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Campylobacter-induced Enteritis and Diarrhea in Captive Cotton-top Tamarins (*Saguinus oedipus*) During the First Year of Life

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A prospective study of 43 cotton-top tamarins, from infancy to 6 to 17 months of age, was conducted to determine the epidemiology of *Campylobacter* spp. infection. Nine infants followed for one year in an isolation unit, where attendants wore protective clothing, did not become infected. In the main facility where 32 of 34 animals had repeated infections with *C. coli*, 6% of the infections developed initially in incubators, 66% in the nursery room, and 28% after transfer to the main colony. Fifteen of these tamarins also were infected with *C. jejuni*. Twenty percent of the infections developed initially in the nursery room and 80% in the colony. Polyacrylamide gel electrophoresis analysis of *C. jejuni* cultures revealed multiple reinfections with different strains. Both types of infections were most prevalent between 3 and 9 months of age. *Campylobacter jejuni* infection developed most frequently between April and June and *C. coli* infection developed between October and December. In the nursery, diarrhea developed most frequently at times when there was no infection with *Campylobacter* spp. Forty percent of animals with diarrhea in the nursery had *C. coli* and none had *C. jejuni*, whereas, in the colony, 49% had *C. jejuni* and 11% had *C. coli* infections. There was no association between these infections and diet or idiopathic colitis.

Colitis and colon cancer are the predominant diseases in laboratory-housed cotton-top tamarins (CTTs) (1-3), and diarrhea is a common clinical finding. At the New England Regional Primate Research Center (NERPRC) *Shigella* sp. is a rare etiologic agent in diarrhea of CTTs. However, bacteriologic culture results indicate that infection with *Campylobacter* spp. is endemic in CTTs at the NERPRC, as has been observed in other colonies of captive primates (4-8).

The CTT is a small primate, native to the forests of northern Colombia. Because they are endangered in the wild, none have been imported to the United States since 1976. Large colonies of up to 400 animals have been achieved through successful breeding in captivity (9, 10). Twins or triplets are produced after a gestation period of 187 days (11). In captivity, there is high neonatal mortality associated with parental neglect and abuse. A program at the NERPRC, in which neglected infants are hand raised in a nursery, has been successful if they survive more than one month (10).

The purpose of the prospective study reported here was to investigate the epidemiology of *C. jejuni* and *C. coli* infections in infant tamarins hand reared under two housing and management regimens. One group (n = 34) was reared under standard nursery conditions, and 19 of these were transferred to the main tamarin colony. The other group (n = 9) was reared in an isolation nursery and adult housing facility. The two groups were studied to determine whether any association exists between infection and husbandry, seasonal occurrence, age, location, diarrhea, diet, or idiopathic colitis.

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Materials and Methods

Animals. Forty-three CTT infants were entered into the study over a period of one year. All animals were housed in accordance with standards of the American Association for Accreditation of Laboratory Animal Care. The investigators adhered to the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council and approved by the Animal Use Committee of the Harvard Medical School.

Animal housing and management. The main facility nursery provided intensive care and artificial rearing for infants of all primate species. Neonates under one month of age were placed in incubators in the nursery room, and were handled by personnel who wore sterile masks, gloves, and clothing. Neonates were hand fed human milk formula (Simulated Milk Alternative, SMA; Wyeth Laboratories, Philadelphia, Pa.). At approximately one month of age, animals were transferred singly or in pairs to cages in the same room, where they were gradually weaned from SMA to one of three adult diets over the course of three to four months. Personnel did not wear sterile clothing while handling animals in these cages.

The 34 CTTs assigned to the study in the main nursery included 11 CTTs, aged one week to four months old, present at the commencement of the study, and 23 CTTs who subsequently entered the nursery shortly after birth. All had been admitted because of perinatal abuse, neglect, or illness. Of these 34, 15 remained in the nursery, where seven died and the other eight were not completely weaned at the end of the study. The remaining 19 were transferred after weaning to a room in the main tamarin colony where they lived through the end of the study.

In the main facility colony, the 19 transferred CTTs were housed in diet groups of two to four individuals in one of seven large cages measuring $1.19 \times 0.9 \times 0.87$ m. Temperature was maintained at

26.6 to 29.4°C, humidity at > 50%, and lighting on a 12/12-h light/dark cycle.

The isolation facility was located in a separate building 300 yards from the main facility where there were no other CTTs. The unit, consisting of a nursery, treatment room, and a room with adult living quarters, was separated from the rest of the building by an air lock. Personnel working in this unit had no contact with other animals and wore sterile protective clothing. Animals were entered into incubators shortly after birth, then were transferred to cages in the same room at one month of age. They were hand-fed SMA, then weaned to the same diets as those fed to CTTs in the main facility. After weaning at 3 to 4 months of age, animals were housed in dietary groups in aviary cages measuring 1.5 × 1.2 × 0.76 m in the adult room. Temperature, humidity, and light/dark cycles were the same as those for the colony, except the adult room had natural daylight.

Seven CTTs, taken from their mothers at birth, were in the isolation nursery at the beginning of the study and an additional 2 were admitted shortly after birth. Of these 9 CTTs, one died at 3.5 months of age in the nursery, and the remaining eight were transferred to an adult room in the isolation unit after weaning, where one died at 8.5 months of age and the remaining seven were followed to the end of the study.

The perinatal experience of these animals was not known because they were removed within one day after birth. All but one appeared healthy and unabused. One was treated for pulmonary congestion on the day of arrival in the nursery.

Diets. After weaning, infants in both facilities were fed one of three diets as follows: canned marmoset food (Zu/preem; Hills, Topeka, Kans.) supplemented with fruits, eggs, yogurt, crickets, and wax worms (standard diet); a semi-purified diet, high in total fat and polyunsaturated fatty acids (fat diet); a semi-purified diet, high in several forms of soluble and insoluble dietary fiber as well as supplemental β-carotene and extra vitamin E (fiber diet) (3). Vitamin D_3 on marshmallows was added to all diets.

Bacteriologic methods. Rectal swab specimens for bacteriologic culture were obtained biweekly from each animal. Swabs were inoculated onto half of an agar plate, and three cross streakings were made, flaming the loop between each streaking. To examine for *Shigella* and *Salmonella* spp., swabs were streaked onto XLD and MacConkey agars (Prepared Media Laboratory [PML] Renton, Wash.). Plates were incubated aerobically at 37°C overnight. Culturing for *Campylobacter* spp. was done by inoculation of swabs onto *Campylobacter* CVA agar (PML) that contained protease peptone (Difco Laboratories, Detroit, Mich.), liver digest, yeast extract, 5% sheep blood, cetoperazone (20 mg/L), vancomycin (10 mg/L) and amphotericin B (2 mg/L) (8). Plates were incubated for 96 h at 42°C in anaerobic jars (BBL Microbiology Systems, Cockeysville, Md.) and an atmosphere of 5% oxygen and 10% carbon dioxide provided by CampyPak envelopes (BBL Microbiology Systems). Isolates were subcultured onto *Brucella* anaerobic agar (PML). Thermophilic *Campylobacter* spp. were identified by use of Gram staining and morphology, and positive results of oxidase and catalase reactions. *Campylobacter jejuni* and *C. coli* were susceptible to nalidixic acid (30 μg/disk). The hippuric acid test was positive for *C. jejuni* and negative for *C. coli* (Rapid Hippurate Hydrolysis test, PML) (8).

A total of 574 cultures were obtained from the 34 animals in the main facility, and 186 from the 9 animals in the isolation unit. Twenty-four cultures of *C. jejuni* from 7 animals who were followed to at least one year of age, were analyzed by use of polyacrylamide gel electrophoresis (PAGE) according to the method described by Russell and co-workers (12).

Diarrheal score of fecal examination. Observation of feces was done daily except on weekends. Fecal consistency was classified into five categories: 1 = normal, formed; 2 = normal semi-formed; 3 = soft, unformed; 4 = mild diarrhea, loose; 5 = severe diarrhea, watery. To determine the diarrheal score that corresponded to the rectal swab specimen, the score on the day a rectal specimen was obtained and plus or minus two days was observed and the highest number was entered into the computer together with the date and outcome of culture.

Pathologic examination. All animals had a colon biopsy beginning at four months of age and repeated every four months until the end of the study. Necropsy was conducted on the nine animals that died during the study. Colitis was graded as mild, moderate, or marked as described (3).

Treatment. Twenty-seven CTTs were treated with antibiotics (erythromycin, Keflex, ciprofloxacin, bactrim) and/or sulfasalazine. Five animals had diarrhea without *Campylobacter* infection; one had *C. jejuni* and one had *C. coli* infection. The remaining 20 animals were treated for conditions other than diarrhea or infection with *Campylobacter* spp.

Biometric analysis. Data were stored in a computerized data base (A Data Analysis Program System, ADAPS, Adaptive Systems, Northampton, Mass.) for retrieval and analysis. Incidence rates were calculated from the number of cases of diarrhea or infection divided by the number of animal days at risk. The days at risk were those from entrance to the study to the first *Campylobacter* infection or episode of diarrhea, or, in those without infection or diarrhea, the total days followed in the study. Prevalence was the number of bacteriologic cultures positive for *Campylobacter* spp. divided by the total number of cultures, or the number of infections divided by the number of animals examined. The difference in proportions and the overall χ^2-test for significance were computed for r × c contingency tables by use of standard methods (13). The level of significance was set at $P \leq 0.05$.

Results

Bacteriologic findings. Of the 34 CTTs studied in the main facility, 32 were infected with *Campylobacter* spp. The two uninfected neonates were less than 10 weeks of age in the nursery room at the end of the study. The percentage of CTTs infected with *C. coli* (94%) was more than twice that of *C. jejuni*

Table 1. *Campylobacter* infection in cotton-top tamarins in the main facility*

	Total	*Campylobacter jejuni*			*Campylobacter coli*		
	No.	No.	%	Mean±SD	No.	%	Mean±SD
Animals	34	15	44.1		32	94.1	
Cultures	574	54	9.4		181	31.5	
Infections	129	35	27.1	2.7 ± 2.1	94	72.9	2.8 ± 2.0
Duration (days)		1 - 51		11.8 ± 9.5	3 - 52		15.4 ± 10.0
Onset, age (mo)		2.4 - 7.8		5.3 ± 1.8	0.5 - 5.3		3.2 ± 1.3

*There was no *Campylobacter infection* in the isolation unit.

(44%) and the percentage of infections with *C. coli* (73%) was more than that of *C. jejuni* (27%) (Table 1). However, the mean number of infections per animal was approximately the same. Mean duration of infection with *C. coli* was longer, and the age of onset was younger than that associated with *C. jejuni*, but these differences were not significant. None of the nine CTTs followed through the end of the study in the isolation unit had *Campylobacter* spp. infection on the basis of results of biweekly culturing. None of the CTTs in the main or isolation facility were infected with *Shigella* or *Salmonella* spp. during the study.

Individual CTTs experienced multiple infections with *Campylobacter* spp. Analysis by use of PAGE of 24 sequential *C. jejuni* isolates revealed seven patterns indicating infections with multiple strains in individual animals. These different strains were isolated consecutively from animals in the main facility, suggesting that the prevalent strain varied temporally and by location.

Age and location. Among CTTs, the percentage of *Campylobacter* infections in age groups from less than one 1 month to 17 months in the main facility is shown in Fig. 1. Of the 35 *C. jejuni* infections, none developed in an animal less than one month old when the CTTs were in incubators and only 5.7% developed in animals less than three months old in the nursery. A small percentage (2.1) of the 94 *C. coli* infections developed in neonates while they were in incubators and this increased to 14.9% for animals less than three months old in the nursery room. Both types of infection increased between 3 and 6 months of age when CTTs were transferred to the colony. *Campylobacter coli* infections started, peaked, and decreased at an earlier age than did *C. jejuni* infections.

The prevalence of *Campylobacter* spp. infection was low in neonates in incubators; only two of 25 CTTs (8.0%) were infected with *C. coli*. Prevalence increased after animals were caged singly or in pairs in the nursery. Of these 34 CTTs, three (8.8%) were infected once with *C. jejuni* and 21 (61.8%) infected one or more times with *C. coli*. After entering the colony, more CTTs were initially infected with *C. jejuni* (12/19, 63.2%) and fewer were infected with *C. coli* (9/19, 47.3%) than were those in the nursery. The incidence of *C. jejuni* infections in the colony was ten times higher (10.5/1,000 days) than that in the nursery (1.8/1,000 days), and the incidence of *C. coli* infections was more than two times higher in the colony (33.0/1,000 days) than in the nursery (14.9/1,000 days).

Seasonality of the year. The prevalence of C. *jejuni*-positive culture results was higher during the first (23/208), compared with the last (12/366, $P < 0.001$) half of the year. The prevalence of *C. coli*-positive culture results, however, was higher during the last (78/366), compared with the first (16/208, $P < 0.0001$) half of the year.

Seasonal differences in the two infections are most clearly documented by a comparison of CTTs in the main colony (Table 2). The 10 CTTs in cages 1-3 were in the colony from July through November and the 9 CTTs in cages 4-7 were in the colony from February to November. For purposes of comparison with cages 1-3, the data from cages 4-7 were analyzed in two groups: those which were observed during the months of February through June and were 3 to < 12 (mean ± SD, 6.9 ± 2.1) months old and the same animals who were observed during the months of July through November and which were 6 to < 17 (mean, 11.4 ± 2.4) months old. This comparison indicates that the only significant difference was between the group in cages 4-7 observed from February to June and the group in cages 1-3 observed from July through November. Significantly more *C jejuni*-positive culture results (32.5% versus 6.3%, $P < 0.0001$) and infections (17.9% versus 5.4%, $P < 0.01$) and fewer *C. coli*-positive cultures (16.3% versus 50.0%, $P < 0.0001$) developed in animals observed from January through June than those observed from July through November. Also, *C. coli* infections were less frequent (13.0 versus 22.3%) in these two groups, but the difference was not significant. Interestingly, the Campylobacter infections observed in CTTs in the two cage groups during the months of July through November were similar despite the difference in ages. These observations indicate the significance of seasonality.

Diarrhea. A total of 5,578 fecal specimens from 43 CTTs (3,258 in the main facility and 2,320 in the isolation unit) were examined. A similar low incidence of diarrhea was observed in the isolation nursery (one case, 1.9/1,000 days at risk) and after weaning in the adult room (three cases, 1.7/1,000 days at risk). In the main nursery, the incidence was higher in aninals in incubators (six cases, 19.8/1,000) than in the nursery room (20 cases, 16.7/1,000) and higher than in the main colony in gang cages (five cases, 2.4/1,000 days at risk).

In both locations, there were multiple episodes, 80% of which lasted for one or two days only. Only two episodes lasted more than one week. Among the 19 CTTs, which were studied for periods up to one year in the main facility, were 17 that had experienced diarrhea in the nursery. After transfer to the colony, only 5 animals in two cages had diarrhea.

The association between diarrhea and *Campylobacter* infection, using fecal scores that were accompanied by simultaneous bacteriologic examinations, is shown in Table 3. Among CTTs in the main nursery, diarrhea was observed in approximately 21% of the fecal specimens that were accompanied by bacteriologic

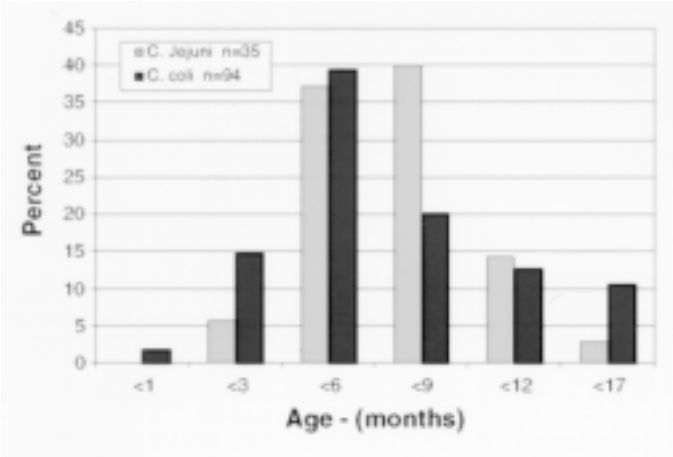

Figure 1. Age distribution of 35 *C. jejuni* and 94 *C. coli* infections.

Table 2. *Campylobacter* infection in cages in the main colony

	Cages 1–3		Cages 4–7			
Animals (No.)	10		9		9	
Season (mo)	7 - 11		2 - 6		7 - 11	
Age (mean ± SD)	5.5 ± 1.2		6.9 ± 2.1		11.4 ± 2.4	
Cultures	No.	%	No.	%	No.	%
Total	112	100.0	123	100.0	120	100.0
C. jejuni	7[a]	6.3	40[a]	32.5	4	3.3
C. coli	56[b]	50.0	20[b]	16.3	49	40.8
Infections						
C. jejuni	6[c]	5.4	22[c]	17.9	4	3.3
C. coli	25	22.3	16	13.0	23	19.3

[a]$P < 0.0001$, [b]$P < 0.0001$, [c]$P < 0.01$.

Table 3. Association of diarrhea and *Campylobacter* culture results in cotton-top tamarins (CTTs) with concomitant fecal and bacterial examinations

	Diarrhea/fecal exam		*C. jejuni*		*C. coli*		No campylobacters	
	No.	%	D/C[a] (No.)	%	D/C (No.)	%	D/C (No.)	%
CTTS in nursery	40/194	20.6	0/3	0.0	16/51	31.4	24/140	17.1
Colony	45/353	12.8	22/51	43.1	5/125	4.0	18/177	10.7
Isolation	4/180	2.2	0	0.0	0	0.0	4/180	2.2

[a]D/C diarrhea /culture.

examination. In the main nursery, none of the three *C. jejuni*-positive cultures was accompanied by diarrhea, whereas, 31.4% of the *C. coli*-positive cultures and 17.1 % of the negative cultures were accompanied by diarrhea. In the main colony, the prevalence of diarrhea was lower than in the nursery (12.8%). Forty-three percent of the *C. jejuni*-positive cultures were accompanied by diarrhea, whereas only 4.0% of *C. coli*-positive cultures and 10.7% of negative cultures were accompanied by diarrhea. Of the 16 *C. coli*- positive cultures accompanied by diarrhea in the main nursery, 13 specimens (83.3%) were obtained during initial infections, whereas, the 5 culture specimens in the main colony accompanied by diarrhea were obtained during recurrent infections. Of the 22 *C. jejuni*-positive cultures in the main colony, 12 specimens (54.5%) were obtained during initial infection. In the isolation unit, where neither infection developed, only 4 (2.2%) of the 180 fecal specimens observed were diarrheic.

Diet. Although fewer of the 35 episodes of *C. jejuni* infection were associated with eating the fiber (22.9%) or standard (28.5%) than the fat (48.6%) diet, the difference was not significant (χ^2 2 df = 5.74, $P > 0.05$). Among the 94 episodes of *C. coli* infection, there was no difference among the adult diets (30.9% fat, 27.7% fiber, and 29.8% standard). However, significantly fewer animals ate SMA (10.6%, χ^2 3 df = 12.09, $P < 0.01$). Diet, however, was associated with diarrhea. Twenty-three percent of those consuming the fat diet, 16.9% fed the fiber diet, and 19.0% eating the SMA had diarrhea, whereas only 2.3% of those eating the standard diet had diarrhea (χ^2 3 df = 26.5, $P < 0.001$)

Colitis. Sixty histologic examinations, including 51 biopsies in the 33 animals over 4 months old and 9 necropsies in those that died during the study, did not reveal association between idiopathic colitis and observation of previous *Campylobacter* spp. infection. Of 14 CTTs with colitis, 9 had one or more *C. coli* infections; of 19 without colitis, 14 had been infected ($P > 0.50$). Similar lack of correlation was seen for *C. jejuni* infection: 4 of 14 CTTs were infected, and 9 of 19 were not infected ($P > 0.20$).

Discussion

Bacteriologic culturing indicated that *Campylobacter* spp. infection was enzootic among infant CTTs in the main facility at the NERPRC, as has been reported in other laboratory-housed tamarins and marmosets (7), patas monkeys (5, 6), and pigtailed macaques and cynomolgus monkeys (8). Infection in newly imported simians (4) and in 7 species of New World monkeys in Peru also has been described (14). In the study at the NERPRC, infection was uncommon in infants in incubators. In a similar prospective study of infection with *Campylobacter* spp. in laboratory-housed *Macaca nemestrina*, neonates reared by the mother were culture positive by rectal swab specimen by 2 months of age, whereas, neonates reared in incubators were culture negative until moved to single cage housing in an infant animal room (12). After the initial infection, all had multiple reinfections with different strains of *C. jejuni* and *C. coli* following successful treatment (12). Recurrence after treatment also was found in patas monkeys (5). In the main facility at the NERPRC, the prevalence of *C. coli* infection was three times higher than that of *C. jejuni* infection. *Campylobacter coli* infections were reported to be more prevalent than those of *C. jejuni* in other laboratory-housed animals (8). Multiple reinfections with *C. jejuni* in CTTs, lasting an average of 12 to 15 days and subsiding without treatment, were documented by results of PAGE analysis to be sequential infections with different strains. Multiple strains of *C. jejuni* and *C. coli* were observed in reinfections in *M. nemestrina* in a nursery facility (12).

In contrast to animals in the main facility, none of the animals in the isolation unit, which were studied up to 12 or more months of age, developed *Campylobacter* infection. Attendants to animals in incubators and in the isolation unit wore masks, gowns, and gloves. Thus, an important finding was that infection can be prevented by isolating animals from the source of infection in other animals and humans. Although perinatal stress in CTTs brought to the main nursery, could have an effect on the immune system, it is more likely that proximity to infected macaques was responsible for infection in CTTs.

The age at which animals were first infected with *C. jejuni* was correlated with location. A high percentage of CTTs were first infected after they were transferred from the infant room to the main colony after 3 months of age. However, the percentage of infections decreased to approximately a third of the peak value after 6 months of age. In a study of *M. nemestrina* (8), location took precedence over age and prevalence was lower in older animals.

Both infections were seasonal in occurrence. *Campylobacter jejuni* was most prevalent in the spring months, which accounted for the higher percentage of infections among CTTs entering the colony from the infant room in the spring than did those entering in the summer and fall. Similarly, *C. coli* was more prevalent in the fall, and those entering the colony during that period had a higher percentage of infection than did those entering in the spring. Season was not observed in relation to infection in previous studies of other non-human primates. In humans, however, *C. jejuni* infections peak in late summer and early fall (15).

Diarrhea is a problem in laboratory-housed primates, particularly in infants in nurseries. In our main nursery, approximately two-thirds of CTTs with diarrhea did not have *Campylobacter* infection and a third had *C. coli*. After removal to the colony, 49% of diarrheal episodes were accompanied by *C. jejuni* infection and 11% by *C. coli* infection.

Mean duration of *Campylobacter*-positive culture results was 12 to 15 days (Table 1), but more than 80% of the episodes of diarrhea continued for only 1 to 2 days, which suggests there may be a reservoir of these bacteria that are shed without causing disease. *Campylobacter jejuni* was isolated from the duodenum, jejunum, ileum, colon, and gall and urinary bladders of a cynomolgus monkey (*M. fascicularis*) which had been found to be shedding bacteria during a routine health check (4). The same

organism was isolated from the jejunum, ileum, cecum, and colon of patas monkeys with chronic diarrhea (5). In two unreported cases from our laboratory, with severe diarrhea and wasting and negative rectal culture results for *C. jejuni*, examination of biopsy specimens at laparotomy revealed ileitis, typhlitis, and colitis associated with *C. jejuni* isolated in culture from these sites.

Previous studies have not indicated consistent relationship between *Campylobacter* infection and diarrhea (4-6, 12). Reinfection with various homologous and heterologous strains of *Campylobacter* species was accompanied by milder diarrhea (16), and was not observed in infants over 4 months old when reinfected with multiple strains, suggesting immune protection against diarrhea. In our study, diarrhea developed on significantly more days of initial infections than on days of subsequent infections with *C. coli*. However, the difference between the number of days of diarrhea during initial and subsequent infections with *C. jejuni* was not significant. This may have been due to the fact that there were more first infections with *C. coli* in the nursery, where diarrhea was more prevalent, than first infections with *C. jejuni* in the colony where diarrhea was less prevalent. Diarrhea was more closely associated with diet than with infection. There was significantly less diarrhea among CTTs consuming the standard diet than among those eating the other two adult diets or SMA.

This study of CTTs has confirmed the findings of studies of other primates: *Campylobacter* infections and reinfections with multiple strains are common among laboratory-housed primates; *C. coli* is more prevalent than *C. jejuni*; age at first infection is dependent on the location where infection is enzootic; the percentage of animals with infection is lower in older animals; and the relationship between diarrhea and infection varies. New findings include: infection can be prevented by removing animals from contamination by humans and animals; season of the year is an important factor; diet plays little or no part in the rate of reinfection, but is associated with diarrhea; and there is no association between infection and colitis in these young animals.

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