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Colonization of Mice by *Campylobacter jejuni*

MARY V. JESUDASON,† DAVID J. HENTGES,* AND PINTIP PONGPECH

Department of Microbiology, Texas Tech University Health Sciences Center, Lubbock, Texas 79430

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Both streptomycin-treated and untreated Swiss white mice were irregularly colonized when challenged orogastrically with between 1 and 10^{11} viable organisms of either of two strains of *Campylobacter jejuni*. The organisms were occasionally recovered from portions of the intestinal tracts of these animals in numbers ranging from 10^1 to 10^3/g when the challenge doses were 10^{10} or more. When germfree mice were challenged with 10^8 organisms of either strain, the entire intestinal tracts of all the animals were colonized with *C. jejuni* in numbers ranging from 10^4 to 10^9/g. The ceca were most heavily colonized. Both strains of *C. jejuni* multiplied anaerobically in brucella broth, except when the broth contained 60.80 μeq of total volatile fatty acids (VFA) per ml at pH 6.75, simulating conditions in the ceca of untreated mice, or when it contained 21.63 μeq/ml at pH 7.04, simulating conditions in the ceca of streptomycin-treated mice. Active multiplication occurred, however, in brucella broth without VFA at pH 7.02 that was incubated microaerobically, simulating conditions in the ceca of germfree mice. The results suggest that VFA operating under anaerobic conditions present in the intestinal tract of both streptomycin-treated and untreated conventional mice interfere with the multiplication of *C. jejuni*. The organisms actively multiply, on the other hand, in the absence of VFA at the higher oxidation-reduction potential of the intestinal tract of germfree mice.

The protective role of the intestinal microflora against colonization of the intestinal tract with potentially pathogenic bacteria, termed colonization resistance by van der Waaij et al. (15), has been well established. Antibiotic administration is known to decrease colonization resistance, thereby increasing the susceptibility of the host to colonization with antibiotic-resistant enteric pathogens (6, 7). Loss of colonization resistance occurs as a result of disruption of the intestinal flora of the host by the antibiotics, thereby resulting in conditions that favor multiplication of nonindigenous organisms. Bohnhoff et al. (3) and Que and Hentges (14) demonstrated that administration of streptomycin to mice resulted in increased susceptibility to *Salmonella* colonization. Additionally, streptomycin was found to be the most effective of five antibiotics tested in decreasing resistance to intestinal colonization with orally administered *Pseudomonas aeruginosa* (9). Que et al. (13) showed that streptomycin administration increased the pH and decreased the concentration of total volatile fatty acids (VFA) of cecal contents of mice, thereby enhancing the susceptibilities of the animals to colonization with *Salmonella typhimurium* and *P. aeruginosa*.

Campylobacter jejuni is an enteric pathogen that has recently been recognized as an important cause of acute diarrheal disease in humans. Field et al. (5) examined the influence of tobramycin administration on the colonization of the intestinal tracts of adult mice with *C. jejuni*. They found that more treated than untreated mice harbored *C. jejuni* in fecal pellets and that the organisms were present in larger numbers in the treated animals. Different results were obtained by Blaser et al. (2), who studied experimental *C. jejuni* infection in adult HA-ICR mice. Pretreatment of these mice with antibiotics was not necessary to induce infection.

The purpose of our study was to determine if orogastric challenge of outbred Swiss white mice with *C. jejuni* resulted in colonization of the intestinal tract with the pathogen and if treatment with streptomycin enhanced susceptibility to colonization. We found that neither streptomycin-treated nor untreated mice were consistently colonized with *C. jejuni* but that the pathogens readily colonized the intestinal tracts of germfree mice and attained large populations in the ceca.

MATERIALS AND METHODS

Bacterial strains. Two strains of *C. jejuni* were used in the study. Strain A, which was originally isolated from a patient, was kindly provided by Martin Blaser of the University of Colorado School of Medicine; strain B was isolated at the Lubbock General Hospital from the stool of a child suffering from gastroenteritis. Both strains produced characteristic grayish, glistening colonies on brucella agar (Difco Laboratories, Detroit, Mich.) enriched with 5% defibrinated sheep blood (BBA) and displayed typical comma-shaped cells. Stock cultures of the strains in brucella broth (BB) (Difco) containing 40% (vol/vol) glycerol were stored at −70°C.

Media and growth conditions. BBA containing Blaser Wang supplement (Difco) (BBA-S) was used as a selective medium for the culture of *C. jejuni* from mouse fecal pellets and organ homogenates. The organisms were also grown in BB. Plates and broth cultures were incubated for 48 h at 42°C in jars into which Microaerophilic Campy Paks (BBL Microbiology Systems, Cockeysville, Md.) were placed.

Mice. Outbred Swiss white mice weighing 25 to 30 g (Harlan Sprague Dawley Inc., Madison, Wis.) were used in the study. These animals were housed in groups of five in cages with wire mesh bottoms and were fed Purina Lab Rodent Diet (Ralston Purina Co., St. Louis, Mo.) and water ad libitum. Germfree BALB/c mice were purchased from the University of Wisconsin, Madison. The animals were kept in Trexler type isolators (Standard Safety Equipment Co., Palatine, Ill.) and were housed in groups of five in plastic cages containing corncob bedding. The mice were maintained on Autoclavable Rodent Chow (Ralston Purina), which is formulated for germfree animals. Food and water were provided ad libitum.

Treatment of conventional mice with streptomycin. Conventional Swiss white mice were given streptomycin sulfate

* Corresponding author.
† Present address: Department of Microbiology, Christian Medical College and Hospital, Vellore 632004, India.

TABLE 1. ID_{50} determinations of *C. jejuni* for mice

Strain and inoculum size	No. of mice colonized/total no. of mice (% positive)	
	Untreated	Streptomycin treated
A		
10^{11}	6/9 (67)	4/10 (40)
10^{9}	6/10 (60)	3/10 (30)
10^{7}	11/20 (55)	2/9 (22)
10^{5}	9/20 (45)	3/9 (33)
10^{3}	6/19 (32)	3/10 (30)
10^{1}	10/20 (50)	3/10 (30)
10^{0}	4/10 (40)	3/10 (30)
B		
10^{11}	10/10 (100)	2/10 (20)
10^{9}	4/10 (40)	6/20 (30)
10^{7}	0/10 (0)	6/20 (30)
10^{5}	1/10 (10)	3/20 (15)
10^{3}	0/10 (0)	4/20 (20)
10^{1}	4/10 (40)	0/20 (0)
10^{0}	0/10 (0)	0/20 (0)

(Sigma Chemical Co., St. Louis, Mo.) in a concentration of 0.5 mg/g of body weight per day. The antibiotic was administered orogastrically in three doses each day for a period of 3 days. Untreated control mice received phosphate-buffered saline at pH 7.0 instead of streptomycin.

ID_{50} determinations. An effort was made to determine the 50% implantation dose (ID_{50}) of *C. jejuni* A and B for streptomycin-treated and untreated conventional mice. Twenty-four hours after completion of the antibiotic regimen, groups of treated and untreated control mice were challenged orogastrically with graded numbers of *C. jejuni* A or B. On day 3 postchallenge, two fecal pellets collected from each mouse were emulsified in 1 ml of sterile saline. The emulsion (0.1 ml) was then plated on BBA-S. Colonies on the plates were randomly picked and identified by standard procedures (1). The presence or absence of *C. jejuni* was recorded after 48 h of incubation at 42°C under microaerophilic conditions.

Colonization of the mouse intestinal tract. Twenty-four hours after completion of streptomycin treatment, groups of 10 treated and 10 untreated control mice were challenged with large numbers of *C. jejuni* A or B. Treated mice received either 1.20×10^{10} organisms of strain A or 4.13×10^{10} organisms of strain B. Untreated control mice received either 7.60×10^{10} organisms of strain A or 1.10×10^{10} organisms of strain B. Additionally, two groups of five germfree mice were challenged with either 1.01×10^{8} organisms of strain A or 2.01×10^{8} organisms of strain B. Three days after challenge, all mice were sacrificed by cervical dislocation and the duodena, jejuna, ilea, ceca, and colons were aseptically removed and separately homogenized in 9 volumes (wt/vol) of sterile phosphate-buffered saline. Serial 10-fold dilutions of the homogenates prepared in phosphate-buffered saline were plated on BBA-S. After 48 h of incubation at 42°C under microaerophilic conditions, randomly picked colonies were identified (1) and counts of *C. jejuni* were reported as viable organisms per gram of organ (wet weight).

Broth culture studies. VFA were added to prereduced BB inside an anaerobic isolator (model 1024; Forma Scientific Inc., Marietta, Ohio) in total concentrations of either 21.63 μeq/ml, simulating conditions in the ceca of streptomycin-treated mice, or 60.80 μeq/ml, simulating conditions in the ceca of untreated mice (8). The pHs of the two types of broth were adjusted to 7.04 and 6.76, respectively. Each broth type and BB without VFA were inoculated separately with approximately 1.0×10^{7} organisms of *C. jejuni* A or B per ml, and the cultures were incubated anaerobically at 37°C. At 6, 12, and 24 h postinoculation, serial 10-fold dilutions of the cultures were prepared with phosphate buffered saline. The dilutions were plated on BBA. Plates were incubated at 42°C for 48 h under microaerophilic conditions, and then colonies were counted. Results were reported as the number of viable bacteria per milliliter of broth. Similar experiments were done by using BB without VFA and adjusted to pH 7.02, simulating conditions present in cecal contents of germfree mice (unpublished data). In this case, the cultures were incubated under microaerophilic conditions at 37°C. All broth culture experiments were repeated twice.

Statistical methods. Statistical evaluations of the significance of differences in *C. jejuni* populations in the organs of mice and in broth cultures were performed with Fisher's least significant difference test (12). The significance of differences in the incidence of colonization with *C. jejuni* between untreated mice and streptomycin-treated or germfree mice was determined by the test of significance of differences between two proportions (4).

RESULTS

ID_{50} values. There was no correlation between inoculum size and incidence of intestinal colonization when groups of untreated conventional mice or streptomycin-treated mice were challenged orogastrically with between 1 and 10^{11}

TABLE 2. Colonization of intestinal tracts of mice with *C. jejuni* A

Organ	Mean ± SEM $\log_{10}$ viable organisms recovered from mice[a]			No. (%) of mice harboring *C. jejuni* in various organs		
	Conventional		Germfree[b,c]	Conventional		Germfree (n = 5)
	Streptomycin treated[d]	Untreated[e]		Streptomycin treated (n = 10)	Untreated (n = 10)	
Duodenum	3.08 ± 0.06	1.74 ± 0.44	4.76 ± 0.55	2 (20)	3 (30)	5 (100)[c]
Jejunum	N	N	4.34 ± 0.40	0 (0)	0 (0)	5 (100)[c]
Ileum	N	2.27	5.37 ± 0.52	0 (0)	1 (10)	5 (100)[c]
Cecum	2.66 ± 0.19	2.47 ± 0.05	9.17 ± 0.13	2 (20)	8 (80)	5 (100)
Colon	3.09 ± 0.02	2.52 ± 0.06	7.60 ± 0.07	3 (30)	8 (80)	5 (100)

[a] Per gram of organ. Counts obtained 3 days after challenge. N, No organisms isolated.
[b] Challenge dose, 1.01×10^{8} viable organisms.
[c] Significantly different from values obtained with untreated conventional mice.
[d] Challenge dose, 1.20×10^{10} viable organisms.
[e] Challenge dose, 7.60×10^{10} viable organisms.

TABLE 3. Colonization of intestinal tracts of mice with *C. jejuni* B

Organ	Mean ± SEM $\log_{10}$ viable organisms recovered from mice[a]			No. (%) of mice harboring *C. jejuni* in various organs		
	Conventional		Germfree[b,c]	Conventional		Germfree (n = 5)
	Streptomycin treated[d]	Untreated[e]		Streptomycin treated (n = 10)	Untreated (n = 10)	
Duodenum	2.72	2.78 ± 0.08	5.03 ± 0.27	1 (10)	4 (40)	5 (100)[c]
Jejunum	N	2.77 ± 0.17	4.80 ± 0.14	0 (0)	4 (40)	5 (100)[c]
Ileum	N	2.64 ± 0.12	5.18 ± 0.37	0 (0)	6 (60)	5 (100)
Cecum	3.01 ± 0.04	3.06 ± 0.22	9.19 ± 0.05	8 (80)	5 (50)	5 (100)
Colon	3.00 ± 0.05	2.71 ± 0.15	8.19 ± 0.19	7 (70)	6 (60)	5 (100)

[a] Per gram of organ. Counts obtained 3 days after challenge. N, No organisms isolated.
[b] Challenge dose, 2.01×10^8 viable organisms.
[c] Significantly different from values obtained with untreated conventional mice.
[d] Challenge dose, 4.13×10^{11} viable organisms.
[e] Challenge dose, 1.10×10^{10} viable organisms.

viable *C. jejuni* (Table 1). When strain A was used, between 32 and 67% of the untreated mice in groups receiving various inocula were colonized; when strain B was used, between 0 and 100% of the mice in different groups were colonized. When the mice were treated with streptomycin, between 0 and 40% of the animals in groups receiving various inocula were colonized when challenged with *C. jejuni* A or B. Because of lack of correlation between inoculum size and incidence of colonization, ID_{50} values could not be calculated.

Colonization of the mouse intestinal tract. The intestines of both streptomycin-treated and untreated conventional mice were irregularly colonized after challenge with inocula of *C. jejuni* A exceeding 10^{10} organisms (Table 2). The organism was not recovered from the jejuna or ilea of treated mice or the jejuna of untreated mice 3 days after challenge. However, it was sometimes isolated from homogenates of the other organs in numbers ranging between 10^1 and 10^3/g of organ homogenate. When germfree mice were challenged with approximately 10^8 *C. jejuni* A, the entire intestinal tracts of all mice were colonized with the pathogen recoverable at levels ranging between 10^4 and 10^9/g of organ homogenate. The mean number of *C. jejuni* recovered from the ceca exceeded the challenge inoculum size by a factor of 10, indicating that extensive multiplication occurred in this organ. These data agree with those reported by Lee et al. (10). Populations of *C. jejuni* in all organs were significantly greater in the monoassociated germfree mice than in conventional mice. Almost identical results were obtained when conventional and germfree mice were challenged with *C. jejuni* B (Table 3).

During dissection of germfree mice monoassociated with *C. jejuni* of either strain, marked congestion and redness of the intestinal tracts were observed, especially in the ceca. The intestinal tracts were also more friable than those observed in conventional mice.

Multiplication of *C. jejuni* in broth. Both strains of *C. jejuni* multiplied in BB when the cultures were incubated anaerobically (Fig. 1). The effect of the addition of VFA to BB in concentrations corresponding to those observed in cecal contents of streptomycin-treated and untreated conventional mice on the multiplication of *C. jejuni* anaerobically was determined. The pHs of the broth were adjusted to those observed in cecal contents in vivo. There was no evidence of active multiplication of strain A or B under these conditions, although the mean populations of the organisms increased very slightly over the 24-h incubation period (Fig. 1). In broth without VFA adjusted to pH 7.02 and incubated microaerobically to simulate conditions in cecal contents of germfree mice, both strains A and B actively multiplied (Fig. 2). Mean counts of these strains obtained at 12 and 24 h after inoculation were significantly greater than corresponding counts of the organisms obtained when the cultures contained VFA (Fig. 1).

DISCUSSION

The results of this study show that the intestinal tracts of untreated adult conventional mice are irregularly colonized

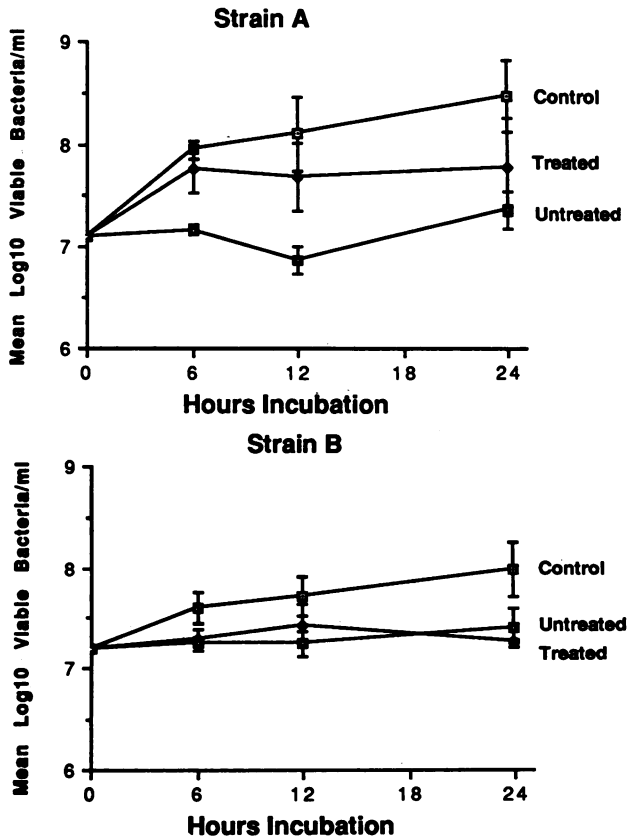

FIG. 1. Growth curves of *C. jejuni* A and B in BB adjusted to simulate conditions present in cecal contents of streptomycin-treated and untreated mice. Results are expressed as the mean $\log_{10}$ of values obtained from three separate experiments. Bars indicate standard errors of mean values.

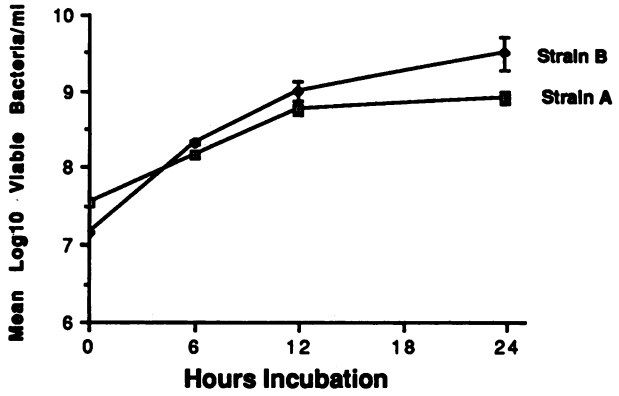

FIG. 2. Growth curves of *C. jejuni* A and B in BB adjusted to simulate conditions present in cecal contents of germfree mice. Results are expressed as the mean $\log_{10}$ of values obtained from three separate experiments. Bars indicate standard errors of mean values.

with *C. jejuni*. Some mice seemed inherently more susceptible to colonization than others. There appeared to be no clear-cut dose relationship, although the incidence of colonization was somewhat greater with higher challenge doses. These findings are similar to those reported by Blaser et al. (2) but different from the results obtained by Field et al. (5). *C. jejuni* strain variation may account for the discrepancies.

Treatment of mice with streptomycin produced no significant increase in the incidence of colonization of the gastrointestinal tract with *C. jejuni*. These results do not correspond with those obtained when streptomycin-treated mice were challenged with *P. aeruginosa* or *S. typhimurium* (3, 9, 14). In those studies, streptomycin treatment greatly increased susceptibility to colonization. The activity of inhibitory factors operating in the intestinal tract was greatly diminished as a consequence of streptomycin treatment, providing conditions that favored colonization by *P. aeruginosa* or *S. typhimurium*. The changes induced by streptomycin treatment had no measurable effect on colonization of the mouse intestine by *C. jejuni*, however.

Since gram-negative enteric pathogens such as *Escherichia coli* and *Salmonella* and *Shigella* species examined to date are inhibited by VFA, we decided to study the influence of the acids on the multiplication of *C. jejuni* in broth culture. Que et al. (13) reported that treatment of mice with streptomycin did not significantly increase the oxidation-reduction potential of cecal contents. However, the oxidation-reduction potential of cecal contents of germfree mice is considerably more positive than that of conventional mice (11). Under anaerobic conditions, VFA present in broth in concentrations equal to those measured in cecal contents of either streptomycin-treated or untreated mice essentially prevented active multiplication of *C. jejuni*. However, the numbers of organisms increased slightly during the 24-h incubation period. In broth without VFA incubated microaerobically, simulating conditions in cecal contents of germfree mice, *C. jejuni* actively multiplied. This suggests that VFA operating at the low oxidation-reduction potential of the ceca of both streptomycin-treated or untreated mice interfere with the multiplication of *C. jejuni*. The organisms multiply, on the other hand, in the absence of VFA at the relatively high oxidation-reduction potential of the ceca of germfree mice. This may explain the irregular colonization of the intestines of conventional mice with *C. jejuni* at low population levels and the consistent colonization of the intestinal tracts of germfree mice at high population levels.

This study provides evidence that ecological conditions in the intestinal tracts of conventional and streptomycin-treated mice impede colonization by *C. jejuni*. Therefore, germfree mice that are easily colonized with *C. jejuni* represent better experimental animal models for the study of *C. jejuni* infections than do conventional or streptomycin-treated mice that resist colonization with this pathogen.

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