

## A Domestic Ferret Model of Immunity to *Campylobacter jejuni*-Induced Enteric Disease

JUDITH A. BELL<sup>1</sup> AND DEAN D. MANNING<sup>2\*</sup>

Departments of Veterinary Science<sup>1</sup> and Medical Microbiology and Immunology,<sup>2</sup>  
University of Wisconsin, Madison, Wisconsin 53706

Received 1 November 1989/Accepted 26 March 1990

Oral or intravenous inoculation of previously unexposed juvenile and adult ferrets with *Campylobacter jejuni* uniformly resulted in intestinal colonization lasting 2 to 12 days. Disease varied from mild to moderate diarrhea, which resolved in 2 to 3 days. Orally infected animals developed agglutinin titers of 8 to 256 within 3 weeks, while those infected intravenously developed titers of 256 to 2,048. Ferrets which had recovered from campylobacteriosis all developed high titers of agglutinating and bactericidal antibodies but were readily colonized by subsequent oral inoculation with the same strain of *C. jejuni*. Orally infected ferret kits 3 to 6 weeks of age exhibited the same general pattern of infection and disease as adults, but diarrhea was somewhat more severe. Kits resolved their diarrhea in 1 to 6 days and developed agglutinin titers in serum of 16 to 32 within 3 weeks. A series of five oral or rectal inoculations of kits during the 5- to 9-week age interval resulted in progressively shorter clearance times and eventual strain-specific resistance against infection, as well as disease. Gnotobiotic adults showed the same pattern of strain-specific accelerated clearance and resistance to disease. Kits born to immune dams with high levels of whey antibodies had passively acquired serum agglutinin titers of 256 to 2,048. These kits showed no resistance to colonization with the homologous strain of *C. jejuni* but were completely refractory to diarrhea. These observations suggest that (i) some form(s) of specific immunity, rather than factors relating solely to age or normal flora, is responsible for resistance to *C. jejuni* colonization and disease production and (ii) humoral immunity at a level that does not prevent colonization can protect against enteric disease caused by this organism.

*Campylobacter jejuni* infection has been reported to cause several forms of human disease, including mild-to-severe diarrhea, colitis clinically indistinguishable from acute ulcerative colitis (10), and abortion (5). The incidence of human abortion induced by this organism is unknown, but *C. jejuni* is among the most common bacterial agents of human diarrhea and colitis (10, 11).

No fully adequate animal model for studying the role of immunity in containing any of these disease entities has been developed. Recently proposed models for studying campylobacteriosis include those using congenitally athymic (nude) mice in a germfree condition (12), hamsters preconditioned by flushing the intestinal tract with saturated magnesium sulfate (6), and intestinally ligated rabbits (3). None of these animals, however, are seriously affected by infection with *C. jejuni* without such manipulations, i.e., under natural conditions. Dogs and cats occasionally develop severe *C. jejuni*-induced diarrhea naturally but do not readily develop disease when infected experimentally (9).

The domestic-ferret model for studying campylobacteriosis (4) may in fact be the model of choice because infection of these animals, without prior manipulation, reproducibly generates two of the three disease manifestations seen in humans. Thus, we have recently shown that abortion occurs quite regularly in experimentally infected ferrets but is not readily prevented by preformed antibody or other specific immune defenses acquired during recovery from previous infection (J. A. Bell and D. D. Manning, submitted for publication). Self-limiting diarrhea can also be induced consistently, but the role of specific immune defenses in limiting this manifestation remains an open question (J. A. Bell and D. D. Manning, Am. J. Vet. Res., in press). The primary

objective of the present study was, therefore, to examine the nature and extent of specific immune involvement in this process of enteric disease limitation.

### MATERIALS AND METHODS

**Animals.** Ferrets for our breeding colony were obtained from Marshall Farms (North Rose, N.Y.). Ferrets 0 to 9 weeks of age were designated kits, and those over 9 weeks old were considered adults. An effort to derive germfree ferrets (8) resulted in the production of two gnotobiotic females intestinally colonized with *Bacillus cereus* at 10<sup>4</sup> CFU/g of feces at the time of incorporation into this study. These two ferrets were maintained under gnotobiotic conditions throughout the investigation.

**Bacterial cultures.** The two principal strains of *C. jejuni* used in this study were D80 and 28209. Strain D80, originally isolated from an outbreak of abortions in Ontario ranch mink (7), was passaged several times in mink and ferrets and once on artificial medium. Strain 28209 was isolated from a human case of colitis and adapted to ferrets by oral passage. Both strains were grown in brucella broth (BBL Microbiology Systems, Cockeysville, Md.), concentrated by centrifugation, suspended in brucella broth containing 10% dimethyl sulfoxide, and frozen at -70°C in 1- or 2-ml inoculum samples.

**Inoculation.** Inocula were thawed, washed once in phosphate-buffered saline (PBS) or brucella broth, and suspended to the desired concentration in PBS. Two oral inoculations were made 12 h apart, each by feeding of 1 ml of a suspension containing 10<sup>8</sup> to 10<sup>10</sup> CFU in 10 ml of homogenized cow's milk. For intravenous inoculation, animals were anesthetized with halothane and 1.0 ml of PBS containing 10<sup>9</sup> to 10<sup>10</sup> CFU was slowly injected into the jugular vein. Controls were sham inoculated with PBS only.

\* Corresponding author.

TABLE 1. Durations of colonization and disease and immune responses of nonimmunized adult ferrets infected with *C. jejuni*

No. of ferrets	Inoculation route	Strain	Dose (CFU) <sup>a</sup>	Duration (days) of:		Range of agglutinin titer in serum (day[s]) <sup>c</sup>
				Colonization	Clinical disease <sup>b</sup>	
8	Oral	D80	$8 \times 10^9$ – $2 \times 10^{10}$	2–8	M, D: 1–2	16–256 (10–23)
2	Oral	28209	$4 \times 10^8$	3–8	M, D: 3	8–32 (17)
2	Intravenous	D80	$3 \times 10^9$	8–12	WD: 1–2; D: 3–6	512–2,048 (25)
3	Intravenous	D80	$3 \times 10^9$ – $2 \times 10^{10}$	5–10	M, D: 1–5	256–1,024 (8–20)

<sup>a</sup> Total no. of CFU given in two doses, 12 h apart.

<sup>b</sup> M, Green mucoid feces; D, diarrhea; WD, watery diarrhea.

<sup>c</sup> Number of days after inoculation.

**Culture methods.** Microbial enumerations of inocula were made by (i) incubation of a series of 10-fold limiting dilutions in brucella broth and (ii) subculture of 50  $\mu$ l of each dilution on chocolate agar. Intestinal colonization of infected animals was monitored by daily culture of fecal samples and/or rectal swabs on Skirrow agar (Bacto Campylobacter Agar Kit Skirrow; Difco Laboratories, Detroit, Mich.) or in 10-fold serial dilutions of brucella broth containing Skirrow antibiotics (vancomycin, 10 mg/liter; polymyxin B, 2,500 U/liter; trimethoprim, 5 mg/liter). Broth cultures were incubated for 24 h at 42°C in sealed plastic bags containing a tank mixture of 85% N<sub>2</sub>, 10% CO<sub>2</sub>, and 5% O<sub>2</sub>. Agar plates were incubated similarly but for 48 h. Cultures were examined for campylobacters by dark-field microscopy.

**Blood sampling.** All ferrets were lightly anesthetized before being bled. Adults were injected with 0.7 ml of a 1:10 mixture of acepromazine-ketamine per kg and bled from the jugular or ventral tail vein. Kits were similarly anesthetized and bled by cardiac puncture (if under 6 weeks of age) or from the jugular vein.

**Serological assays.** Anti-*C. jejuni* agglutinin titers were determined by a tube assay by using as the antigen formalinized organisms suspended in PBS to an A<sub>540</sub> of 0.15. Equal quantities (0.2 ml) of twofold saline dilutions of serum or colon washings and antigen were mixed, incubated overnight at 37°C, centrifuged for 3 min at 1,000  $\times$  g, and read macroscopically.

For the bactericidal assay (4), target *C. jejuni* cells were grown for 24 h on chocolate agar and harvested in PBS; the resulting suspension was standardized to an A<sub>540</sub> of 0.50. This stock suspension was diluted by a factor of 10<sup>-5</sup> immediately before use. Twofold serial dilutions of serum (25  $\mu$ l) were made with PBS in a microtiter plate. Each well then received 25  $\mu$ l of the diluted *C. jejuni* suspension plus 15  $\mu$ l of a 1:20 dilution of guinea pig complement (Colorado Serum Co., Denver, Colo.). The plates were incubated for 45 min at 37°C in plastic bags containing the same gas mixture used for *C. jejuni* culture. A 25- $\mu$ l sample from each well was then inoculated onto blood agar and incubated for 48 h at 42°C in the standard gas mixture. Controls contained PBS in place of diluted serum. Any dilution showing a 50% reduction in the number of colonies compared with the control level was considered positive for bactericidal activity.

## RESULTS

Ten adult ferrets inoculated orally with  $4 \times 10^8$  to  $2 \times 10^{10}$  CFU of either of two strains of *C. jejuni* became intestinally colonized for 2 to 8 days (Table 1). Clinical signs ranged from very mild diarrhea (loose stools for a day or less) to moderate diarrhea, often accompanied by copious green mucus in the stool. In every case, the disease resolved in 1 to 3 days. Agglutinating antibodies were detected at titers of

8 to 256 in the serum of every infected animal 10 to 23 days after inoculation (Table 1). No animal had a detectable level of anti-*C. jejuni* agglutinins before inoculation. All uninoculated, age-matched controls failed to develop detectable *Campylobacter*-specific agglutinins throughout the study (data not shown).

Five adult ferrets inoculated intravenously with  $3 \times 10^9$  to  $2 \times 10^{10}$  CFU of *C. jejuni* became intestinally colonized for 5 to 12 days (Table 1). Two developed severe watery diarrhea for 1 or 2 days, and all had at least mild diarrhea which resolved within a week. Anti-*C. jejuni* agglutinin levels in serum, undetectable in all cases before inoculation, rose to titers of 256 to 2,048 within 25 days after inoculation (Table 1).

To examine the possible role of specific immunity in the containment of either infection or diarrhea in *C. jejuni*-infected ferrets, seven additional adults were actively immunized by one of several protocols, all involving prior infection, and then challenged orally. These animals generated agglutinin titers of 128 to 4,096 and bactericidal antibody titers of 16 to 256 in a pattern showing no correlation between the two antibody activities and no clear relationship of either type of antibody to the length or frequency of antigenic exposure (Table 2). Despite the presence of these antibodies, all seven ferrets were readily colonized for 2 to 17 days when subsequently challenged orally with the homologous strain. Four of these animals, however, developed no accompanying signs of enteric disease, and the other three developed only mild diarrhea lasting 1 day. There was no evident relationship between the level of either type of antibody activity and the length of colonization or absence of disease (Table 2).

Oral infection of 20 kits 3 to 6 weeks of age resulted in the same general pattern of colonization and disease seen in adults (data not shown). Colonization lasted 5 to 10 days, with diarrhea and mucoid stools for 1 to 6 days. Although the duration of disease was similar to that of adults, diarrhea was more severe in kits, with more fluid stools, often accompanied by anorexia for a day or two. Of nine kits assessed for agglutinins in serum 10 or 18 days after infection, seven had a titer of 16 and two had a titer of 32.

A concerted effort was made to induce functional immunity against both infection and disease by administering a series of five inoculations (the first four orally, the last rectally) of strain 28209 to a group of 6-week-old kits. The first low-level inoculum (containing only 10<sup>3</sup> CFU) colonized all kits but produced no discernible diarrhea (Table 3). Colonization persisted until a second oral inoculation was made 5 days later. The second inoculation resulted in continued colonization lasting 2 to 7 days and diarrhea lasting 2 to 6 days. The third oral inoculation, 1 week later, resulted in colonization of all kits but no diarrhea. No

TABLE 2. Colonization and disease of adult ferrets previously infected with *C. jejuni* D80 upon subsequent oral challenge<sup>a</sup>

Ferret no.	Previous exposure			Titer at inoculation		Duration (days) of:	
	Nature	Frequency	Total time (days) <sup>b</sup>	Agglutinin	Bacteriocidin	Colonization	Clinical disease
201	Oral infection, recovery	Once	32	256	32	2	None
202	Oral infection, recovery	Once	32	128	32	2	None
2	Intravenous infection, recovery	Once	274	512	16	9	Mild diarrhea (1)
9	Intravenous infection, recovery	Twice	291	4,096	16	17	Mild diarrhea (1)
14	Intravenous infection, recovery	Twice	236	256	256	3	None
18	Vaccination four times, intravenous infection, recovery <sup>c</sup>	Once	273	2,048	128	7	Mild diarrhea (1)
17	Vaccination four times, intravenous infection, recovery <sup>c</sup>	Once	310	2,048	128	7	None

<sup>a</sup> Oral challenge was with  $2 \times 10^9$  to  $6 \times 10^9$  CFU of strain D80 in two doses, 12 h apart.

<sup>b</sup> Total time elapsed since first contact with *C. jejuni* (immunological response time).

<sup>c</sup> Vaccination was with four doses of  $10^9$  CFU equivalents, formalinized, administered once intravenously and three times intramuscularly.

diarrhea resulted from a fourth (oral) or fifth (rectal) inoculation. The fourth inoculation with strain 28209 produced colonization in four of five kits for 1 day only. By the fifth inoculation, all five kits had become refractory to recolonization. The efficacy of the rectal route of inoculation was verified by (i) colonization and diarrhea in two kits of this group that were subsequently challenged rectally with a serologically distinct strain of *C. jejuni* (M113; Table 3) and (ii) uniform infection of an age-matched group of six kits by rectal inoculation with  $10^8$  CFU of strain 28209 (data not shown). Production of agglutinins appeared to be strain specific, because after two infections with strain 28209, all kits had anti-28209 agglutinin titers of 16 or 32 in serum (Table 3) and 4 or 8 in colon washings (data not shown), but none had detectable anti-M113 antibodies.

To assess the role of normal gut microflora in colonization and disease production, we inoculated the only two available gnotobiotic ferrets with two strains of *C. jejuni* on three occasions and compared the resulting infections with those of age-matched, conventionally reared controls. The only discernible difference between the gnotobiotics and conventional controls (no. 3 and 4) during primary infection with strain D80 was a somewhat longer period of diarrhea in the gnotobiotics (4 to 5 days versus 1 to 2 days) (Table 4). Upon reinoculation with the same strain 33 days later, both groups displayed similarly reduced colonization times, compared with either their own primary infections or those seen in a

new group of age-matched controls (no. 5 to 7). Protection against diarrhea appeared complete in the original controls (no. 3 and 4) but only partial in the gnotobiotics, their disease period having been reduced to that of the new controls rather than being longer than control times, as in the primary infection. The last inoculation, 9 days after the second, was made with a serologically distinct strain (28209). At this time, both gnotobiotics and controls 3 and 4 had demonstrable agglutinin titers against strain D80 in serum but none against 28209. Inoculation with the new strain resulted in colonization and diarrhea of comparable durations in gnotobiotics and controls (no. 3 and 4). Infections in both groups were similar to their own primary infections and to those of a final age-matched control group (no. 8 and 9). The agglutinin responses of the gnotobiotics to both strains were similar to those of the appropriate controls in all cases (Table 4).

The role of humoral antibodies in resistance to campylobacterial infection and disease was examined in two groups of 5- to 6-week-old ferrets born to dams having high levels of agglutinins against strain D80 in both serum and whey. These kits had passively acquired serum titers of 1,024 (group I) and 2,048 (group II) (Table 5). Group I remained with the dam for the first week after inoculation and therefore continued to nurse milk with a whey titer of 1,024. Group II was weaned on the night before being infected and were fed pasteurized cow's milk. Three other groups (III to

TABLE 3. Acquisition of resistance to *C. jejuni*-induced diarrhea in ferret kits<sup>a</sup> upon repeated inoculation<sup>b</sup>

Inoculation no.	Inoculation time (day of expt)	Approximate dose (CFU)	Strain	Route	No. colonized/total (duration [days])	No. with diarrhea/total (duration [days])	Agglutinin titer(s) in serum
1	0	$10^3$	28209	Oral	4/4 <sup>c</sup> ( $\geq 5$ )	0/4 (NA) <sup>d</sup>	<4
2	5	$10^{10}$	28209	Oral	5/5 <sup>c</sup> (2-7)	5/5 (2-6)	ND <sup>e</sup>
3	12	$10^{10}$	28209	Oral	5/5 (1-3)	0/5 (NA)	<4-32
4	19	$10^9$	28209	Oral	4/5 (1)	0/5 (NA)	16-32
5	24	$10^9$	28209	Rectal	0/5 (NA)	0/5 (NA)	16-32
6A	29	$10^9$	M113 <sup>f</sup>	Oral	2/2 (4, $\geq 5$ ) <sup>g</sup>	2/2 (1)	
6B	29	$10^9$	M113	Rectal	2/2 ( $\geq 5$ )	2/2 (1)	
6C	29	$10^9$	M113	Oral and rectal	1/1 ( $\geq 5$ )	1/1 (1)	

<sup>a</sup> Kits were 6 weeks of age when first inoculated.

<sup>b</sup> Three sham-inoculated kits remained uncolonized, diarrhea-free and devoid of detectable anti-*C. jejuni* antibodies throughout the study.

<sup>c</sup> One kit was added to the test group after the first inoculation.

<sup>d</sup> NA, Not applicable.

<sup>e</sup> ND, Not done.

<sup>f</sup> Strain M113, isolated from a mink, is serologically distinct from strain 28209.

<sup>g</sup> All kits were sacrificed 5 days after inoculation.

TABLE 4. Colonization, disease, and immune responses of gnotobiotic and conventionally reared adult ferrets orally inoculated with *C. jejuni*

Ferret no.	Inoculation 1, strain D80, day 0 <sup>a</sup>			Inoculation 2, strain D80, day 33 <sup>b</sup>				Inoculation 3, strain 28209, day 42 <sup>c</sup>			
	Time of colonization (days)	Time of diarrhea (days)	Anti-D80 titer in serum (10 days p.i.) <sup>d</sup>	Time of colonization (days)	Time of diarrhea (days)	Anti-D80 titer in serum (12 days p.i.) <sup>d</sup>	Anti-28209 titer in serum (12 days p.i.) <sup>d</sup>	Time of colonization (days)	Time of diarrhea (days)	Anti-D80 titer in serum (17 days p.i.) <sup>d</sup>	Anti-28209 titer in serum (17 days p.i.) <sup>d</sup>
<b>Gnotobiotics<sup>e</sup></b>											
1	21	5	64	3	1	512	<4	11	2	128	32
2	8	4	128	3	1	128	<4	6	2	64	32
<b>Age-matched conventional controls</b>											
3	8	2	32	2	0	256	<4	3	2	128	8
4	8	1	64	2	0	512	<4	6	2	32	8
5	NI <sup>f</sup>	NI	NI	6	1	256	<4	Sac <sup>g</sup>	Sac	Sac	Sac
6	NI	NI	NI	6	1	256	<4	Sac	Sac	Sac	Sac
7	NI	NI	NI	6	1	64	<4	Sac	Sac	Sac	Sac
8	NI	NI	NI	NI	NI	NI	NI	8	2	<4	32
9	NI	NI	NI	NI	NI	NI	NI	3	2	<4	8

<sup>a</sup> Inoculation 1 was given in two oral doses of about  $6 \times 10^9$  CFU of *C. jejuni* D80.

<sup>b</sup> Inoculation 2 was given in two oral doses of about  $10^{10}$  CFU of *C. jejuni* D80.

<sup>c</sup> Inoculation 3 was given in two oral doses of about  $4 \times 10^8$  CFU of *C. jejuni* 28209.

<sup>d</sup> Titers of agglutinating antibodies in serum are shown. p.i., Days postinoculation, referring to current inoculation.

<sup>e</sup> Gnotobiotic ferrets were colonized with about  $10^4$  CFU of *B. cereus* per g of feces.

<sup>f</sup> NI, Not inoculated.

<sup>g</sup> Sac, Sacrificed.

V) lacking anti-*C. jejuni* antibodies served as controls. All kits in this experiment were culture negative for *C. jejuni* before inoculation.

Upon oral challenge with strain D80, all test and control kits became colonized (Table 5). All control kits developed diarrhea, which lasted for 1 to 6 days. In contrast, none of the kits with antibody titers in serum before inoculation showed any sign of diarrhea (Table 5) but two kits in group II showed prolonged colonization (24 and  $\geq 38$  days; the latter was terminated with chloramphenicol).

Although several immunization protocols involving infection and recovery led to protection against redevelopment of enteric disease upon subsequent oral challenge (Table 2), some attempts to immunize ferrets by repeated intravenous exposure produced fatally untoward reactions. Thus, four adults with high agglutinin titers ( $\geq 2,048$ ) died 6 to 8 h after intravenous injection of homologous *C. jejuni* cells. Two received approximately  $10^9$  CFU of living organisms, while the other two received a comparable number of formalinized cells. Necropsy revealed gross splenomegaly, peritoneal fibrin tags, and accumulation of peritoneal fluid. Histologic

examination revealed extensive congestion of the liver and spleen and liver necrosis; there was no evidence of renal cortical necrosis. In the two animals assessed, agglutinin titers in serum dropped four- and eightfold, respectively, during the period between injection and death. Among 55 ferrets of all ages which did not have homologous antibodies when injected with comparable numbers of living or killed *C. jejuni* cells, only one death occurred.

## DISCUSSION

The normal course of events for ferrets inoculated with *C. jejuni* was shown to be self-limitation of both colonization and enteric disease, accompanied by substantial strain-specific antibody responses against the organism. This combination of events suggests that specific immunity is involved in the acquisition of resistance to campylobacteriosis. Ferrets that recovered from infection with *C. jejuni* were shown to produce substantial levels of strain-specific agglutinating and bactericidal antibodies, both of which might reasonably be expected to participate in limitation of

TABLE 5. Colonization and diarrhea of ferret kits with passively acquired specific antibodies and subsequent oral infection with *C. jejuni* D80

Group (no. of ferrets)	Age <sup>a</sup> (wks)	Agglutinin titer <sup>b</sup>	Inoculum size (CFU) <sup>c</sup>	No. colonized/total (duration [days])	No. with diarrhea/total (duration [days])
I (5)	5	1,024	$6 \times 10^9$	5/5 ( $\geq 5-6$ ) <sup>d</sup>	0/5 (NA) <sup>e</sup>
II (4)	6	2,048	$10^{10}$	4/4 (10-38) <sup>f</sup>	0/4 (NA)
III (1)	5	<4	$10^9$	1/1 (10)	1/1 (6)
IV (3)	6	<4	$10^9$	3/3 (4-8)	3/3 (2-3)
V (4)	4	<4	$10^7$	4/4 ( $\geq 9$ )	4/4 (1-2)

<sup>a</sup> Age at time of inoculation.

<sup>b</sup> Agglutinin titer in serum on the day before inoculation.

<sup>c</sup> The inoculum was given in two doses, 8 to 12 h apart.

<sup>d</sup>  $\geq$ , Animal sacrificed on the day listed.

<sup>e</sup> NA, Not applicable.

<sup>f</sup> Colonization was terminated at 38 days with chloramphenicol.

future infections. Acquired immunity against recolonization with this organism has been reported for rabbits, with immunoglobulin A antibodies considered the best indicator of protection (2), as well as for experimental human infection (1). Any role of cell-mediated immunity in resistance to *C. jejuni* remains unknown.

In ferrets, the extent of immunizing exposure needed to limit recolonization proved to be considerably greater than that required to generate protection against disease. Thus, resistance to colonization developed gradually, with progressively shorter periods of colonization after each of the first three or four inoculations, whereas protection against enteric disease was generally complete, or nearly so, after a single exposure. The strain specificity of resistance to both colonization and disease clearly supports participation of specific immunity.

Further evidence that campylobacteriosis is contained immunologically was provided by study of the gnotobiotics. It might be argued that *C. jejuni* is imperfectly adapted to ferrets, so that resistance against it could be due at least in part to competitive overgrowth by normal gut microflora. Such competitive elimination by a complex normal flora has been demonstrated in gnotobiotic (*C. jejuni*-monoassociated) mice (12). Inasmuch as only two gnotobiotics were available, any conclusion based upon their study must be guarded. However, the facts that both animals, which were virtually devoid of competing microflora, cleared an initial *C. jejuni* infection within the control time frame, cleared a second infection with the same strain in accelerated fashion, yet showed no resistance to subsequent infection with a serologically distinct strain strongly suggest participation of specific immune clearance rather than displacement by normal microfloral organisms alone. The apparent lack of relationship between levels of agglutinating or bactericidal antibodies and resistance to colonization observed for all ferrets suggests that some other form(s) of immunity is involved.

Although we found no solid evidence for humoral immune protection against colonization with *C. jejuni*, a better case can be made for humoral protection against enteric disease. The finding that previously uninfected kits with passively acquired maternal anti-*C. jejuni* antibodies were all protected against diarrhea supports a protective role for antibody. Moreover, it does not appear that the protective antibodies need to be exogenously resupplied throughout the period of infection, because kits withdrawn from immune mother's milk before inoculation but having high levels of antibodies of maternal origin in serum were as fully protected as those that continued to be nursed by an immune dam. Our observation (unpublished) that the oral-anal transit time for ferrets at this age is only about 1.5 h does not support a major role for residual antibody which might remain in the lumen after immune milk withdrawal.

None of the antibodies which may be of consequence to the course of campylobacteriosis have been identified. There is no reason to believe, for example, that the antibodies that provide protection against diarrhea are those we assessed (agglutinins and bactericidins). Indeed, in view of the rather limited effects of immunity on colonization with *C. jejuni* observed, it seems likely that protection is related more to antibodies directed against some toxic product or cytoadhesin of this pathogen than to those responsible for destruction of the agent itself. Similarly, the gross pathologic pattern seen in ferrets with high levels of anti-*C. jejuni*

antibodies that died after injection with living or killed homologous cells, together with the accompanying drop in demonstrable free antibody levels in serum and the absence of similar reactions to comparable injections in nonimmune animals, suggest some sort of immunopathologic reaction. A number of less striking immunopathologic consequences of *C. jejuni* infection, including reactive arthritis, Reiter's syndrome, and hemorrhagic nephritis, have been reported in humans (11). The observations presented here also do not eliminate the possible effects of maternal immune cells which might have been transferred to the kits. It seems likely, however, that such cells would function more to contain colonization than disease, and passively immune kits were readily colonized. Despite a lack of details, the overall role of the immune system in containing campylobacteriosis in ferrets thus seems to be twofold. Some unidentified aspect of immunity provides at least partial resistance to infection, manifested mainly by shortened colonization times, and antibodies, which do not appear to prevent colonization, do generate protection against diarrheal illness.

#### ACKNOWLEDGMENT

This study was supported by grant 135-4462 from the University of Wisconsin Graduate School.

#### LITERATURE CITED

- Black, R. E., M. M. Levine, M. L. Clements, T. P. Hughes, and M. J. Blazer. 1988. Experimental *Campylobacter jejuni* infection in humans. *J. Infect. Dis.* 157:472-479.
- Burr, D. H., M. B. Caldwell, A. L. Bourgeois, H. R. Morgan, R. Wistar, Jr., and R. I. Walker. 1988. Mucosal and systemic immunity to *Campylobacter jejuni* in rabbits after gastric inoculation. *Infect. Immun.* 56:99-105.
- Caldwell, M. B., R. I. Walker, S. D. Stewart, and J. E. Rogers. 1983. Simple adult rabbit model for *Campylobacter jejuni* enteritis. *Infect. Immun.* 42:1176-1182.
- Fox, J. G., J. I. Ackerman, N. Taylor, M. Claps, and J. C. Murphy. 1987. *Campylobacter jejuni* infection in the ferret: an animal model of human campylobacteriosis. *Am. J. Vet. Res.* 48:85-90.
- Gilbert, G. L., R. A. Davoren, H. E. Cole, and N. J. Radford. 1981. Midtrimester abortion associated with septicemia caused by *Campylobacter jejuni*. *Med. J. Aust.* 1:585-586.
- Humphrey, C. D., D. M. Montag, and F. E. Pittman. 1985. Experimental infection of hamsters with *Campylobacter jejuni*. *J. Infect. Dis.* 151:488-493.
- Hunter, D. B., J. F. Prescott, J. R. Pettit, and W. E. Snow. 1983. *Campylobacter jejuni* as a cause of abortion in mink. *Can. Vet. J.* 24:398-399.
- Manning, D. D., and J. A. Bell. 1990. Derivation of gnotobiotic ferrets: perinatal diet and hand-rearing requirements. *Lab. Anim. Sci.* 40:51-55.
- Prescott, J. F., and M. A. Karmali. 1978. Attempts to transmit *Campylobacter* enteritis to dogs and cats. *Can. Med. J.* 119:1001-1002.
- Selkon, J. B. 1988. *Campylobacter*, p. 57-69. In D. P. Jewell and R. W. Chapman (ed.), *Topics in gastroenterology*. Blackwell Scientific Publications, Ltd., Oxford.
- Walker, R. I., M. B. Caldwell, E. C. Lee, P. Guerry, T. J. Trust, and G. M. Ruiz-Palacios. 1986. Pathophysiology of *Campylobacter* enteritis. *Microbiol. Rev.* 50:81-94.
- Yrios, J. W., and E. Balish. 1986. Colonization and infection of athymic and euthymic germfree mice by *Campylobacter jejuni* and *Campylobacter fetus* subsp. *fetus*. *Infect. Immun.* 53:378-383.