Colonization and Infection of Athymic and Euthymic Germfree Mice by Campylobacter jejuni and Campylobacter fetus subsp. fetus

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Human clinical strains of Campylobacter jejuni and Campylobacter fetus subsp. fetus colonized the gastrointestinal tracts of both athymic (nu/nu) and euthymic (+/nu) germfree mice (BALB/c). Viable Campylobacter spp. (10^6 to 10^10 CFU/g [dry weight] of cecum and colon contents) were isolated on day 3 after oral challenge, and similar large numbers of viable cells were evident at several intervals during a 10-month experiment. The stomachs and upper small intestines of nu/nu and +/nu mice that were monoassociated for 224 days with C. jejuni 45100 contained 3 to 4 logs fewer viable bacteria than did their ceca or colons. Athymic mice that were monoassociated for 224 days with C. fetus subsp. fetus had 2 to 3 logs more viable Campylobacter spp. in their upper gastrointestinal tracts than did their +/nu littermates. Large viable populations (10^7/g of contents) of C. fetus subsp. fetus were in the ceca and colons of both nu/nu and +/nu mice. All C. jejuni strains used in this study chronically infected the mesenteric lymph nodes of both nu/nu and +/nu mice. C. jejuni strains 24 and INN 73-83, which were cytotoxic for Chinese hamster ovary cells in vitro, were also more frequently isolated from the livers, spleens, and kidneys of nu/nu mice than was the weak cytotoxin-producing strain 45100. Additionally, heat-labile-enterotoxin-producing C. jejuni INN 73-83 was recovered more frequently from the internal organs of monoassociated +/nu mice than were any other Campylobacter spp. tested. Natural gastrointestinal colonization of neonatal nu/nu and +/nu mice (born to Campylobacter-colonized mothers) with Campylobacter spp. appeared to be delayed until approximately 1 to 2 weeks after birth. Conventionalization of C. jejuni 45100-monoassociated BALB/c mice with a complex mouse fecal microflora eliminated viable C. jejuni from the mesenteric lymph nodes by day 14 and from the cecum by day 78. These findings show that the gnotobiotic BALB/c mouse is a new model for studying acute and chronic host-Campylobacter spp. interactions.

Campylobacter spp. are major causes of enteric disease in humans (4, 6). The formulation of a selective plating medium (34) and the use of microaerophilic incubation resulted in increased recoveries of Campylobacter jejuni from clinical stool specimens. Currently, the isolations of C. jejuni from clinical specimens sometimes surpass those of Salmonella or Shigella spp. (6).

Bacteremia, cellular infiltration, and bloody diarrhea all point to an invasive process in Campylobacter pathogenesis; however, the epithelial cell damage and tissue invasion associated with human campylobacteriosis could be due to the production of cytotoxin(s) (24). The role of a classical enterotoxin in the pathogenesis of C. jejuni is controversial. Some investigators have not been able to detect enterotoxin production by Campylobacter spp. (6, 17, 24, 39). Other investigators have used various in vitro cytotoxic assays and rodent intestinal perfusion models and reported that a "cholera-like" cytotoxin enterotoxin is produced by some strains of Campylobacter jejuni (12, 21, 23, 32, 41). Considerable controversy and many unanswered questions remain about the exact mechanism(s) of C. jejuni pathogenesis.

An appropriate animal model that mimics the disease seen in man has not yet been developed to study campylobacteriosis. The following animals have been tested as models for studies on C. jejuni pathogenesis: calves and lambs (1, 15, 40), monkeys (16), chickens (24, 31, 33; S. Welkos, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, B110, p. 41), gnotobiotic chickens (24) and dogs (29), hamsters (19), guinea pigs (5, 37, 38), rabbits (7, 14), rats (14), mice (3, 11, 13, 14, 20, 22, 23, 25), and dogs and cats (30). Most of the latter studies were conducted in conventional animals that have a very complex gut flora. The presence of a complex intestinal microflora poses several problems for studying Campylobacter pathogenesis. The normal bacterial flora may inhibit the growth of C. jejuni or prevent it from attaching to epithelial cells and colonizing the gut. Intestinal microbes may also interfere with the pathogenesis of C. jejuni by producing toxin-degrading enzymes or acids. Conversely, some intestinal bacteria can act synergistically with C. jejuni to enhance Campylobacter pathogenesis.

Campylobacter fetus subsp. fetus can also cause diarrhea (10, 18, 28), as well as systemic infections in the immunocompromised host (4), indicating that the intestinal tract may be its portal of entry. To date, no one has compared the pathogenesis of C. fetus subsp. fetus with that of C. jejuni in an animal model.

The aims of this study were to determine whether human clinical strains of C. jejuni and C. fetus subsp. fetus can colonize, infect, and cause overt disease (42) in gnotobiotic athymic and euthymic BALB/c mice.

**MATERIALS AND METHODS**

**Mice.** BALB/c athymic (nu/nu) and euthymic (+/nu) germfree (GF) mice (8 weeks old) were used in these studies. They were reared, housed, and colonized with a pure culture of Campylobacter sp. in GF isolators at the Gnotobiotic Research Laboratory, University of Wisconsin, Madison. Neonates were obtained from matings of heterozygous (+/nu) females and nude (nu/nu) males. Adult GF mice, including GF pregnant females, were introduced into the Campylobacter-associated isolators, and the latter were allowed to deliver their young. Thereafter, the mice were

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allowed to procreate within the monoassociated (MA) environment of each isolate.

**Bacterial strains.** Human clinical isolates of *C. jejuni* and *C. fetus subsp. fetus* were obtained from the Wisconsin State Laboratory of Hygiene, Madison (strains 45100, 24, and 255) and from Frederick Klopstein, University of Rochester Medical Center, Rochester, N.Y. (strain INN 73-83). The two human fecal isolates of *C. jejuni* from the Wisconsin State Laboratory of Hygiene were from individuals (2-month- and 30-year-old females) with diarrheal illness and were selected to monoassociate GF mice because cell-free broth supernatants of strain 45100 (from the infant) demonstrated a weak (1:1 titer) in vitro cytotoxic response for Chinese hamster ovary (CHO) cells, while strain 24 produced a stronger (1:4 titer) cytotoxic effect in the same in vitro assay. Strain INN 73-83 was included in this study because it is a documented heat-labile enterotoxin-producing strain originally isolated from a Mexican child with diarrhea (21). Cultures of strains 45100 and 24 were passaged twice and once, respectively, on Skirrow agar before being frozen at -70°C in buffered (pH 7.2) glycerol brain heart infusion broth. The culture of strain 25 was a blood isolate of *C. fetus subsp. fetus* which, along with the culture of strain INN 73-83, had undergone an unknown number of passages on plating media before being received, identified, and stored (-70°C).

**Oral inoculation.** Initially, all mice in an isolator were inoculated orally with 1 drop of a brucella broth (Difco Laboratories, Detroit, Mich.) suspension (~10^6 viable organisms) of *Campylobacter* spp. All other GF animals were colonized with these bacteria by allowing a natural fecal-oral inoculation to occur after GF mice were placed into cages that contained fresh feces from *Campylobacter*-MA mice. Cultures of mouse fecal pellets demonstrated that colonization occurred very rapidly (within 24 h).

**Quantitation of bacteria in tissues.** At various times after oral inoculation, three *nu/nu* and three *+/nu* mice were anesthetized (ether) and exsanguinated by cardiac puncture, and the internal organs were aseptically excised. Tissues cultured for viable *Campylobacter* spp, included heart blood, liver, spleen, kidneys, mesenteric lymph nodes (MLNs), stomach, upper small intestine, lower small intestine, cecum, and colon. The MLNs and ceca from individual animals were assayed separately, whereas the other organs from three mice were pooled by type and assayed at each sampling time.

Aseptically removed tissues were added to 5 ml of sterile phosphate-buffered saline in a tissue homogenizer, homogenized, and serially diluted in phosphate-buffered saline (10-fold), and duplicate 0.05-ml amounts were plated on 5% sheep blood agar (GIBCO Diagnostics, Madison, Wis.). A separate 1.0-ml aliquot of each organ homogenate was added to 9 ml of bruccella broth for enrichment and to a preweighed aluminum pan for subsequent drying and weighing.

Plating media and enrichment broths were incubated at either 37°C (*C. fetus subsp. fetus*) or 42°C (*C. jejuni*) for 48 h in a microaerophilic atmosphere produced inside vented jars by evacuation (25 lb/in^2) and replacement (80% N_2, 10% CO_2, 10% H_2). Enrichment broth cultures were then subcultured onto sheep blood agar plates and incubated as described above to determine the presence or absence of viable *Campylobacter* spp.

**Conventionalization.** *C. jejuni* 45100-MA mice, born to *C. jejuni*-MA mothers, were removed from the isolator at 4 to 7 weeks of age and housed in groups of five in cages that had been prepared with dirty bedding from conventional pathogen-free laboratory mice. Food and water for these mice were not sterilized. At various times (7, 14, 21, 37, and 78 days) after removal from the plastic isolator, groups of five mice were killed, and the various internal organs and ceca were aseptically removed, homogenized, diluted, and plated on Campy agar (GIBCO) to determine the numbers of viable *C. jejuni* present. Cecal dilutions plated onto sheep blood agar plates at the various sampling times showed an increase in the complexity of the flora present; however, no attempt was made to quantitate or speciate the microorganisms in the cecal contents during this study.

**Scanning electron microscopy.** Tissue specimens obtained from various areas of the gastrointestinal (GI) tract were immediately frozen (-70°C) and stored until processed. Frozen tissues were thawed, fixed in 2.5% glutaraldehyde, dehydrated through a series of alcohols, dried to the critical point, gold coated, and viewed with a no. JSM-U3 scanning electron microscope (Japanese Electron Optics Laboratories).

**Quantitative bacteriology.** Counts of viable bacteria in the alimentary tract or tissues of MA mice are presented as mean log_{10} ± standard error of the mean. Statistical analysis of viable counts from MLNs was carried out by one-way analysis of variance with Fisher's least-significant-difference test.

**RESULTS**

**GI colonization.** In the GI tract, large numbers (10^{10.0} to 10^{10.9} CFU/g) of viable *C. jejuni* strains 45100, 24, and INN 73-83 were in the ceca of both *nu/nu* and *+/nu* mice by day 3 after oral inoculation. These viable counts were maintained for 42 days but decreased slightly to 10^{8.7} CFU/g in mice that were monoassociated for 224 days. *C. fetus subsp. fetus* 255 also rapidly colonized the ceca of both *nu/nu* and *+/nu* mice; viable counts of 10^{9.5} to 10^{10.0} CFU/g of cecum were observed from 3 to 42 days after oral inoculation. At 224 days after colonization, the ceca from *nu/nu* or *+/nu* mice with *C. fetus subsp. fetus* still yielded 10^{9.5} and 10^{8.3} CFU/g of cecal contents, respectively.

<p>| Table 1. Viable <em>Campylobacter</em> spp. in the GI tracts of gnotobiotic mice that were monoassociated for 224 days |
|---------------------------------|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th>Section of GI tract and BAL/Bc genotype* (dry wt)^b</th>
<th>Log_{10} CFU of viable bacteria/g</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>nu/nu</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>+/nu</td>
<td>6.0</td>
</tr>
<tr>
<td>Upper small intestine</td>
<td>nu/nu</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>+/nu</td>
<td>5.6</td>
</tr>
<tr>
<td>Lower small intestine</td>
<td>nu/nu</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>+/nu</td>
<td>8.1</td>
</tr>
<tr>
<td>Cecum</td>
<td>nu/nu</td>
<td>9.7</td>
</tr>
<tr>
<td></td>
<td>+/nu</td>
<td>9.7</td>
</tr>
<tr>
<td>Colon</td>
<td>nu/nu</td>
<td>10.1</td>
</tr>
<tr>
<td></td>
<td>+/nu</td>
<td>9.6</td>
</tr>
</tbody>
</table>

* nu/nu, Athymic; +/nu, euthymic.

**a** Obtained by culturing dilutions of pooled homogenates (three mice) from each section of the GI tract.
Viable counts of *C. jejuni* 45100 or *C. fetus* subsp. *fetus* 255 in various sections of the GI tract of *nu/nu* and *+nu* BALB/c mice that were monoassociated with *C. jejuni* or *C. fetus* subsp. *fetus* for 224 days are presented in Table 1. The *nu/nu* and *+nu* mice had similar populations of *C. jejuni* 45100 within the different sections of their GI tracts. Approximately 10^3.4 to 10^6.9 CFU of viable *C. jejuni* per g were in the stomachs and upper small intestines, 10^8.1 CFU/g were in the lower small intestines, and 10^6.5 to 10^9.0 CFU/g were in the ceca and colons of these MA mice. *C. fetus* subsp. *fetus* 255 was isolated from the upper levels of the GI tract in the *nu/nu* mice in higher numbers (1 to 3 logs greater) than in their *+nu* littermates. Overall, the largest viable populations of *C. fetus* subsp. *fetus* occurred in the ceca and colons of the mice (Table 1).

**MLN cultures.** Lymphadenopathy was evident in the MLNs (a size increase from less than 1 by 1 mm to approximately 3 by 5 mm) of mice (*nu/nu* and *+nu*) that were monoassociated for 7 days or more. Viable *C. jejuni* strains 45100, 24, and INN 73-83 were consistently recovered from the MLNs of all mice at each sampling time. Conversely, we were unable to consistently recover viable *C. fetus* subsp. *fetus* 255 from the MLNs of mice of that were killed at the two early sampling times after inoculation (2/6 positive on day 4 and 4/6 positive on day 9). The number of viable *Campylobacter* spp. cultured from MLNs of *nu/nu* and *+nu* BALB/c mice at several time intervals over a 36-day period of monoassociation is given in Table 2. The number of viable *Campylobacter* spp. recovered ranged from 10^3.0 to 10^6.0 CFU/g (dry weight) of MLNs. The MLNs from *nu/nu* and *+nu* mice contained similar populations of viable *C. jejuni* 45100 or *C. fetus* subsp. *fetus*. The MLNs of the *nu/nu* mice that were monoassociated for up to 36 days with the more cytotoxic *C. jejuni* strains 24 and INN 73-83, however, had significantly more viable organisms than the MLNs of their *+nu* littermates had (10^5.7 and 10^8.1 CFU/g versus 10^6.6 and 10^10.4, respectively). The MLNs of *nu/nu* mice monoassociated with *C. jejuni* 45100 contained about 10^2 to 10^5 fewer *C. jejuni* than did MLNs from strain 24- or INN 73-83-MA *nu/nu* mice (Table 2).

**Systemic infection.** The number of times and the days after oral challenge on which viable *Campylobacter* spp. were recovered by direct plating or by enrichment broth from pooled internal organs are shown in Table 3. Generally, viable *Campylobacter* spp. were not consistently recovered from the pooled internal organs (kidneys, liver, or spleen) that were cultured at different times after oral colonization. The *Campylobacter* sp. that was most consistently recovered from the internal organs of MA *nu/nu* and *+nu* mice was the cytotoxin-producing strain INN 73-83. The other cytotoxic strain, *C. jejuni* 24, was more frequently isolated from the internal organs of *nu/nu* mice than from those of their *+nu* littermates (Table 3). The weakly cytotoxin-producing *C. jejuni* 45100 and *C. fetus* subsp. *fetus* 255 were only occasionally recovered from the internal organs of *nu/nu* and *+nu* mice. The number of viable *Campylobacter* recovered from the internal organs (livers, kidneys, and spleens) of the MA *nu/nu* and *+nu* mice generally ranged from 10^2 to 10^3 viable bacteria per g of tissue. The spleen appeared to be the most common site for recovering viable *Campylobacter* spp. from colonized *nu/nu* and *+nu* mice (Table 3). All blood cultures for viable *C. jejuni* 45100 and *C. fetus* subsp. *fetus* 255 were negative at the times sampled (days 3, 7, 10, and 42) in the initial monoassociation experiments.

**Conventionalization.** The effect of conventionalization on

### Table 2. Recovery of viable *Campylobacter* spp. from MLNs of MA mice

<table>
<thead>
<tr>
<th>BALB/c genotype</th>
<th>Log_{10} mean CFU ± SEM of viable bacteria/g (no. of positive cultures/total no. cultured)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>C. jejuni</em> 45100</td>
</tr>
<tr>
<td><em>nu/nu</em></td>
<td>4.6 ± 0.2 (9/9)</td>
</tr>
<tr>
<td><em>+nu</em></td>
<td>5.1 ± 0.1 (9/9)</td>
</tr>
</tbody>
</table>

* a MLNs from individual mice (three *nu/nu* and three *+nu* mice at each time interval) were cultured for viable *Campylobacter* spp. on the indicated days after oral inoculation: strain 45100 on 7, 22, and 36; strain 24 on 3, 7, 8, 14, 15, and 22; strain INN 73-83 on 3, 7, 14, and 36; and strain 255 on 4 and 9.

* +Inu, Athymic; +nu, euthymic.

* d Dry weight.

* Significant higher (*P* < 0.001) when compared with counts for *nu/nu* mice monoassociated with *C. jejuni* 45100 and with counts for *+nu* mice associated with strains 24 and INN 73-83.

### Table 3. Systemic infection after intestinal colonization of athymic (*nu/nu*) and euthymic (*+nu*) BALB/c mice with *Campylobacter* spp.

<table>
<thead>
<tr>
<th>Colonizing <em>Campylobacter</em> spp.</th>
<th>No. of assays</th>
<th>Day(s) on which organs from different mice were culture positivea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>nu/nu</em></td>
</tr>
<tr>
<td><em>C. jejuni</em> 45100</td>
<td>8</td>
<td>42</td>
</tr>
<tr>
<td><em>C. jejuni</em> 24</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td><em>C. jejuni</em> INN 73-83</td>
<td>6</td>
<td>3b</td>
</tr>
<tr>
<td><em>C. fetus subsp. fetus</em> 255</td>
<td>7</td>
<td>7b, 10</td>
</tr>
</tbody>
</table>

* a Individual organs from three *nu/nu* and three *+nu* mice were pooled, homogenized, diluted, and cultured by direct plating and enrichment for the following viable *Campylobacter* spp. on the indicated days after oral challenge: *C. jejuni* 45100 on 1, 3, 7, 10, 22, 36, 42, and 224; *C. jejuni* 24 on 3, 7, 8, 14, 15, and 22; *C. jejuni* INN 73-83 on 3, 7, 14, and 36; and *C. fetus subsp. fetus* 255 on 3, 4, 7, 9, 10, 42, and 224.

* b Positive in enrichment culture only.

* d Dry weight.

* Repeat experiment with a mouse-adapted strain.
TABLE 4. Elimination of C. jejuni from MLNs and ceca by intestinal flora

<table>
<thead>
<tr>
<th>Days after conventionalization</th>
<th>Log_{10} viable population of C. jejuni 45100 ± SEM (no. positive/total no.) in:</th>
<th>MLN</th>
<th>Cecum</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>4.1 ± 0.1 (5/5)</td>
<td>6.8 ± 0.7 (5/5)</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>— (0/5)</td>
<td>5.3 ± 0.7 (5/5)</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>3.7 (1/5)</td>
<td>6.7 ± 0.6 (5/5)</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>— (0/5)</td>
<td>5.5 ± 0.1 (4/5)</td>
<td></td>
</tr>
<tr>
<td>78</td>
<td>— (0/5)</td>
<td>— (0/5)</td>
<td></td>
</tr>
</tbody>
</table>

* a — None detected by enrichment and plating onto Campy agar.

the number of viable C. jejuni 45100 in the ceca and the MLNs of the previously MA mice is shown in Table 4. The number of viable C. jejuni 45100 in the ceca of these mice decreased approximately 3.5 logs (from $10^{10.5}$ to $10^{6.5}$ CFU/g) by day 7 after the MA mice were removed from the plastic isolator and allowed to acquire a more complex intestinal flora. Viable C. jejuni (10^5 CFU/g) were still in the ceca of the mice at 37 days after their removal from the isolator; however, by day 78, no viable C. jejuni 45100 were detected in the cecal contents by plating or by enrichment broth. Conventionalization also resulted in a clearance of Campylobacter organisms from the MLNs of C. jejuni-colonized mice. At day 7 after their removal from the plastic isolator, all mice still had viable C. jejuni 45100 in their MLNs (10^4.1 CFU/g); thereafter, the MLNs were free of culturable C. jejuni, and only one mouse (at day 21) had MLNs that were positive for viable Campylobacter spp.

Neonatal colonization. GI colonization of neonates by C. fetus subsp. fetus 255 was first detected in the lower GI tracts (colon) of 13-day-old nu/nu and +/nu pups delivered by mothers colonized by this strain. C. jejuni 45100 was isolated from the colons of nu/nu and +/nu pups at 7 and 13 days of age, respectively. Viable C. jejuni 45100 was recovered from the upper GI tracts of both nu/nu and +/nu mice at 18 days after birth. Viable C. fetus subsp. fetus was not detected in the upper GI tracts of nu/nu mice until 24 days after birth. Adult levels (i.e., $10^5$ to $10^{10} 	ext{ CFU/g}$) of viable Campylobacter ssp. in the alimentary tract of neonatal mice were not attained until approximately 2 to 3 weeks after birth. Campylobacter colonization of the GI tracts of infant mice coincided with their eating of solid food. No visible signs of disease were observed in any of the offspring that were naturally monoassociated as neonates; however, the percent mortality among pups born in the C. jejuni 45100 isolator compared with that of those born in the C. fetus subsp. fetus 255 isolator was 15.6% and 5%, respectively. The average neonatal mortality in our GF BALB/c mouse breeding colony is ~5%.

Scanning electron microscopy. Scanning electron microscopy of cecal and colon segments from adult GF BALB/c mice that were colonized for 7 days with C. jejuni 45100 showed large numbers of these organisms closely associated with the epithelial cell brush border (Fig. 1). The bacteria extended down into the mucosal crypts and glands. The association was at the microvillus surfaces, and the C. jejuni did not appear to penetrate into the microvillus brush border. In +/nu mice that had been colonized with C. jejuni 45100 for 7 days or in nu/nu mice that had been monoassociated for more than 7 days, the organisms were observed primarily in the lumen and the mucus layer. The upper regions of the alimentary tracts (stomach and duodenum) in nu/nu or +/nu MA mice also did not contain C. jejuni 45100 in close association with the brush border epithelium.

DISCUSSION

These data show that a rapid (by day 3), persistent (224 day) GI colonization occurred after GF BALB/c nu/nu and +/nu mice were orally inoculated with Campylobacter ssp. These organisms were most prevalent ($10^{5.5}$ to $10^{10.5} 	ext{ CFU/g}$) in the lower GI tract (cecum and colon) of nu/nu and +/nu mice. The upper alimentary tracts of these MA mice also were colonized, but 2 to 3 logs fewer (per gram [dry weight])

FIG. 1. Scanning electron micrograph of colonic mucosa from an adult athymic BALB/c mouse that had been colonized by C. jejuni 45100 for 7 days. This bacterial strain is closely associated with the epithelial cell brush border (A) and the mucosal glands (B). Bars, 1.0 μm.
viable bacteria were present. The GI colonization was stable since large viable populations were detected in the alimentary tract over a 224-day period.

Campylobacter spp. are also invasive for gnotobiotic nu/nu and +/nu BALB/c mice. The MLNs of all mice tested (with the exception of some C. fetus subsp. fetus-MA animals) consistently contained 4 to 5 logs of viable Campylobacter spp. A persistent adenopathy of the MLNs occurred in conjunction with the intestinal colonization. Our observations on Campylobacter spp. in gnotobiotic BALB/c mice resemble the observations of Collins and Carter (8), who used two avirulent (to mice) salmonellae strains to colonize TRU:ICR GF mice. During a 3-week experimental period, Collins and Carter (8) consistently recovered viable salmonellae (10^5 CFU/g by day 4) from the MLNs and noted a sporadic, low (first dilution) recovery of viable bacteria from liver and spleen homogenates. We also observed a low (11 to 29%) sporadic recovery of viable Campylobacter spp. from the internal organs (livers, spleens, and kidneys) of BALB/c mice after oral inoculation with these bacteria. Recently Fauchère and co-workers (11) reported that GF and Clostridium perrenae-MA C5H mice were intestinally colonized (up to 23 days) after intragastric administration of 10^7 to 10^8 viable C. jejuni. C. jejuni was recovered from blood, spleen, liver, and bile on only day 1 after an oral challenge and from the MLNs (of ~66% of the mice tested) of MA mice for up to 23 days after the challenge; however, no significant tissue (intestinal) damage or inflammation was reported. Lee and his colleagues (22) recently reported that C. jejuni colonized a “limited number” of GF BALB/c mice, which remained monoassociated with these bacteria in their cecal mucosa for several weeks. Lee et al. (22) also demonstrated that gnotobiotic mice which were colonized with a Lactobacillus sp., a Bacteroides sp., and a Fusobacterium sp. were susceptible to colonization with C. jejuni, as were spiral-free BALB/c mice after oral treatment with a combination of antibiotics (ampicillin, kanamycin, and vancomycin) and magnesium sulfate; however, no signs of tissue invasion or disease were reported in these models (22). In our studies, a comparison of C. jejuni strains 45100, 24, and INN 73-83, which differed in their cytotoxic effects on CHO cells in vitro, showed that the cytotoxic strains (24 and INN 73-83) were found in significantly higher numbers (~1 log) in the MLNs of the nu/nu mice than in those of their +/nu littermates. Also, strain 24 was more consistently isolated from the internal organs of the nu/nu mice than from those of the heterozygotes (+/nu), while strain INN 73-83 was isolated with equal frequency from the internal organs of nu/nu and +/nu mice. Therefore, with the limited number of strains tested in our experiments, it appeared that strains with a greater cytotoxin-producing capacity could establish an infection in the MLNs, cause lymphadenopathy, and disseminate to the internal organs better than a weakly cytotoxic strain could; however, MLN infection did not correlate with diarrheal disease. Thymus-matured T cells may play a role in controlling the spread of some Campylobacter strains to internal organs, since dissemination occurred more frequently in nu/nu than in +/nu mice monoassociated with C. jejuni 24. Owens and Berg (27) also observed that several different species of indigenous aerobic and anaerobic bacteria translocate to the internal organs of nu/nu conventional BALB/c mice (~50% positive) more frequently than in +/nu BALB/c mice (~5% positive). Therefore, there may be a fine distinction between passive translocation and a true invasiveness.

Several investigators have attempted to colonize conventional mice with C. jejuni (3, 13, 14, 20, 25). Field and her associates (13, 14) could not colonize adult mice with C. jejuni unless antibiotics were given to the mice before an intragastric inoculation. Merrell et al. (25) found it necessary to perform laparotomies and directly inject C. jejuni into the ilea and colons of NMRI mice to establish a transient colonization. Blaser and co-workers (3) reported that infection of conventional adult mice can be established if a relatively large inoculum (10^6) of C. jejuni is introduced intragastrically. The latter investigators (3) could not recover viable C. jejuni from the ceca of these mice 60 days after inoculation; however, 1 to 3 log_10 CFU were still in the stomachs and small intestines at that time. We showed that colonization of Campylobacter-MA mice with a complex mixture of bacteria from the intestinal tracts of conventional mice gradually eliminated the C. jejuni from the ceca by day 78. Others have shown that the intestinal microflora can inhibit the capacity of C. jejuni to colonize the GI tracts of mice (3) and chickens (35). Apparently, C. jejuni can not compete well against a complex gut flora and is gradually eliminated from the alimentary tract. Recently, Kazmi and co-workers (20) have reported success in inducing an enteritis in neonatal (1 to 10 day old) BALB/c mice after an intragastric challenge with 2 × 10^6 CFU of a virulence-enhanced (intraperitoneal mouse passage with mucin or iron dextran) strain of C. jejuni. They (20) suggested that the virulence enhancement procedures resulted in a more efficient colonization.

Field and co-workers (14) produced a transient GI colonization in neonatal mice by gavage with a large inoculum of C. jejuni (10^7 to 10^8 CFU) but did not observe dissemination of the organisms to internal organs. In our study, neonatal mice (<1 week old) had an innate or maternally supplied mechanism of resisting GI colonization by the Campylobacter spp. These gnotobiotic neonates did, however, rapidly acquire high levels of Campylobacter spp. immediately after adult feeding patterns were established (2 to 3 weeks of age). Similar findings of delayed alimentary tract colonization were observed in gnotobiotic neonates exposed to pure cultures of other microbes (2, 9, 26, 36).

In summary, a rapid, persistent GI colonization by Campylobacter spp. occurred in GF BALB/c nu/nu and +/nu mice after either an experimental or a natural oral route of inoculation. Intestinal colonization was associated with an invasion of the MLNs and occasionally of the internal organs. The fact that colonization and infection were not lethal to either the athymic or euthymic host will facilitate studies of the host-parasite interactions during Campylobacter infections and provide basic information on acute and chronic aspects of the pathogenesis of this prevalent human pathogen.

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LITERATURE CITED


CAMPYLOBACTER SPP. IN GNUTOBIOTIC MICE


