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Experimental Infection of Pig-Tailed Macaques (*Macaca nemestrina*) with *Campylobacter cinaedi* and *Campylobacter fennelliae*

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***Campylobacter cinaedi* and *C. fennelliae* have been associated with proctocolitis, bacteremia, and asymptomatic rectal infection, primarily in homosexual men. To more directly assess the pathogenic role of these organisms, we studied their disease-producing potential in 12- to 25-day-old pig-tailed macaques (*Macaca nemestrina*). Four infant monkeys were challenged with 10^8 to 10^9 *C. cinaedi*, three were challenged with *C. fennelliae*, two were challenged with *C. jejuni*, and one received no microorganisms. Watery or loose stools without associated fever or fecal leukocytes developed 3 to 7 days postinoculation in all of the animals given *C. cinaedi*, *C. fennelliae*, and *C. jejuni*, but not in the control animal. Stool cultures were simultaneously positive and remained so in the animals challenged with *C. cinaedi* or *C. fennelliae* for 3 weeks after inoculation despite the resolution of clinical illness. All of the animals challenged with *C. cinaedi* and *C. fennelliae* became bacteremic, and three had clinical evidence of septicemia. Histopathologic evaluation of rectal biopsies (five animals) and necropsy (one animal) showed no evidence of mucosal disruption. Specific immunoglobulin M and immunoglobulin G antibody responses occurred in all of the animals challenged with *C. cinaedi* and *C. fennelliae*, as determined by enzyme-linked immunosorbent assay and immunoblotting. We conclude that *C. cinaedi* and *C. fennelliae* consistently produce a diarrheal illness accompanied by bacteremia and followed by prolonged gastrointestinal colonization in *M. nemestrina*.**

Campylobacter cinaedi and *C. fennelliae* are fastidious species of the genus *Campylobacter* that have been isolated primarily from homosexual men. Recently, *C. cinaedi* has also been isolated from the feces and blood of children and women, suggesting that infection with this species may be more widespread than previously thought (22, 25). *C. cinaedi* and *C. fennelliae* have been associated with proctocolitis, with bacteremic illness in immunocompromised patients, and with asymptomatic rectal infection (4, 14–16). The pathogenic role of these organisms has been supported by their association with symptoms and signs of infection in homosexual men (16), by their association with increased leukocytes on rectal gram stains of culture-positive men (23), by an associated immune response (8), and by their isolation from blood cultures of febrile immunocompromised patients (4, 14, 15). Nevertheless, a more direct assessment of the pathogenic role of these organisms in an animal model would help to clarify their disease-producing potential in humans. For this reason, we have experimentally infected the pig-tailed macaque, *Macaca nemestrina*, with these organisms. We selected this model because of the lack of reliable small animal models for studying infection with *C. jejuni* (6, 10, 20) and because these animals develop natural infection with *C. jejuni*, a closely related pathogen (18, 19).

MATERIALS AND METHODS

Bacterial strains. The three *C. cinaedi* and three *C. fennelliae* strains used in this study were isolated from rectal swabs or blood cultures obtained from homosexual men, and the two *C. jejuni* strains were isolated from human infants with diarrhea (Table 1). All campylobacter strains were isolated on a selective medium made from a brucella agar base (Difco Laboratories, Detroit, Mich.) containing vancomycin (10 mg/liter), polymyxin B (2,500 U/liter), trimethoprim (5 mg/liter), and amphotericin B (2 mg/liter) supplemented with 10% defibrinated sheep blood (5). After inoculation, specimens were incubated at 37°C for 48 h in a microaerophilic environment provided by an anaerobe jar containing a GasPak envelope (BBL Microbiology Systems, Cockeysville, Md.) without the catalyst (5). Isolates were preserved in Trypticase soy broth (TSB; BBL) and 50% inactivated horse serum and stored at −70°C.

Source and care of *M. nemestrina*. We used 10 infant pig-tailed macaques (6 females and 4 males) ranging in age from 12 to 25 days and in weight from 400 to 900 g. Pregnant dams were treated orally with erythromycin (50 mg/kg twice a day) for 10 days prior to delivery to eliminate possible perinatal campylobacter infection. Immediately after natural delivery, the infant was removed from the dam and placed in an isolette housed in the nursery facility of the Infant Primate Research Laboratory at the Regional Primate Research Center of the University of Washington. All animals were housed in individual cages suspended over a stainless steel drop pan filled with a waste-absorbent material (Pel-e-Cel). The cages were placed with sufficient distance apart so that animals had no direct contact with each other. The infants were fed SMA-R (Wyeth Laboratory, Philadelphia, Pa.) human infant formula with a mild protein base from birth through 4 months of age. Weaned infants and dams were fed Purina monkey chow (Ralston Purina Co., Richmond, Ind.) and had access to water ad libitum.

Oral challenge. For experimental infection, organisms were grown on brucella agar supplemented with 10% defibrinated sheep blood for 48 h as described above. The bacteria were harvested in 2 ml of TSB, and the suspensions

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TABLE 1. *Campylobacter* strains and infecting doses

Campylobacter sp. and animal no.	Strain	Source	Age (days)	Dose (CFU)
C. cinaedi				
2	CC930	Homosexual man, blood	19	1.2×10^9
5	CC1785	Homosexual man, rectum	21	2.2×10^8
7	CC1785	Animal 5, blood	12	4.0×10^9
9	ATCC 35683	Homosexual man, rectum	14	4.2×10^9
C. fennelliae				
1	CF897	Homosexual man, rectum	12	1.5×10^8
3	CF74	Homosexual man, rectum	12	3.8×10^9
8	ATCC 35684	Homosexual man, rectum	22	1.1×10^9
C. jejuni				
4	B16	Human infant, feces	25	1.8×10^9
6	B258	Human infant, feces	21	9.8×10^9
None				
10			15	0

were adjusted to yield 10^8 to 10^9 viable organisms per ml. The control animal was challenged with 2 ml of TSB with no microorganisms. Animals were entered in the study on an availability basis. Usually one animal was challenged, but never were more than two animals challenged and studied during a given period. When two animals were studied concurrently, different species of campylobacter were given to monitor the potential cross-contamination of animals.

Prior to challenge, the infant was given 1 ml of sodium bicarbonate via a number 5 French feeding tube introduced through the mouth into the stomach to neutralize the gastric pH. Fifteen minutes after administration of the bicarbonate, the infant monkey was inoculated intragastrically with 2 ml of TSB containing the challenge organism or sterile TSB.

Baseline determinations for all animals were made shortly after birth and on the day of inoculation. Blood (1 to 2 ml) was obtained from each infant for a complete blood count, and serum for serological tests was collected and stored at −70°C until use. Rectal swabs of both the infant and dam were obtained to screen for *Cryptosporidium* sp., *Campylobacter* sp., and other enteric gram-negative pathogens. Only those monkeys which were healthy, were afebrile, had normal stools, and had no previous infection or colonization with *Campylobacter* sp. were used in the study.

Clinical evaluation of challenged monkeys. Daily monitoring of feeding patterns, rectal temperature, stool consistency, and behavioral changes was initiated 3 days prior to challenge and continued for 21 days after challenge. For the duration of the study, one veterinary technician cared for and provided consistent and unbiased evaluations of the animals. Stool consistencies were categorized as normal (for specimens which had a toothpastelike consistency), soft, or watery. At daily intervals for the first week and subsequently every other day for 3 weeks, fresh stool specimens were collected for culturing and were examined for fecal leukocytes and erythrocytes. Complete blood counts were performed at 3 days and at 14 or 21 days postchallenge.

Laboratory methods. For improved isolation of *Campylobacter* sp., we modified the selective agar medium described above by the substitution of Mueller-Hinton agar base (Difco) and by the addition of cefoperazone (10 mg/liter) at a concentration which was inhibitory to most enteric gram-negative rods but not inhibitory to *C. cinaedi*, *C. fennelliae*, and *C. jejuni* (1, 7, 9). Each fresh fecal specimen was weighed and suspended in 2 ml of TSB. The suspension was inoculated directly on both types of selective agar media for isolation of *Campylobacter* sp. In addition, a membrane filter technique was used (21). Briefly, the suspension was applied to a 0.45-μm (pore size) membrane filter (Millipore Corp., Bedford, Mass.) which was placed on the surface of a nonselective blood agar medium. The suspension was allowed to dry, the filter was discarded, and the agar medium was incubated for 7 days as previously described. The identity of spiral or wavy, slender gram-negative organisms which were oxidase positive was confirmed by biochemical tests and susceptibilities to nalidixic acid and cephalothin disks (5) and by Coomassie blue-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels (8).

Culturing for other enteric pathogens was performed biweekly for 2 weeks postchallenge. Stool specimens were inoculated directly onto selective agar media for isolation of *Salmonella* sp., *Shigella* sp., and *Yersinia* sp. (11). An acid-fast stain for detection of *Cryptosporidium* sp. was performed on watery specimens (3).

Stool specimens were also examined for fecal leukocytes by Gram stain and wet mount. Occult blood was detected by using a Guaiac test. The presence of organisms with "corkscrew" motility was detected by dark-field microscopy.

Rectal and tissue biopsies were cultured and examined in the same manner as stool specimens except that each biopsy specimen was weighed, macerated with a sterile surgical blade, and homogenized in 1 ml of TSB in a tissue homogenizer. The homogenate was inoculated directly on selective media for isolation of *Campylobacter* sp. Dilution samples of the homogenate were also plated for quantitation of growth. Blood samples (2 to 4 ml) for culture were collected in VACUTAINER tubes containing sodium polyanethol-sulfonate (Becton Dickinson and Co., Rutherford, N.J.), inoculated into radiometric aerobic blood culture medium (Bactec 6B; Johnston Laboratories, Cockeysville, Md.), and incubated at 37°C for 7 days. Positive cultures were indicated by a positive growth index (>30) and subsequent subculture of the infecting campylobacter strain.

Histologic studies. Rectal biopsies were obtained on the third day after challenge. The infant was sedated intramuscularly with a mixture of ketamine hydrochloride and xylazine at a dose of 0.1 ml/kg of body weight. After the infant was fully sedated, the rectal area was cleaned with a Betadine Surgical Scrub. A small biopsy forceps was intro-

duced through the anus, and two pieces of rectal tissue (5 to 8 mm^2) were excised. Following the biopsy, there was minimal bleeding and the infant showed no discomfort upon awakening from the procedure.

Tissue samples were placed in a sterile container for culturing and in the appropriate fixative for staining: Hollandes fixative for rectal biopsy specimens and 70% ethanol for necropsy specimens. The samples were processed and embedded in paraffin blocks, and serial sections were cut. Necropsy tissues were stained with hematoxylin and eosin, whereas rectal biopsies were stained with alcian blue, hematoxylin, safranin, and eosin.

Serology. We used an enzyme-linked immunosorbent assay (ELISA) to measure serum immunoglobulin M (IgM) and IgG titers. Sera from animals inoculated with *C. cinaedi* strains were tested against antigen prepared from *C. cinaedi* ATCC 35683, whereas sera from animals inoculated with *C. fennelliae* or *C. jejuni* were tested against homologous antigen. Serum samples from the control animal were tested against a representative antigen from each species. Aliquots (0.2 ml) of a 5-μg/ml sonicated suspension of bacterial cells (1 μg of antigen) in 0.5 M carbonate buffer (pH 9.6) were adsorbed to wells of flat-bottom microtiter plates (Immulon 2; Dynatech Laboratories, Inc., Chantilly, Va.) overnight at 4°C. Excess antigen was aspirated from the wells and replaced with 0.3-ml aliquots of carbonate buffer containing 2 mg of gelatin per ml. After 2 h of incubation at 35°C, the wells were aspirated and then washed three times with 0.01 M phosphate-buffered saline containing 0.05% Tween 80 (PBST). Twofold serial dilutions of sera were prepared in PBST containing 2 mg of gelatin and 15 mg of bovine gamma globulin per ml. A total of 0.2 ml of each dilution was added to the wells, incubated for 1 h at 35°C, and washed three times. Bound antibody was detected by peroxidase-conjugated goat anti-human IgG (1:4,000; Tago, Inc., Burlingame, Calif.) and goat anti-human IgM (1:1,000; Tago). The substrate consisted of 1 mg of 2,2′-azinobis(3-ethylbenzthiazoline sulfonic acid) per ml of McIlvain buffer (pH 4.6) and 0.005% H_2O_2. The optical density at 412 nm was measured on a Titertek Multiscan (Dynatech) immediately following 30 min of incubation at room temperature in the presence of substrate. Titers were expressed as the highest reciprocal serum dilution for which the optical density was greater than twice the optical density of the control well containing no serum.

SDS-PAGE and immunoblotting. Whole cell extracts were prepared from 48-h cultures solubilized in 2× SDS-PAGE sample buffer and heated at 100°C for 3 min. Twenty-five micrograms of the extracts was separated by SDS-PAGE (12.5% acrylamide) in a discontinuous buffer system (12). Proteins were stained with Coomassie blue or were transferred to 0.45-μm (pore size) nitrocellulose paper (Millipore) by the method described by Towbin et al. (24) at 10 V for 18 h at 4°C in a Transphor unit (Hoefer, San Francisco, Calif.). Because *C. cinaedi* and *C. fennelliae* have distinct protein profiles (8), Coomassie blue-stained gels were used to confirm the identity of the campylobacter species isolated from the animals. Immunoblotting was performed as previously described (8). The nitrocellulose paper was blocked in a buffer containing 0.15 M NaCl, 5 mM Na_4–EDTA, 10 mM TES [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid], 0.25% gelatin, and 0.1% Tween 20 (pH 7.3) at 37°C for 1 h, followed by incubation with either rabbit antisera (1:800) or monkey sera (1:50) for 1 h at room temperature. The nitrocellulose strips were washed in phosphate-buffered saline, pH 7.2, containing 0.1% Tween 20 and then incubated with ^{125}I-labeled staphylococcal protein A (Dupont, NEN Research Products, Boston, Mass.) for 1 h at room temperature. The blots were washed, dried, and exposed to Cronex MRF 32 film (Dupont) with intensifying screens for 18 h at −70°C. To identify specific immunoblot profiles, rabbit antisera to *C. cinaedi*, *C. fennelliae*, and *C. jejuni* were tested in parallel with monkey sera. Serum from the uninfected monkey was used as a negative control.

RESULTS

Clinical and microbiological evaluation of challenged *M. nemestrina*. We experimently infected nine infant *M. nemestrina* monkeys: four were challenged with *C. cinaedi* strains, three were challenged with *C. fennelliae* strains, and two were given *C. jejuni* strains (Table 1). One control monkey received sterile TSB.

Two of the four monkeys challenged with *C. cinaedi* (animals 2 and 5) developed stools which were clearly abnormal; animal 5 had watery stools, and animal 2 had soft stools with mucus over days 4 to 8 postchallenge (Table 2). *C. cinaedi* was recovered from the stools of both monkeys, but fecal leukocytes were not detected at any time. In the stool of animal 5, occult blood was detected 9 days postchallenge. Both animals had a drop in rectal temperature, were lethargic, and had positive blood cultures for *C. cinaedi*. In addition, animal 5 experienced labored breathing during an episode of low body temperature (Table 3).

Two of the three monkeys challenged with *C. fennelliae* (animals 1 and 8) developed watery stools at day 3 and over days 7 to 11 postchallenge. Animal 3 developed soft stools at day 6 when 2+ colonization was observed, but it never developed high concentrations of organisms and watery stools or mucus. All of the monkeys given *C. fennelliae* had stools that were negative for leukocytes and for occult blood, and only one of the monkeys (animal 8) manifested any systemic signs of infection (labored breathing on day 2 and lethargy on days 3 and 4 postinoculation) (Table 3).

Both of the monkeys given *C. jejuni* (animals 4 and 6) developed watery stools over days 2 and 3 and days 2 to 9, respectively. Neither had fecal leukocytes, but both had stools positive for occult blood 3 and 4 days postchallenge (Table 2). One of the monkeys (animal 6) developed lethargy on days 7 to 9, but no other systemic signs of illness were observed in either monkey.

In eight of the nine monkeys, stool cultures were positive by the first to third day postchallenge, and presence of the infecting *Campylobacter* sp. in the stool usually coincided with a change in stool consistency (Table 2). Watery stools consistently yielded 4+ quantities of the infecting strain, while the recovery rate of organisms from soft stools ranged from 1+ to 4+. In monkey 7, the challenge organism was not detected until the seventh day following inoculation. The consistency of stools in this animal remained normal, and the yield of the infecting organism recovered was lower (2+ to 3+) throughout the screening period.

Prolonged rectal colonization (≥3 weeks) with the challenge microorganism was demonstrated in all three monkeys given *C. fennelliae*, in both monkeys given *C. jejuni*, and in both monkeys given *C. cinaedi* for which prolonged follow-up was possible. The observation period for the other two monkeys infected with *C. cinaedi* was curtailed because one animal was euthanized on day 4 and one developed an infection with *C. jejuni* on day 11. Qualitatively, prolonged colonization was usually heavy, being characterized by 3+ to 4+ growth of organisms (corresponding to 5×10^6 to 10^9 CFU/g of feces; data not shown) in many instances.

TABLE 2. Description of feces and quantitation of *Campylobacter* spp. isolated from monkeys

Campylobacter sp. and animal no.	Description of feces (infection) on day postchallenge[a]													
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
C. cinaedi															
2	N (−)	N (−)	N (−)	N (3+)	SM (4+)	S	SM (4+)	S	SM (3+)	N	N (4+)	N	N		N (2+)
5[b]	N (−)	S (−)	S (4+)	S (4+)	W (4+)	W	W	S (4+)	N	N (4+)	N	N (4+)	S	S	S
7	N (−)	N (−)	N (−)	N (−)	N (−)	N (−)	N (−)	N (2+)	N	N	N (2+)	N (2+)	N	N	N (3+)
9[c]	N (−)	S (4+)	S (4+)	S (4+)	S (4+)										
C. fennelliae															
1	N (−)	N (−)	N (−)	W (4+)	N	N	N	N (2+)	N (2+)	N (2+)	N	S (1+)	N	N (2+)	N
3	N (−)	S (1+)	N (−)	N (−)	N		S (2+)	N	N	N	N (1+)	N	N	N (1+)	N
8	N (−)	S (1+)	S (2+)	S (3+)	S (4+)			W (4+)	W	S (4+)	S	W	S	S	S (4+)
C. jejuni															
4	N (−)	S (4+)	W (4+)	W (4+)	S (4+)	S (4+)	S (4+)	N (4+)	N	S (4+)	N	N (4+)		N	N (4+)
6	N (−)	N (4+)	W (4+)	S (4+)	S (4+)	S	S	W (4+)	W (4+)	W	S	S (4+)	S	S	S
None	N (−)	N (−)	S (−)	S (−)	S (−)	N (−)	N (−)	N (−)	N	N (−)	N	N (−)	N	N	N (−)

[a] N, Normal; W, watery; S, soft; SM, soft with mucus. *Campylobacter* infection was quantitated as follows: −, not isolated; 1+ to 4+, degree of infecting organism recovered.
[b] Animal 5 developed an infection of *C. jejuni* on day 11.
[c] Animal 9 was euthanized on day 4.

All of the animals had one or more blood cultures positive for the infecting organism (Table 3). Blood cultures were most frequently positive on the 1st, 2nd, or 3rd day postchallenge. Four monkeys had two or more positive blood cultures over a period of several days.

The control monkey remained well throughout the study period, and all cultures of feces and blood were negative for campylobacter species (Tables 2 and 3).

Histopathologic features. Rectal biopsies were obtained in five of the animals and were normal in four animals. One monkey infected with *C. fennelliae* (animal 8) had acute mucosal inflammation, demonstrated in a portion of the biopsy. Cultures of the biopsied tissues were negative in all but one monkey which was challenged with *C. jejuni* (animal 6). The biopsy from this monkey yielded 2+ *C. jejuni*.

One monkey (animal 9) inoculated with *C. cinaedi* was euthanized. Histopathologic evaluation of the small intestinal and colonic mucosa in the monkey were normal, and there was no evidence of either acute mucosal inflammation or of adherent microorganisms. Marked lymphoid hyperplasia was noted in Peyer's patches, however, consistent with immune stimulation. By culture of necropsy material, large numbers of *C. cinaedi* (7.5×10^5 to 3.6×10^6 CFU/g of tissue) were found in the transverse and descending colon, with a smaller number of organisms found in the small intestine ($\leq 6.7 \times 10^2$ CFU/g). Cultures of the spleen, liver, and kidneys were negative.

Serological studies. Table 4 shows the mean IgG and IgM antibody titers as measured by ELISA in infected and control monkeys. No increases in antibody titers were seen in the uninfected control animal. Mean IgM antibody titers rose after infection with *C. cinaedi* and *C. fennelliae*, but an IgM response was not seen in either monkey challenged with *C. jejuni*. Mean IgG titers rose after infection in the monkeys given *C. cinaedi* and *C. fennelliae*, but not in the monkeys given *C. jejuni*.

We used immunoblotting to confirm that the antibody titers demonstrated by ELISA were specific for *C. cinaedi*, *C. fennelliae*, or *C. jejuni* antigens. Immunoblotting results revealed that serum antibodies from prechallenged animals were not specific for the major antigenic proteins of the challenge organism and that postchallenge sera recognized all or most of the major determinants recognized by rabbit polyclonal antiserum (Fig. 1).

DISCUSSION

Although *C. cinaedi* and *C. fennelliae* have both been associated with proctocolitis in homosexual men, their pathogenic role in these patients has been difficult to evaluate since many were simultaneously infected with other potential pathogens (16). Because the isolation of these species from stool or rectal swabs requires use of a selective medium and techniques not commonly employed in clinical laboratories, the reported experience with them is small (5, 13). The recent description of bacteremias caused by *C. cinaedi* and *C. fennelliae* in immunocompromised homosexual men from centers where these species have never been isolated from stool cultures suggests that they are probably underrecognized and that they sometimes produce serious systemic infection (4, 14, 15). The recent isolation of *C. cinaedi* from the blood and feces of children and adult women further supports this hypothesis and indicates that the spectrum of hosts infected is broader than previously appreciated. For these reasons, we undertook studies in an animal model to better evaluate the pathogenic role of these

TABLE 3. Clinical and laboratory evaluation of challenged monkeys

Campylobacter sp. and animal no.	Condition of monkey (day postchallenge)[a]					
	Rectal temp (°C)	Lethargy	Labored breathing	Occult blood	Rectal colonization	Bacteremia
C. cinaedi						
2	35	+ (3)	–	–	+ (9 wk)	+ (8)
5[b]	34.5	+ (2–6)	+ (2–4)	tr (9)	+ (11)	+ (1, 2, 3, 7)
7	N[c]	+ (3–4)	–	–	+ (4.5 wk)	+ (1)
9[d]	N	–	–	–	+ (4)	+ (4)
C. fennelliae						
1	N	–	–	–	+ (3 wk)	+ (2, 3, 5)
3	N	–	–	–	+ (7 wk)	+ (3)
8	N	+ (3–4)	+ (2)	–	+ (9 wk)	+ (3)
C. jejuni						
4	N	–	–	tr (3)	+ (5 wk)	+ (1, 7)
6	N	+ (7–9)	–	+ (4)	+ (4 wk)	+ (1, 2, 7)
None[e]	N	–	–	–	–	–

[a] Numbers in parentheses indicate the day(s) postchallenge, except where indicated in weeks. Fecal leukocyte counts were 0 in all cases.
[b] Animal 5 developed an infection of *C. jejuni* on day 11.
[c] Normal range (N), 36.9 to 38.9°C.
[d] Animal 9 was euthanized on day 4 postinoculation.
[e] Sterile broth.

species. We selected *M. nemestrina* for use in these studies because a satisfactory animal model has not been developed for campylobacter infections (6, 10, 20) and because *M. nemestrina* naturally acquires infections with *C. jejuni*, usually in the newborn period (18, 19).

Our data suggest that both *C. cinaedi* and *C. fennelliae* produced gastrointestinal infection. Watery or loose stools devloped 3 to 6 days after inoculation in the majority of animals challenged with these organisms, and their stool cultures were simultaneously culture positive. Furthermore, the illness observed after challenge with *C. cinaedi* or *C. fennelliae* was clinically indistinquishable from that caused by *C. jejuni*, an established enteric pathogen, whereas the control inoculum produced no signs of illness. The development of IgM and IgG antibodies to specific *C. cinaedi* and *C. fennelliae* antigens is also consistent with acute infection. Increased numbers of fecal leukocytes, a marker of extensive mucosal destruction, was not seen in any of the animals, and evidence of hemorrhage was found in only three animals (one challenged with *C. cinaedi* and two challenged with *C. jejuni*). These findings suggest that neither *C. cinaedi* nor *C. fennelliae* caused extensive mucosal damage in these animals, a finding corroborated by the histopathologic studies performed in the one animal which was sacrificed. Cultured sections of bowel in this animal and the negative rectal biopsy cultures from all of the animals indicated that the distal ileum and the colon were the portions of the bowel that were infected.

Despite the lack of evidence of extensive mucosal damage associated with *C. cinaedi* and *C. fennelliae* infections, both organisms unexpectedly caused bacteremia in all of the challenged monkeys. Furthermore, three of the monkeys developed systemic evidence of bacteremia, including labored breathing, hypothermia, and lethargy. The histopathologic evaluation of the one monkey which was sacrificed showed marked hyperplasia in Peyer's patches, also consistent with transmucosal invasion with *C. cinaedi*. The occurrence of bacteremia in the newborn monkeys, which can probably also be regarded as immunosuppressed, is consistent with the isolation of these species from blood cultures in immunocompromised humans. *C. fetus* subsp. *fetus* has been previously recognized as a cause of bacteremia in immunodeficient hosts, and, more recently, *C. jejuni* bacteremia has been described in patients with acquired immunodeficiency syndrome (2). Thus, organisms of this genus may have a propensity to cause bacteremia in this setting, even in the absence of diarrheal disease.

All of the infant monkeys demonstrated IgG antibody titers to the various campylobacter species ranging from 1:50 to 1:200 before being challenged. Infection with *C. jejuni* is

TABLE 4. Acute and convalescent reciprocal mean antibody titers of challenged monkeys tested by ELISA

Time postinfection[a]	Titer (no. of monkeys)[b]							
	Control (1)		Challenge					
			C. cinaedi		*C. fennelliae*		*C. jejuni*	
	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
Day 0	<12.5	200	6.25 (3)	150 (3)	25 (3)	200 (3)	6.25 (2)	150 (2)
Wk 3–4	12.5	100	50 (3)	400 (3)	100 (3)	600 (3)	12.5 (2)	150 (2)
≥9 wk	12.5	<100	600 (2)	3,200 (2)	400 (1)	1,600 (1)	<25 (1)	200 (1)

[a] Observation and serum collection period for the first four animals studied was 4 weeks.
[b] Control sera were tested against a representative antigen of *C. cinaedi*, *C. fennelliae*, and *C. jejuni*, whereas sera from animals infected with *C. cinaedi* were tested against antigen prepared from *C. cinaedi* ATCC 35683 and sera from animals infected with *C. fennelliae* or *C. jejuni* were tested against homologous infecting antigen.

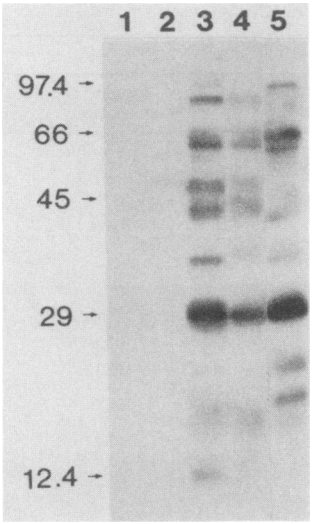

FIG. 1. Immunoblot of sera from a monkey infected with *C. cinaedi* (animal 5), demonstrating specificity of IgG antibodies to *C. cinaedi* antigens. Monkey sera were tested at 1:50 dilution, and rabbit anti-*C. cinaedi* serum was tested at 1:800. Bound antibody was detected with ^{125}I-labeled protein A and exposed to X-ray film for 18 h. Molecular mass standards are expressed in kilodaltons. Lanes: 1, monkey serum collected prechallenge (IgG titer by ELISA, 400); 2, monkey serum collected 21 days postchallenge (IgG titer, 200); 3, monkey serum collected 90 days postchallenge (IgG titer not tested); 4, monkey serum collected 127 days postchallenge (IgG titer, 12,800); 5, rabbit polyclonal anti-*C. cinaedi* serum.

known to be common among *M. nemestrina*, and we presume this represents maternal antibody developed as a result of prior *Campylobacter* sp. infection and passively transferred to the infants. Interestingly, neither of the two infant monkeys challenged with *C. jejuni* developed rises in either IgM or IgG antibody titers to *C. jejuni*, while the development of IgM antibody titers and increases in IgG antibody titers was observed in the animals infected with *C. cinaedi* and *C. fennelliae*. One possible explanation for this phenomenon may be that the passively acquired maternal antibody was directed primarily against *C. jejuni* and was partially protective against *C. jejuni* infection. Of interest in this regard, Russell et al. recently described the experimental infection of four *M. nemestrina* with *C. jejuni* (17). The resulting gastrointestinal illness described in their studies was generally more severe than we observed in that all four of their animals developed acute diarrhea, bloody stools, and fecal leukocytes. We observed acute diarrhea in both of the animals we infected with *C. jejuni*, but neither had fecal leukocytes. Both had Guaiac-positive stools but not frankly bloody stools. The animals we infected were considerably younger than those infected by Russell et al. (12 to 25 days versus 3 to 4 months) and may have been protected by maternal antibody to a greater extent, as mentioned above. The inoculum we used (10^9 bacteria) was also lower than that used by Russell (10^{11} bacteria) and may have contributed to the differences observed.

A final observation of interest was the development of prolonged colonization with *C. cinaedi* and *C. fennelliae* despite the resolution of acute signs of infection and the presence of specific antibody. Colonization persisting for ≥3 weeks at relatively high concentrations was observed for both organisms. If a similar colonization state persists in humans, this may explain the propensity for these organisms to be transmitted sexually among homosexual men (16) and may also explain the occurrence of bacteremia when immunosuppression develops.

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