against one of the most important causes of neonatal scours (*E. coli* infections) in piglets.

Résumé

Il a été précédemment rapporté que l'administration à des porcs nouveau-nés d'une culture (PCF-1) provenant de la microflore du caecum d'un jeune porc en santé et maintenue dans un système de fermentation en flux continu permettait, par compétition exclusive, de réduire l'incidence d'excrétion fécale et la colonisation du caecum par Salmonella choleraesuis *au sevrage. Les effets de l'administration du dérivé RPCF de la culture PCF-1 furent évalués lors d'une infection par* E. coli *entérotoxinogène chez des porcelets nouveau-nés élevés sans leur mère. L'administration de RPCF 12 et 24 h après la naissance entraîna une diminution significative* ($P < 0,05$) *de la mortalité, de l'incidence de l'excrétion fécale, et de la colonisation de l'intestin par* E. coli *en comparaison avec un groupe témoin. La mortalité fut réduite de 17,5 % à 4,4 % chez les recevant le RPCF. L'excrétion fécale de* E. coli *fut réduite de façon significative* ($P < 0,05$) *chez les animaux inoculés entre les jours 1 et 3. Les résultats démontrent que l'administration de la culture RPCF est efficace contre les infections à* E. coli, *une des principales causes de diarrhée néo-natale chez les porcelets.*

(Traduit par docteur Serge Messier)

Introduction

Escherichia coli has been described as the most important cause of neonatal and post-weaning diarrhea in pigs (1–3). Disease caused by *E. coli* in neonatal pigs can be fatal, with mortality reaching high levels during the first few days after birth (1–3). Serotypes of *E. coli* causing disease in neonatal pigs include O8, 9, 20, 101, 141, 147, 149, and 157. Fimbrial types F4 (K88), F5 (K99), and F6 (P987) are commonly associated with enterotoxigenic strains causing disease in young pigs (4,5). Traditional control measures for *E. coli* in neonatal and young pigs involve the use of antibiotics and the control of environmental conditions, such as ambient temperature and hygiene (1,5).

Prevention of *E. coli* scours, and other infectious diseases, would be beneficial to producers, saving both time and costs associated with outbreaks of disease. In addition, public and scientific requests for the removal of antibiotics as subtherapeutics in feeds are forcing researchers and producers to investigate possible disease prevention alternatives. One such alternative measure is the use of competitive exclusion cultures. The theory of competitive exclusion technology is to colonize the neonatal gastrointestinal tract with beneficial/commensal bacteria considered to be the normal flora of healthy adult animals of a particular species. Prior to birth, the intestinal tract of the animal is considered to be a sterile environment. Upon birth, the establishment of a healthy gut flora can take a week or longer, providing a window of opportunity for enteric pathogens to colonize the neonatal gastrointestinal tract (6,7).

The mechanism of action for competitive exclusion of enteropathogens has not been established; however, several hypotheses have been proposed: exclusion of enteropathogens by competition

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Table I. Mortality during the *Escherichia coli* infection study

Group	Mortality rate
Control	17.5% (7/40)
RPCF	4.4% (3/68)[a]

[a] significant difference from control value ($P < 0.05$)

for attachment sites and/or for nutrients; stimulation of the local immune mechanisms, which preclude colonization/invasion by enteric pathogens; and the production of various antimicrobial substances that either have direct action on pathogenic bacteria or that produce conditions within the gut that are unfavorable for the growth and colonization by pathogens (8).

Using the knowledge gained in the development of a defined competitive exclusion culture for use in poultry (CF-3/Preempt), our laboratory has developed a swine-derived competitive exclusion culture (9–11). Experiments in our laboratory have shown that the administration of a porcine-derived continuous flow culture (PCF-1; Nisbet et al, US Patent 5,951,997) to neonatal pigs enhanced colonization resistance to *Salmonella choleraesuis* during weaning (11). The enhanced resistance to *S. choleraesuis* colonization was manifested in decreased numbers of PCF-1-treated piglets shedding *S. choleraesuis* and in decreased numbers of pigs culturing positive for *S. choleraesuis* in cecal contents (11).

The objectives of the current studies were to determine the possible effects of a swine-derived bacterial competitive exclusion culture on the fecal shedding, gut colonization, and mortality associated with enterotoxigenic *Escherichia coli* (ETEC) infection in neonatal pigs raised off-sow.

Materials and methods

Animals

Three replicate experiments had a combined total of 108 crossbred piglets from the same source farm weaned at less than 12 h of age. Piglets were ear-tagged, checked for overall health, and randomly placed into farrowing crates (10 pigs/crate), which served as nursery units for these studies. Piglets were provided heating pads and heat lamps for added warmth. A commercial, non-medicated milk replacer (MS Bioscience, Dundee, Wisconsin, USA) was provided ad libitum, using milking units from commercial nursery units (Kane Manufacturing Company, Des Moines, Iowa, USA). All procedures involving animals and their care were approved and monitored by the Animal Care and Use Committee of the Southern Plains Agricultural Research Center location of the United States Department of Agriculture, Agricultural Research Service (USDA ARS).

Bacteria

A porcine isolate of enterotoxigenic *Escherichia coli*, originally isolated from diseased neonatal pigs and designated EC 987 (O9:NM, 987P, STa, STb), was generously provided by Nancy Cornick, Iowa State University, Ames, Iowa, USA. EC 987 was selected for resistance to 25 μg/mL of novobiocin and 20 μg/mL of nalidixic acid (NN) in our laboratory and were maintained in tryptic soy broth

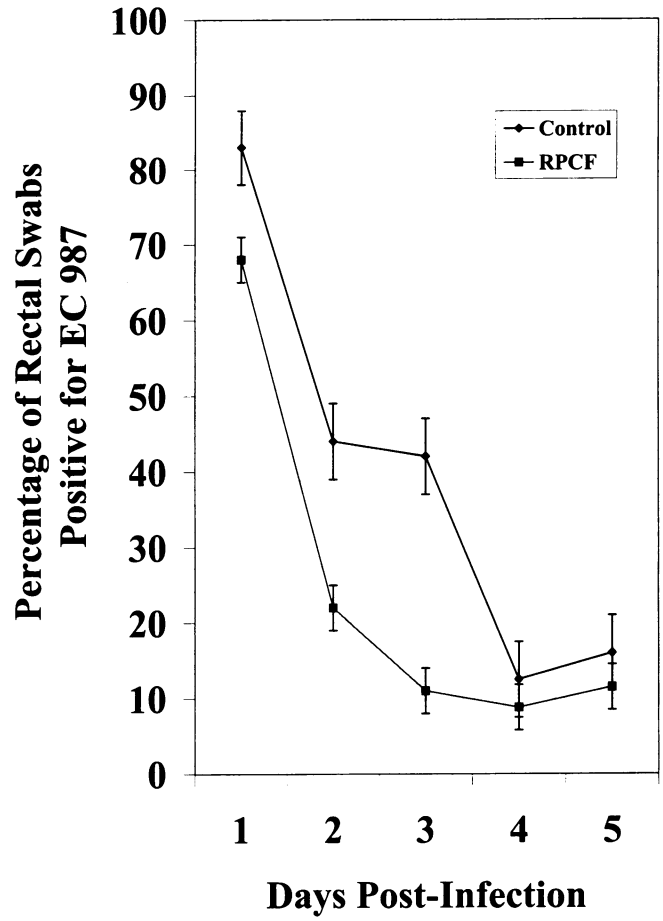

Figure 1. Percent of rectal swabs positive for EC 987 over five day study period. Data shown represent the average of 3 replicate experiments ± SEM. Asterisk represents a significant ($P < 0.05$) difference between control and RPCF group swabs positive for EC 987.

(TSB) NN. An overnight culture of EC 987, transferred 3 times previously, was used as a 10^9 colony forming unit (cfu)/mL stock and was diluted to a challenge concentration of 1×10^8 cfu/mL. Challenge doses were verified by colony formation of serial dilutions of the 10^8 cfu/mL dose spread onto MacConkey's agar NN plates.

Porcine competitive exclusion culture

A porcine competitive exclusion (CE) culture (RPCF), derived from a culture originally developed from the cecal contents of a healthy 6-week-old pig, was used in this study (11). The RPCF culture is maintained in a continuous-flow (CF) system using modified Viande Levure (VL) medium, as described for avian cultures (12). Aliquots for administration to neonatal pigs were taken directly from the CF system and given to the piglets.

Procedures

Pigs in the RPCF group received 5 mL of the RPCF culture by oral gavage at 12 and again at 24 h of age. Control pigs were administered 5 mL of sterile VL broth by oral gavage at 12 and 24 h of age. At 48 h of age, all pigs were challenged by oral gavage with 1×10^8 cfu of EC 987. Rectal swabs were taken daily from individual pigs and dead pigs were necropsied and cultured for the presence of EC 987. Five days post-challenge, the pigs were euthanized and tissue

Table II. Reduction of *E. coli* in neonatal pigs

	Ileocecal lymph node	Ileum	Colon	Cecum
Control	15% (6/40)[a]	30% (12/40)	30% (12/40)	25% (10/40)
RPCF	0% (0/68)[b]	3% (2/68)[b]	3% (2/68)[b]	10.3% (7/68)[b]

[a] Numbers in parentheses are the total EC positives/total number

[b] Indicates a significant difference from control values ($P < 0.05$)

samples (ileocecal lymph nodes, ileum, cecum, and colon) were obtained and cultured for the presence of EC 987. All procedures, health, and care of pigs involved in these studies were approved and monitored by the Animal Care and Use Committee at the Southern Plains Agricultural Research Center research location.

Statistical analysis

Data from 3 replicate experiments were pooled and analyzed by using a computer software (Sigma Stat; Jandel Scientific Software, San Rafael, California, USA). Significant differences between groups were determined using the *t*-test.

Results

Mortalities recorded during these experiments are presented in Table I. The mortality rate observed in control pigs that received EC 987 was 17.5%. The RPCF group had a 4-fold reduction in mortality, with only 4.4% mortality. Figure 1 shows rectal swab data over the 5-day experimental time period. Pigs in the RPCF group shed less often than control pigs, especially during Days 1 through 3 post-infection, in which a significant ($P < 0.05$) reduction in the percentage of pigs shedding EC 987 was observed. Preliminary observations in our laboratory of rectal swab data in both control and RPCF-treated pigs showed that by Days 7 to 9, both control and RPCF-treated pigs had negative rectal swabs (data not shown). In addition, significant ($P < 0.05$) reductions in the recovery of EC 987 from RPCF-treated pigs were observed in samples cultured at necropsy (combined results for mortalities and termination of study; Table II).

Discussion

Previously, our laboratory developed a porcine-derived CF culture (PCF-1) that was found to reduce the incidence of *S. choleraesuis* in weaned pigs (11). The present studies describe the use of a derivative culture of PCF-1, RPCF, which protects neonatal pigs from an enterotoxigenic strain of *E. coli* known to cause disease in neonatal pigs. The RPCF-treated pigs showed significant reduction in the incidence of *E. coli* shedding and gut colonization, and showed reduced mortalities when compared with untreated pigs. Earlier studies by other researchers using *Streptococcus faecium* have demonstrated that bacterial competitive exclusion could be effective against ETEC infections in gnotobiotic, colostrum-deprived neonatal pigs, reducing diarrhea, *E. coli* colonization of the gut, and mortality associated with an ETEC infection (13,14).

The PCF-1 culture has been shown to protect both neonatal and weaned pigs against *S. choleraesuis* infection (11). The present study demonstrates that the RPCF culture protects against *E. coli* challenge and further studies in our laboratory are considering other bacterial pathogens, including those of host animal health and human food safety concerns.

The mode of action of CE cultures has not been fully characterized, but may include a variety of factors that negatively affect the well-being and/or colonization of the host gastrointestinal tract by enteropathogens. Data presented in this study suggest that there is less invasion and/or uptake of *E. coli* into the lymph tissues of the gastrointestinal tract of RPCF-treated pigs. This observation may have several explanations, including: 1) the blocking/exclusion of *E. coli* by competing bacteria for attachment sites lining the gut; 2) the reduced levels of *E. coli* recovered from the lymph nodes of RPCF-treated pigs, due to the production of conditions or substances that inhibit the growth of or the direct killing of *E. coli*; or 3) the administration of CE into the relatively sterile environment of the neonatal gastrointestinal tract causes a stimulation and/or maturation of the gut epithelium and the mucosa-associated lymph tissue, resulting in the production of humoral and cellular immune defense mechanisms and the subsequent killing or clearance of *E. coli*. Experiments designed to define the possible effects of the CE culture on intestinal immunity are ongoing.

Evidence supporting the hypothesis that the administration of a CE culture to neonates results in competition for binding sites and the production of substances detrimental to enteropathogens was shown in chicks treated with the Preempt CE culture (15). Electron micrographs of the ceca after CE administration showed extensive colonization of intestinal crypts by the Preempt bacteria, while micrographs of ceca from untreated control chicks had very few bacteria colonizing the gut. In addition, volatile fatty acid production in CE-treated chicks was found to be much higher in treated chicks compared to levels observed in control chicks. Volatile fatty acids are indicators of anaerobe growth and may act bacteriostatically against enteropathogens, such as *Salmonella* spp. (9). Whether these same mechanisms occur upon the administration of RPCF to neonatal pigs is not known at this time, however, studies are currently underway to elucidate the mechanism of RPCF protection against enteropathogens in pigs.

Information regarding fecal shedding of pathogenic *E. coli* strains in swine is lacking, with very few studies measuring *E. coli* in feces or rectal swabs (16–18). Data presented in the current report show that shedding in both the control and RPCF groups drops off precipitously between Days 1 and 3 post-challenge. However, the RPCF-treated pigs show a steeper decline in the number of pigs shedding EC 987 between Days 1 and 3 post-challenge. The drop in fecal shedding in both groups with increasing age corresponds with earlier reports that state that particular *E. coli* serotypes and virulence determinants are associated with disease in pigs of a certain age (19–21). In addition, laboratory observations indicate that EC 987 is not found in rectal swabs from either control or RPCF-treated pigs

by Days 7 to 9 post-challenge. The ability of strains of *E. coli* to colonize/attach to the intestinal epithelium and cause disease is age-related and most likely has to do with receptors used for bacterial attachment on the luminal surface of the intestinal epithelium (19–21). RPCF seems to enhance the age-related resistance to infections by neonatal *E. coli* and may bind or block the receptors necessary for attachment/colonization on the intestinal epithelium. Further research in this area must be done to determine the mechanism of pathogen exclusion by CE cultures.

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