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Rabbit Model To Evaluate Enterovirulence of *Bacteroides fragilis*†

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Enterotoxigenic *Bacteroides fragilis* elicited fluid accumulation in the intestinal tract and exfoliation of epithelial cells in the proximal colon of the 2-week-old rabbit. The rabbit model was employed to screen isolates of *B. fragilis* for enterovirulence and to study the pathogenesis of disease caused by enterotoxigenic *B. fragilis*.

Enterotoxigenic isolates of *Bacteroides fragilis* are enteropathogenic in laboratory rabbits (6, 8; L. L. Myers, D. S. Shoop, and J. E. Collins, U.S.-Jpn. Joint Conf. Cholera, **25**:34, 1989) and gnotobiotic pigs (J. R. Duimstra, J. E. Collins, L. L. Myers, and D. A. Benfield, Conf. Res. Workers Anim. Dis., **67**:50, 1986). They are also associated with diarrheal diseases in livestock (1, 2, 4, 5, 7) and humans (8; R. B. Sack, L. L. Myers, J. Almeido-Hill, and D. S. Shoop, U.S.-Jpn. Joint Conf. Cholera, **25**:33, 1989). The latter report indicated that enterotoxigenic isolates were found significantly ($P = 0.036$) more often in the feces of diarrheic humans than in those of control persons. The enterotoxin of *B. fragilis* is heat labile (7) and causes fluid accumulation in the lamb or calf ileal loop test (2). In vitro tests that detect enterotoxin produced by *Escherichia coli* and *Vibrio cholerae* have given negative results for *B. fragilis* enterotoxin (2, 7). Enterotoxin production among *B. fragilis* isolates is not correlated with strain differences (3).

A total of 56 14- to 16-day-old New Zealand White rabbits were employed 2 to 4 h after their removal from the does. Rabbits were anesthetized with an intramuscular injection of 0.8 mg of xylazine (Mobay Corporation Animal Health Division, Shawnee, Kans.), followed immediately by an intramuscular injection of 20 mg of ketamine hydrochloride (Parke Davis & Co., Morris Plains, N.J.). A 4-cm abdominal (midline) incision was made after injection of a local anesthetic (10 mg of lidocaine hydrochloride; Fermenta Animal Health Co., Kansas City, Mo.), and a short section of small intestine was exteriorized. A 1-ml sample of whole-broth culture containing 5×10^9 CFU of *B. fragilis* was inoculated into the small intestine with a 3-ml syringe fitted with a 30-gauge needle. Bacteria for inoculation were grown anaerobically (GasPak anaerobe system; BBL Microbiology Systems, Cockeysville, Md.) for 24 to 48 h at 37°C in brain heart infusion broth (Difco Laboratories, Detroit, Mich.). Each bacterial isolate was usually evaluated in three rabbits (from two or three litters). Control rabbits (inoculated with nonenterotoxigenic *B. fragilis*) were from the same litters as the rabbits inoculated with enterotoxigenic *B. fragilis* were. Inoculated rabbits were housed together (separate from the does) without food or water and were euthanized (T-61 Euthanasia solution; Taylor Pharmacol Co., Decatur, Ill.) at 44 h postsurgery. The intestinal tract (duodenum to anus) from each rabbit was placed in a separate 30-ml centrifuge tube, minced with scissors to release fluid, and centrifuged at 8,000 × *g* for 20 min. The volume of supernatant fluid was measured, and the mean volume of fluid for rabbits inoculated with the same bacterial isolate was calculated. At necropsy, a small section of tissue was excised from the proximal colon of 16 rabbits in litters 4 and 5. Tissues were immersed in 10% neutral buffered Formalin solution. Formalin-fixed tissues were embedded in paraffin, sectioned 4 μm thick, and stained with hematoxylin and eosin by standard techniques (9).

All 15 isolates of enterotoxigenic *B. fragilis* elicited a greater mean value of intestinal fluid than the nonenterotoxigenic strains did (Table 1). Of 42 rabbits given an enterotoxigenic isolate, 30 had an intestinal fluid volume of at least 1.5 times the mean volume of the littermate controls; none of the 10 control rabbits reached this volume of fluid. Fluid accumulated primarily in the cecum and proximal colon (Fig. 1). The proportions of rabbits given enterotoxigenic *B. fragilis* that gave a positive response (mean fluid volume of ≥1.5 times the volume of littermate controls) were four of seven in litter 1, four of five in litter 2, five of six in litter 3,

TABLE 1. Accumulation of fluid in intestinal tracts of 14- to 16-day-old rabbits inoculated with *B. fragilis*

Isolate[a]	Bacterial source (presence of enterotoxin[b])	No. of rabbits tested[c]	Mean intestinal fluid vol (ml)
86-3682-1	Piglet (−)	2	1.13
86-3217-2	Piglet (+)	3	2.03
3-101-5	Piglet (+)	3	4.23
20664-1-2	Calf (+)	2	1.58
20766-1-3	Calf (+)	1	2.40
20799-1-4	Foal (+)	3	1.32
078320-3	Human (+)	3	1.98
078044-1	Human (+)	3	1.88
20784-3-1	Foal (−)	4	1.26
20794-1	Foal (+)	3	3.33
86-3209-3	Foal (+)	3	3.40
20661-2-2	Calf (+)	3	2.77
20793-3	Human (+)	3	4.30
20490-4	Human (−)	4	0.51
079298-3	Human (+)	3	0.93
86-3552-1	Piglet (+)	3	2.22
20797-2-4	Foal (+)	3	1.12
20769-2-5	Calf (+)	3	2.03

[a] The first eight isolates listed were tested on the same day in litters 1, 2, and 3; the next five isolates were tested on the same day in litters 4 and 5; the last five isolates were tested in litters 6 and 7 on the same day.
[b] Based on the lamb ileal loop test.
[c] Numbers less than 3 appear because four rabbits died, apparently for surgery-related reasons, during the 44-h postsurgical period.

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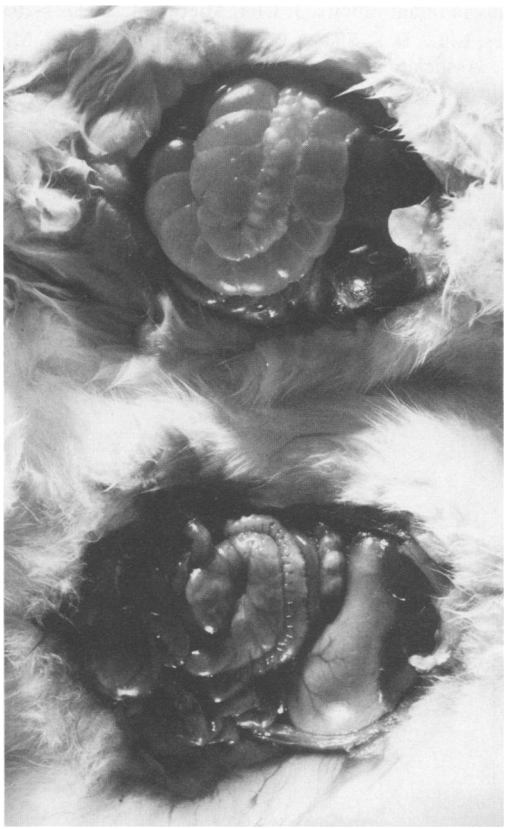

FIG. 1. Accumulation of fluid in the cecum and proximal colon of a rabbit inoculated with enterotoxigenic *B. fragilis* (top), compared with that in a control rabbit.

zero of five in litter 4, seven of seven in litter 5, five of six in litter 6, and five of six in litter 7. Marked interlitter variability in results indicated that littermate controls were needed and that isolates should be tested in rabbits representing at least two and preferably three litters. An isolate should be retested if it elicits fluid accumulation of at least 1.5 times the mean littermate control value in at least one of three rabbits. Isolates that give a mean fluid volume of 1.3 to 1.5 times the mean fluid volume of the littermate control group may also be enterotoxigenic in the lamb ileal loop test. Exfoliation of surface epithelial cells and crypt hyperplasia were observed in six of seven rabbits given enterotoxigenic *B. fragilis* in litter 5 (Fig. 2), whereas lesions were not observed in four of five rabbits in litter 4 given enterotoxigenic *B. fragilis*. The latter rabbits gave false-negative reactions for fluid accumulation. Lesions were also not observed in the four rabbits in litters 4 and 5 given nonenterotoxigenic *B. fragilis* (Fig. 3).

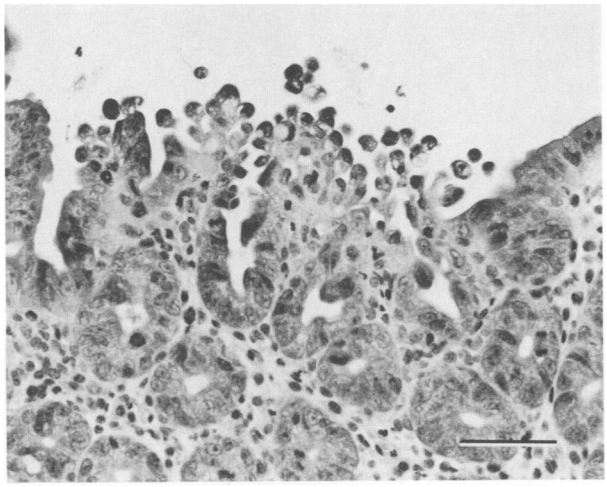

FIG. 2. Colonic mucosa of a rabbit inoculated with enterotoxigenic *B. fragilis* with a focal area of intense cellular swelling and detachment of surface epithelial cells. Bar = 50 μm.

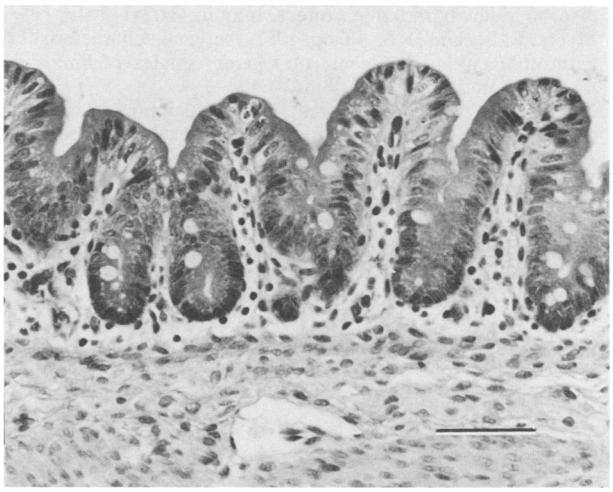

FIG. 3. Colonic mucosa of a rabbit inoculated with nonenterotoxigenic *B. fragilis*, lined by a uniform layer of tall columnar epithelial cells. Bar = 50 μm.

Our preliminary studies (unpublished) indicated that the bacterium did not elicit fluid accumulation when inoculated into the large intestine. Unlike in the model of the adult rabbit with ligated cecum (8), cecal ligation did not appear to improve test results. A 24-h postsurgical period may be adequate for accumulation of fluid. As in an adult-rabbit model (5), it appeared that some isolates of enterotoxigenic *B. fragilis* were more virulent (on the basis of volume of fluid in the intestine) than others. The two tests gave similar results regarding the relative virulence of the isolates.

Intestinal lesions occurred only in rabbits with increased intestinal fluid volume. The lesions were reminiscent of lesions associated with enterotoxigenic *B. fragilis* in infant rabbits (6), adult rabbits with ligated ceca (4, 5, 8), gnotobiotic piglets (Duimstra et al., Conf. Res. Workers Anim. Dis., 1986), and conventional piglets (1).

The 2-week-old rabbit model (or a modification thereof) appears to be useful for studying disease pathogenesis and, as an alternative to the lamb ileal loop test, for screening isolates for enterovirulence. A bacterial toxin(s) that causes fluid accumulation and sloughing of epithelial cells may be important in the pathogenesis of enteric disease caused by enterotoxigenic *B. fragilis*.

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