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Prairie Dog Model for Antimicrobial Agent-Induced *Clostridium difficile* Diarrhea

E. L. MULLER,[1,2] H. A. PITT,[1,2†] AND W. L. GEORGE[2,3*]

Surgical Service, Sepulveda Veterans Administration Medical Center,[1] and Medical and Research Services, Wadsworth Division, West Los Angeles Veterans Administration Medical Center,[3] Los Angeles, California 90073, and Departments of Medicine and Surgery, University of California at Los Angeles School of Medicine, Los Angeles, California 90073[2]

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We have noted that prairie dogs given cefoxitin develop diarrhea and lose weight yet survive for periods of up to 4 weeks. Therefore, we tested the hypothesis that cefoxitin causes *Clostridium difficile* cecitis in prairie dogs. Six prairie dogs were given a single intramuscular dose of 100 mg of cefoxitin per kg of body weight, and six control animals received saline; both groups were sacrificed 1 week later. Controls had no diarrhea and lost 2% of their body weight, whereas cefoxitin-treated animals had diarrhea ($P < 0.001$) and lost 16% of their body weight ($P < 0.001$); one animals died 6 days after cefoxitin challenge. None of the controls yielded *C. difficile* or had cecal cytotoxin or pseudomembranes detected. Cecal contents from all cefoxitin-treated animals, however, yielded *C. difficile* ($P < 0.01$) and had cecal cytotoxin present ($P < 0.01$). Four of five surviving animals also had cecal pseudomembranes present ($P < 0.01$). These results demonstrate that in prairie dogs cefoxitin induces *C. difficile* cecitis. We conclude that the prairie dog is another model for the study of antibiotic-induced diarrhea. The disease in prairie dogs may have a more chronic course than in other animal models of *C. difficile*-induced diarrhea and may be useful as a model for studying certain aspects of *C. difficile*-induced diarrhea.

Clostridium difficile has become recognized as a major cause of antimicrobial agent-induced diarrhea in humans. Laboratory studies of antibiotic-induced colitis have used several different species of animals, including Syrian hamsters, guinea pigs, and rabbits (1, 5, 12, 13). A variety of antibiotics, including penicillins, cephalosporins, and clindamycin, have been found to produce lethal intestinal disease in these animals (1, 5, 12, 13). To our knowledge, *C. difficile*-induced diarrhea cannot be produced in conventional rats and mice, however. When a susceptible species of animal is challenged with an antibiotic, essentially all animals become anorexic, dehydrated, and hypothermic, have evidence of diarrhea, and usually die 3 to 5 days after antibiotic challenge. Upon sacrifice, the animals are invariably found to have hemorrhagic ileocecitis (1, 5, 12, 13). Gnotobiotic rats and mice may survive for long periods when monoassociated with *C. difficile* (3, 11). These animals, however, lack a normal intestinal flora, a fact that may limit their usefulness in certain areas of research. In our laboratory, we have used prairie dogs in studies of gallstone formation (4, 9) and biliary tract motility (10) because their bile composition and extrahepatic biliary anatomy are similar to those of humans. Cefoxitin has been used in our laboratory as a preoperative prophylactic antibiotic for the past 5 years. Initially, no adverse effects were noted following antibiotic prophylaxis. More recently, however, animals given cefoxitin developed weight loss and diarrhea yet survived for periods of several weeks. Therefore, we tested the hypothesis that *C. difficile* causes cecitis in prairie dogs.

* Corresponding author.
† Present address: Department of Surgery, Johns Hopkins Hospital, Baltimore, MD 21205.

MATERIALS AND METHODS

Prospective study. Twelve adult male prairie dogs (*Cynomus ludovicianus*) trapped in the wild (Otto Marten Locke, New Braunfels, Tex.) were used in this study. The animals were maintained on a cholesterol-free diet in a temperature-regulated room in individual cages. Each prairie dog was anesthetized with ketamine (100 mg of drug per kg of body weight intramuscularly) and then weighed. Six prairie dogs were given a single intramuscular injection of cefoxitin (100 mg of drug per kg of body weight) (Merck Sharp & Dohme, Rahway, N.J.). Six animals served as controls and received an equal volume (3 ml) of normal saline intramuscularly. Each animal was then returned to its individual cage. The cages were cleaned daily, and both animals and cages were examined daily for evidence of diarrhea. One week after cefoxitin or saline treatment, all animals were again anesthetized and weighed. A midline laparotomy incision was made, and the abdominal contents were examined. The cecum was isolated proximally and distally, the mesentery was divided and ligated, and the cecum was incised. Cecal contents were removed by using sterile techniques and transferred into sterile vials.

Cecal contents. All cecal specimens were analyzed for total bacterial counts and counts of *C. difficile* (7) and for *C. difficile* cytotoxin titer (8) by previously reported methods.

Pathologic examination. Two 1-cm^2 specimens for the anterior cecal wall were removed and placed in a vial of Bouin solution. The following morning, the specimens were rinsed with 70% ethyl alcohol. An independent evaluator examined each specimen for evidence of pseudomembrane formation. Pseudomembranes were defined as collections of inflammatory tissue overlying the mucosa but directly attached to the lamina propria.

TABLE 1. Bacterial counts and titers in cefoxitin-treated and control animals

Animal	Counts[a]		*C. difficile* toxin titers
	Total bacterial	*C. difficile*	
Cefoxitin treated			
1	9.30	5.18	1:128
2	9.89	8.26	1:256
3	10.04	8.34	1:16,384
4	10.48	7.72	1:32
5	10.04	7.75	1:1,024
6[b]			
Control			
1	9.57	—[c]	—
2	9.38	—	—
3	10.30	—	—
4	9.49	—	—
5	9.34	—	—
6	9.11	—	—

[a] Expressed as the $\log_{10}$ number of organisms per gram of cecal material.
[b] This animal was not included in the statistical evaluation because it died 6 days after cefoxitin challenge. The corresponding control animal, (number 6) was also not included.
[c] —, Negative or not detected.

Retrospective study. To elucidate further the survival time following challenge with cefoxitin, we retrospectively reviewed the survival of a second group of 12 prairie dogs that had received cefoxitin during a 2.5-month period. All 12 animals had received cefoxitin (100 mg of drug per kg of body weight intramuscularly) prior to a sham laparotomy and were monitored for periods of 1 to 4 weeks until their sacrifice. Data were reviewed with regard to weight loss, diarrhea, and survival time.

Statistical analysis. Student's unpaired *t* test was used to evaluate weight loss and total bacterial counts. The Wilcoxon test was used to compare *C. difficile* counts, toxin titers, and pseudomembrane formation.

RESULTS

Prospective study. None of the control animals developed diarrhea, and all survived until sacifice 1 week later. Control animals lost a mean of 2% of their body weight. All cefoxitin-treated animals had watery or brown, mucoid diarrhea that was intermittently blood flecked in two animals and that developed 3 to 5 days after challenge ($P < 0.001$); one animal died on day 6 after challenge. Animals given cefoxitin lost a mean of 16% of their body weight ($P < 0.001$); none was moribund at the time of sacrifice.

Total bacterial counts (number of bacteria per gram of cecal material) were similar for the two groups (Table 1). The animals given cefoxitin had an average total bacterial count of 9.95 $\log_{10}$, while the control prairie dogs had a mean total bacterial count of 9.53 $\log_{10}$.

Each animal given cefoxitin had *C. difficile* isolated from its cecal contents; the mean count was 7.41 $\log_{10}$ (Table 1). None of the control animals had *C. difficile* isolated from its cecal contents ($P < 0.01$).

Each prairie dog given cefoxitin had *C. difficile* cytotoxin detected in its cecal contents. The toxin titers ranged from 1:32 to 1:16,384 (Table 1). However, none of the control animals had *C. difficile* cytotoxin detected ($P < 0.01$).

Four of the five surviving prairie dogs that had received cefoxitin had pseudomembranes present upon histologic examination of their ceca. The pseudomembrane was directly attached to the lamina propria (Fig. 1). For comparison, a normal cecum is shown in Fig. 2. None of the control animals had pseudomembranes or other histologic changes present ($P < 0.01$).

Retrospective study. The clinical course and survival of a group of 12 prairie dogs that were given an identical dose of cefoxitin (prior to our recognition of the role of *C. difficile* in diarrhea in prairie dogs) was retrospectively reviewed. These animals lost a mean of 13% of their body weight. Four prairie dogs also developed diarrhea; two of these four had nonbloody, brown, mucoid diarrhea, whereas the other two had blood-flecked, mucoid diarrhea. Of the 12 animals, 2 died, 1 at 1 week and the other at 3 weeks following antibiotic challenge; neither of these 2 animals had been noted to have diarrhea. The remaining 10 prairie dogs survived until the time of their scheduled sacrifice at 1 to 4 weeks following cefoxitin injection as part of another study. Thus, 4 of these 10 surviving animals were alive at 1 week, 2 were alive at 2 weeks, 3 were alive at 3 weeks, and 1 was alive at 4 weeks after antibiotic challenge. Of the four animals with diarrhea, three were sacrificed at 1 week and one was sacrificed at 3 weeks following cefoxitin challenge. None of the animals was moribund at the time of sacrifice.

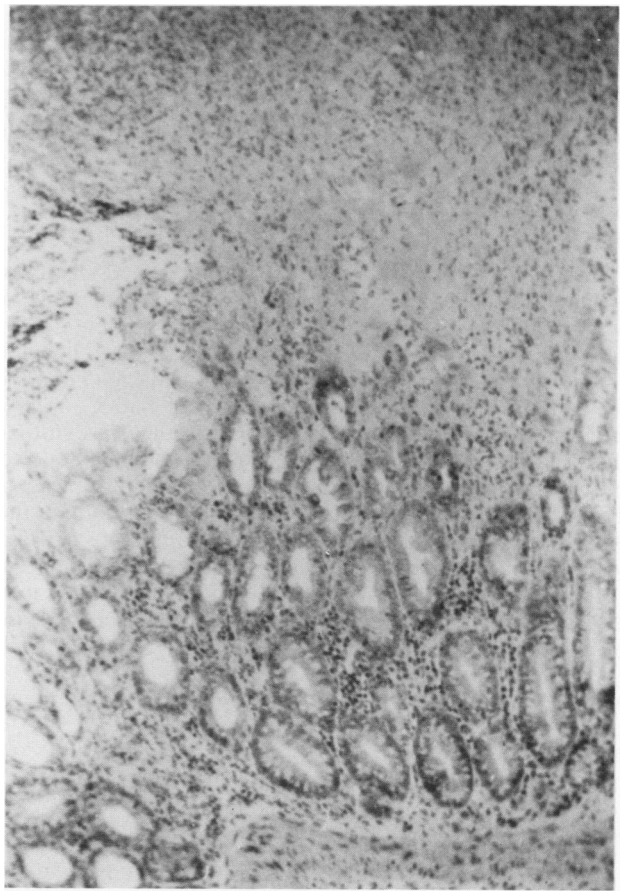

FIG. 1. Histologic examination of the cecum from a prairie dog given cefoxitin; pseudomembrane formation is evident.

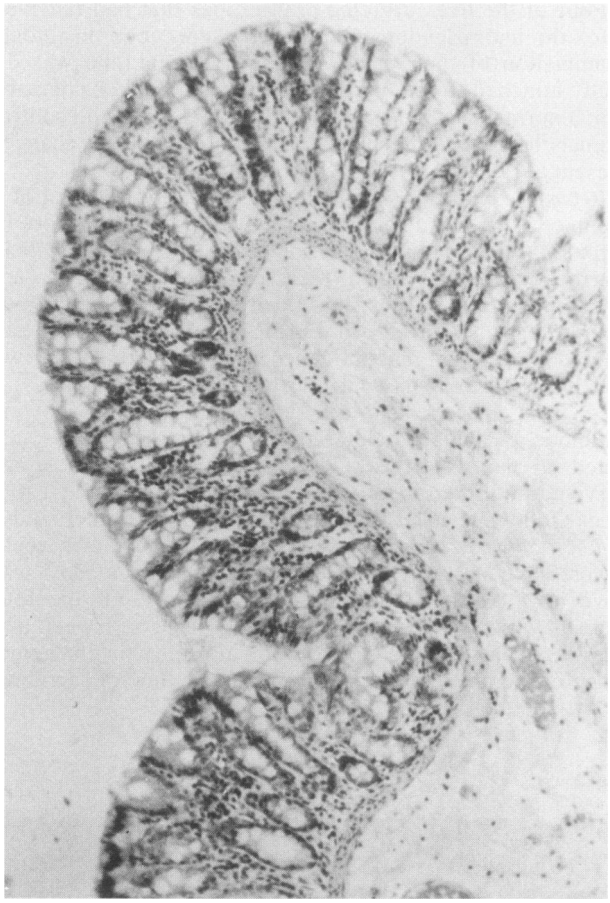

FIG. 2. Pathologic examination of the cecum from a control animal. No pseudomembranes or other histologic changes are evident.

DISCUSSION

In summary, prairie dogs given cefoxitin had significant weight loss and developed diarrhea which was either mucoid or blood flecked or both. While the cecal contents of the cefoxitin and control groups had similar total bacterial counts, the cefoxitin-treated animals all had high counts of *C. difficile* in their cecal matter, and all had *C. difficile* cytotoxin detected. There was a broad range in cytotoxin titer (1:32 to 1:16,384), a finding that has been reported previously in studies of both humans and hamsters (2, 6). The significance, if any, of the differences in cytotoxin titer is unclear; in humans, the titer does not appear to correlate with the severity of disease (6). Four of five prairie dogs that received cefoxitin also had pseudomembranous cecitis upon histology examination of their ceca. A retrospective review of another group of animals revealed that 10 of 12 prairie dogs survived challenge with cefoxitin and sham laparatomy for up to 4 weeks and that 4 of the 10 survivors had diarrhea.

Ebright *et al.* (5) administered a single dose of cefoxitin (approximately 125 mg/kg, which is similar to the dose we used) to 10 hamsters and noted that the median time to death was 2 days. It is possible that the apparent differences in mortality were due either to the species of animals studied or to the infecting strains of *C. difficile*. We believe that our prairie dog model of cecitis may be a useful tool for the study of antimicrobial agent-associated diarrhea. Our preliminary data suggest that prairie dogs typically develop *C. difficile*-induced diarrhea (and usually pseudomembranous cecitis) following a single dose of an antibiotic; the animals appear to tolerate the disease relatively well and may survive for a relatively long period of time. Further examination of this animal model seems warranted.

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