



Short communication

Prevention of porcine *Clostridium difficile*-associated disease by competitive exclusion with nontoxigenic organisms

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Abstract

Clostridium difficile is widely known as a cause of disease in humans, and has emerged as an important problem in neonatal swine. No commercial product is available for immunoprophylaxis of *C. difficile*-associated disease, but success in preventing experimental infections in hamsters by use of nontoxigenic strains to competitively exclude toxigenic strains led us to try this method in neonatal pigs. Spores were administered orally to newborn pigs or were sprayed onto perineum and teats of dams. Significantly more piglets were weaned among litters receiving spores orally, and average weaning weights were significantly higher for both treatment groups than for controls. Toxins A and B were detected in 44.8% of litters and 16.5% of piglets born to sprayed sows and 58.3% of litters and 15.4% of piglets in the control group. However, toxins were detected in only 13.8% of litters and 3.4% of piglets given spores orally. These data support a contention that precolonization by a nontoxigenic strain can ameliorate the pre-weaning growth retardation associated with *C. difficile* infection in piglets.

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1. Introduction

Clostridium difficile is a widely known cause of disease in humans (Moyenuddin et al., 2002), occurring most often as antibiotic-associated diarrhea or pseudomembranous colitis (Bartlett, 2002).

Recently, *C. difficile*-associated disease (CDAD) has emerged as an important problem in neonatal swine (Waters et al., 1998; Songer et al., 2000; Post et al., 2002; Post and Songer, 2004). Typically affected piglets have pasty-to-watery yellow stools, although some are non-diarrheic and even constipated (Yaeger et al., 2002). Among piglets less than 1-week-old and submitted for diagnosis of enteritis, ~35% were affected by CDAD alone; an additional 25% had CDAD plus one or more other agents associated with

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neonatal diarrhea (Songer et al., 2000; unpublished results). The standard for diagnosis is detection of toxins A (TcdA) and B (TcdB) (Alfa et al., 2002) by cytotoxicity or commercial enzyme immunoassays. About 6% of isolates from affected piglets are nontoxicogenic (unpublished results).

Antimicrobial treatment of neonatal pigs (Post and Songer, 2004) is expensive and labor-intensive, given the common need to repeatedly handle every piglet. No commercial product is available for immunoprophylaxis of CDAD in any species. However, observations in the hamster model of CDAD suggest that toxigenic *C. difficile* might be competitively excluded by nontoxicogenic strains (Merrigan et al., 2003; Sambol et al., 2002; our unpublished results). Hamsters can be protected against disease and death by precolonization with nontoxicogenic strains, even in the face of continuing clindamycin therapy (Sambol et al., 2002). We evaluated competitive exclusion as a prevention strategy in piglets, and report here the successful exclusion of toxigenic strains and inhibition of toxin production in a large proportion of treated animals.

2. Materials and methods

2.1. Swine farm

The study was conducted in a 3500 sow farrow-to-wean operation in North Carolina. During the course of the study, pigs were managed and treatments were administered by farm staff and veterinarians. Thus, the study was not subject to regulation by an animal care and use committee.

Sixty-six sows were farrowed each week in separate rooms (33 sows per room). Rooms were disinfected on a rotational basis with quaternary ammonium compounds, chlorine bleach, and Virkon S (Farnam Livestock Products, Phoenix, AZ). Pregnant animals were washed with Virkon S at label dosage prior to placement in farrowing crates. Clipping of milk teeth, docking of tails, castration of males, and injection of iron were performed routinely at 3 days of age.

The farm had experienced ~12 months of piglet scours, beginning at ~1 day of age, with pre-weaning mortality of 13–15%. Sow and gilt litters were affected equally, and diseased piglets surviving to

weaning averaged 1.44 lb less than unaffected counterparts. Sows and gilts were vaccinated routinely before farrowing against enterotoxigenic *E. coli*, *C. perfringens* type C, and porcine respiratory and reproductive syndrome. Autogenous vaccination against *C. perfringens* type A and *C. difficile* failed to resolve the scour problem.

2.2. Nontoxicogenic *C. difficile*

Strain JGS753 was obtained from a piglet in North Carolina. PCR assays for *tcdA* and *tcdB* were negative, and toxin production was not detected in 7 day dialysis bag cultures in brain heart infusion (BHI; Difco, Detroit, MI).

2.3. Production of spores

JGS753 was cultivated as lawns on BHI agar, incubated for 7 days at 37 °C in an atmosphere of 5% CO₂:5% H₂:90% N₂. Growth was harvested into 2 ml phosphate buffered saline (PBS; pH 7.2, 0.01 M), incubated at 80 °C for 10 min to kill vegetative cells, diluted with PBS to 15 ml, and stored at –80 °C until use. Titrations consistently revealed ~1.5 × 10⁶ spores per ml.

2.4. Administration of spores

Pregnant sows and gilts (*n* = 97) were randomly assigned to three groups of 29, each housed in a separate farrowing room. In Group 1, piglets were given 10⁶ spores orally within 24 h of birth; spores were administered at the beginning of the day to piglets farrowed during the night and at day's end to piglets farrowed during the workday. The equivalent of 15 piglet doses, in 15 ml total volume, were sprayed onto the teats and perineum of Group 2 sows immediately before placement into crates. Group 3 sows and piglets served as untreated controls.

2.5. Observation of piglets

Fecal samples from five pigs per litter were collected and pooled on day 5 post-farrowing. These were examined for TcdA and TcdB by enzyme immunoassay (ToxA/B, Techlab, Blacksburg, VA). Other data collected included number of piglets born

alive and weaned, number of diarrhea days, and litter weaning weights.

2.6. Statistical analysis

Data were examined by analysis of variance.

3. Results and discussion

No ill effects of spore administration were observed in sows or piglets. As expected, there was no difference on average across groups in number of piglets born live (data not shown).

There were no statistically significant differences in numbers of piglets weaned per litter or average weaning weight (Tables 1 and 2), although the raw averages for each of these parameters were greater for Groups 1 and 2 than for Group 3 (controls). The numbers of litters included in each treatment group was, in the end, too small to overcome the inherent variability among pigs weaned and weaning weights. In spite of this, it seems likely that both treatments had positive impact. Neonatal deaths and subsequent cross-fostering reduced the number of litters in Group 3 to 26, with

Table 1
Effect of spore administration on average piglet weaning weight

	Weaning weight by treatment (291 per group)		
	Oral ^a	Spray ^b	Control ^c
Average per piglet	11.75	11.39	10.1
S.D.	2.24	2.17	3.94

^a Piglets administered 10^6 spores orally.

^b Perineum and teats of sows sprayed with 1.5×10^7 spores (15 piglet doses).

^c No spores administered.

Table 2
Effect of spore administration on number of pigs weaned per litter

	Pigs weaned per litter by treatment		
	Oral ^a	Spray ^b	Control ^c
Average per piglet	9.38	9.07	8.21
S.D.	1.24	1.28	3.25

^a Piglets administered 10^6 spores orally.

^b Perineum and teats of sows sprayed with 1.5×10^7 spores (15 piglet doses).

^c No spores administered.

Table 3
Impact of spore administration on rate of CDAD diagnosis^a

	Treatment		
	Oral	Spray	Control
Litter diagnoses	4 (13.8%)	13 (44.8%)	14 (58.3%)
Piglet diagnoses	5/145 (3.4%)	24/145 (16.5%)	20/130 (15.4%)

^a As determined by toxin testing by enzyme immunoassay. Data reported as number of EIA positive samples.

predictable negative impact on the standard deviations; cross-fostering was not required in Groups 1 and 2. When it is possible, this work should be repeated with more litters per treatment group.

TcdA and TcdB were detected in 13 litters, (44.8%) and 24/145 piglets (16.5%) in Group 2 and 14 litters, (58.3%) and 20/130 piglets (15.4%) in Group 3 (Table 3). However, toxins were detected in only 4 litters, (13.8%) and 5/145 piglets (3.4%) in Group 1. Given the >90% correlation between detection of toxins in rectal swab samples and occurrence of typhlocolitis (Post et al., 2002; Songer et al., 2000), it seems likely that piglets in Group 1 received substantial benefit from administration of the spores of nontoxigenic *C. difficile*.

Taken together, these results suggest that it may be possible to prevent effects of *C. difficile* toxin accumulation by precolonization with a nontoxigenic strain. We have considered the possible benefits of environmental contamination with nontoxigenic; however, the suggestion of lower performance among Group 2 piglets implies that a direct oral dose is more likely to provide a useful effect. It remains to apply this method to greater numbers of litters.

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