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Evidence of Reinfection with Multiple Strains of *Campylobacter jejuni* and *Campylobacter coli* in *Macaca nemestrina* Housed under Hyperendemic Conditions

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A prospective bacteriologic study of 18 infant pig-tailed macaques (*Macaca nemestrina*) housed in a nursery facility in which *Campylobacter* spp. are endemic was undertaken to determine the epidemiology of infection and reinfection. The isolates of *Campylobacter jejuni* and *C. coli* cultured from 8 of the 18 infants were characterized by serotyping, DNA hybridization, and polyacrylamide gel electrophoresis protein profiles. The chronology of infection was indicative of multiple reinfections with different strains of *C. jejuni* and *C. coli* during the 12-month study of each infant. The duration of infection with a particular strain was 3 to 4 weeks. Infants were also infected with nalidixic acid-resistant campylobacters. These observations indicated that long-term infections under endemic conditions are caused by continual reinfection. *C. jejuni* or *C. coli* infection correlated with diarrhea in 5 of the 18 infants at 1 to 4 months of age.

We have recently reported (38) that the clinical course of diarrhea and presence of colitis in pig-tailed macaques (*Macaca nemestrina*) after experimental challenge with *Campylobacter jejuni* is similar to the diarrheal disease in humans with natural or experimental infections (1, 5, 6). Also, the 3- to 4-week duration of excretion after challenge of susceptible macaques (38) is comparable to the duration observed in humans after foodborne infections in western countries (5, 20, 32). This epidemiologic and clinical picture of *C. jejuni* infection is different from that observed in developing countries, where under endemic conditions, infections are often asymptomatic and the organism may be isolated repeatedly over extended periods (3, 34, 35). The reasons proposed for these differences in the clinical expression of *C. jejuni* infection include variation in the virulence of the organisms, acquired immunity, a long-term carrier state, and/or reinfection (3, 16, 34). Preliminary studies with laboratory primates (41) and follow-up weekly cultures from children in Thailand (43) have indicated that reinfection occurs commonly under endemic conditions. This prospective study of *M. nemestrina* infants was conducted in a nursery facility in which enteric *Campylobacter* sp. infection is endemic (39, 40). The objectives were to determine the course of infection with individual strains of *Campylobacter* sp. and to determine whether there was an association between infection with *Campylobacter* sp. and clinical episodes of diarrhea. The study was undertaken to provide objective clinical data on which to base treatment and management for the health care of laboratory-housed primates and to contribute to an understanding of the factors involved in the pathogenesis of diarrhea caused by *C. jejuni* and *C. coli*. Also, because of the similarities in the clinical and epidemiologic aspects of *Campylobacter* infections in humans and macaques, this prospective study may provide data of possible relevance to an understanding of the epidemiology of infection in humans under endemic conditions.

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MATERIALS AND METHODS

Animals. Eighteen *M. nemestrina* macaques in an infant primate nursery (Infant Primate Research Laboratory [IPRL]) were studied from birth to 1 year of age. Because infants were assigned to the study at different times in the first year, the total duration of the study was 23 months. Of the 18 infants, 6 were born at the IPRL from pregnant females sent to the IPRL from a Primate Field Station (PFS) of the Washington Regional Primate Research Center, Seattle. Five of the macaques from these mothers were born naturally. They were separated from the mothers immediately after delivery, which was monitored by 24-h closed-circuit television observation. The sixth macaque was delivered by cesarean section. The remaining 12 macaques were born naturally at the PFS. The mothers were moved from group housing to individual cages at 1 to 3 weeks before parturition. The neonates born at the PFS remained with their mothers for 1 to 12 days, until they were sent to the IPRL.

Facilities. Neonates in the IPRL were kept in isolettes until 2 weeks old. They were then moved to individual cages located in a large animal room (800 square feet) housing approximately 90 to 100 infants. Infants were allowed to interact in social groups for 1 h daily. The individual cages in which the infants were maintained during the remaining 23 h when they were not in socialization were located so that the infants were separated from other members of the same social group. The neighbors in immediately adjacent cages were changed at approximately 6-week intervals. All of the social groups comprised four infants. Ten of the infants were assigned to five different social groups which remained constant for the entire study. The remaining eight infants were in four groups, the composition of which changed one to three times during the study as a result of movement of infants in the study to new groups or introduction of new infants into groups. Socialization took place in a designated room (53 square feet). The room was washed and disinfected (Roccal-D; Sterling Drug, Inc., New York, N.Y.) between

groups. Neonates were fed a milk formula (SMA-R; Wyeth Laboratories, Philadelphia, Pa.). At 4 months of age, the infants were weaned onto a commercial monkey diet (Purina Monkey Chow 5045; 25% protein diet; Ralston Purina Co., Richmond, Ind.).

Microbiologic examination. Rectal swabs were obtained weekly from each monkey starting at 1 week of age or upon arrival at the IPRL. Each infant was studied to monitor natural infection from birth until 1 year of age. Specimens were inoculated onto *Campylobacter* CVA agar (Prepared Media Laboratory, Renton, Wash.) which contained Protease Peptone (Difco Laboratories, Detroit, Mich.), liver digest, yeast extract, 5% defibrinated sheep blood, cefoperazone at 20 mg/liter, vancomycin at 10 mg/liter, and amphotericin B at 2 mg/liter. The swabs were inoculated onto half of the plate, and three streaks were made without flaming the loop. Plates were incubated at 42°C for 96 h in an atmosphere of 5% oxygen plus 10% carbon dioxide provided by CampyPak II envelopes (BBL Microbiology Systems, Cockeysville, Md.) with anaerobic jars (BBL). Isolates were subcultured onto brucella anaerobic agar (Prepared Media Laboratory).

Thermophilic *Campylobacter* sp. were identified by colony morphology, typical microscopic appearance of gram-negative curved rods, and positive oxidase and catalase reactions (17, 36, 47). Isolates were further examined for nalidixic acid sensitivity (Nal^s; 30 μg per disk) and by the rapid hippurate test (17, 36, 42, 47). The relative numbers of colonies on the primary isolation plates were semiquantitated by using a scale from 1+ to 5+. A score of 1+ designated <10 colonies per plate. A score of 2+ was used for >10 colonies in the initial area of inoculation, and 3+ to 5+, respectively, were used for each of the three subsequent streaks. This method of quantitation correlates with the titration of *C. jejuni* in stool samples (38).

Isolates were selected for storage from each individual infant by using the following criteria: (i) different colony morphology (spreading versus discrete) and/or biochemical characteristics from the isolate obtained in the preceding weeks, (ii) when an animal with low numbers (1+ or 2+) of organisms on the primary plate showed substantial escalation in the number of organisms recovered, and (iii) when an animal that was negative for a time became positive. Occasionally, additional isolates were stored, for example, when there was continued isolation of *Campylobacter* sp. organisms with similar biochemical profiles and/or when *Campylobacter* sp. numbers were similar over several weeks, also, when qualitative numbers declined on the primary plate. Isolates were stored in tryptic soy broth with 15% gelatin at −70°C.

In addition, *Campylobacter* sp. isolates cultured by the diagnostic laboratory at the Washington Regional Primate Research Center from infants during diarrheal episodes were obtained and stored. These isolates were cultured and characterized by methods and criteria similar to those described above.

Protein profile characterization of isolates by PAGE. Of the 18 infants studied, the isolates from infants no. 1 to 4 and no. 15 to 18 were analyzed by polyacrylamide gel electrophoresis (PAGE). Isolates were grown from frozen stocks and subcultured onto brucella anaerobic agar at 42°C for 48 h in GasPak jars with a CampyPak II gas generator envelope (BBL). They were harvested in 1.0 ml of sterile distilled water, centrifuged at 11,600 × *g* for 10 min at 4°C, and washed twice in sterile distilled water. The bacterial pellet was suspended in 600 ml of double-strength lysis buffer (20% [vol/vol] glycerol, 10% [vol/vol] 2-mercaptoethanol, 4% [wt/vol] sodium dodecyl sulfate, 66% 0.5 M Tris hydrochloride buffer [pH 6.8]). The bacterial sample in lysis buffer was mixed thoroughly and boiled for 5 min. An equal quantity of sterile distilled water was added, mixed, and boiled for a further 5 min. The sample was centrifuged at 11,600 × *g* for 10 min (10). The supernatant was analyzed by sodium dodecyl sulfate-PAGE by the method of Laemmli (21) with 4.5% stacking and 10.0% running gels. Samples of 100 μl per lane were electrophoresed at a constant current of 20 mA per gel and compared with molecular weight markers (Sigma Chemical Co., St. Louis, Mo.) and a standard strain (*C. laridis* NCTC 11352). Protein bands were visualized with Coomassie blue stain.

For PAGE comparison of the isolates from a particular infant, all of the stored isolates were cultured and processed together. All isolates were regrown at least once and run on gels approximately four to six times. Gels were rerun to obtain uniformity of band density between lanes in a gel.

Serotyping. Rabbit antisera to 15 *C. jejuni* and 5 *C. coli* Penner serotypes commonly found in humans were prepared (15). For the passive hemagglutination test, the titers of reactivity of the sera were determined and adjusted to a concentration four times the endpoint titer. Saline-extracted antigens from test isolates were used in passive hemagglutination assays against each antiserum (30, 31). Isolates of *C. jejuni* and *C. coli* recovered from eight infant macaques (no. 1 to 4 and 15 to 18) were serotyped. These isolates were also characterized by PAGE as described above.

DNA hybridization. Whole-cell DNAs from reference strains *C. jejuni* NCTC 11351 and *C. coli* NCTC 11366 were isolated as described previously (46), with modifications. The resultant DNA was collected by ethanol precipitation. The whole-cell DNA was nick translated by using the nick translation system supplied by Bethesda Research Laboratories, Inc., Gaithersburg, Md., and [^{32}P]dATP (800 Ci/mmol; Dupont, NEN Research Products, Boston, Mass.). Removal of unincorporated nucleotides was done by the spun-column procedure of Maniatis et al. (25).

Isolates from frozen stocks were initially grown on Columbia agar plates with 5% sheep blood (Remel, Lenexa, Kans.) for 24 h at 37°C in an anaerobic jar with a CampyPak (BBL) and a catalyst. Secondary cultures were then grown similarly. Bacterial cultures were harvested from these plates in 1 ml of sterile phosphate-buffered saline. The concentration of each isolate was standardized in phosphate-buffered saline to the density of a McFarland no. 5 nephelometer barium sulfate standard. A series of three doubling dilutions were made for each isolate, and 5-μl samples were placed on a GeneScreen Plus nylon membrane (Dupont, NEN) through a Bio-Dot apparatus (Bio-Rad Laboratories, Richmond, Calif.) in duplicate. Three control isolates, *C. jejuni* NCTC 11351, *C. coli* NCTC 11355, and *C. laridis* NCTC 11352, were included on each membrane.

The DNAs were bound to the GeneScreen Plus. The GeneScreen Plus membrane was sequentially placed colony side up onto four pieces of Whatman 3MM paper saturated with the following solutions: 10% sodium dodecyl sulfate for 3 min, 0.5 M NaOH–1.5 M NaCl for 5 min, 1.5 M NaCl–0.5 M Tris buffer (pH 8.0) for 5 min, and 1× SSC (0.15 M NaCl, 0.015 M sodium citrate) for 5 min. The membrane was then allowed to air dry for 60 min. For prehybridization, the membranes were incubated at 42°C for at least 1 h in a solution containing 50% formamide, 1% glycine, 50 mM sodium phosphate buffer (pH 6.5), 5× SSPE (43.8 g of NaCl, 6.9 g of $NaH_2PO_4 \cdot H_2O$, and 1.8 g of EDTA per liter [pH

7.4]), 5× Denhardt solution, and 10 mg of denatured calf thymus DNA per ml in a sealed bag. The prehybridization solution was replaced with a solution containing the appropriate labeled probe DNA, 50% formamide, 1% glycine, 20 mM phosphate buffer (pH 6.5), 5× SSPE, 1× Denhardt solution, and 10 mg of denatured calf thymus DNA per ml. The membranes were incubated at 42°C overnight. Excess probe DNA was removed by washing (twice for 5 min each time) the membranes in 2× SSPE–0.1% sodium dodecyl sulfate at 65°C. Dry membranes were exposed to X-OMAT XR film (Eastman Kodak Co., Rochester, N.Y.) with an intensifying screen at −70°C for 2 to 3 days.

Clinical episodes of diarrhea. A daily medical record was initiated by an animal technician or veterinary technician when an animal was sick. An episode of diarrhea was the period for which an animal was treated for diarrhea. The clinical signs, medical treatments, clinical diagnostic bacteriologic culture results, other laboratory results, and starting and ending dates of treatment were recorded on a PRIME 750 computer at the Washington Regional Primate Research Center. These computer records were reviewed at the completion of the study to identify episodes of diarrhea that occurred either concurrently with the initial infection or upon reinfection with a different *Campylobacter* sp. strain.

Analysis of data. The age of the animal at the time of initial infection was determined from culture records. For the *Campylobacter* strains (from infants 1 to 4 and 15 to 18) characterized by Nal^s, hippurate hydrolysis, PAGE, and serotyping, the duration of infection with each strain was calculated as the number of weeks of consecutive positive weekly cultures until the rectal swab culture became negative or a different strain was recognized. To determine the incidence of *Campylobacter*-associated diarrhea, animal days (defined as the cumulative number of days for which each animal was in the colony during the period of study for *Campylobacter*-associated diarrhea) were used to measure the number of cases of diarrhea per population at risk (39). *Campylobacter*-associated diarrhea was defined as an episode of diarrhea that coincided with (i) the initial isolation of *Campylobacter* sp. or (ii) reinfection with a different strain.

RESULTS

Sixteen infants completed the study. Infants 8 and 14 died during the study at 7.6 and 8.5 months of age, respectively. *Campylobacter* sp. organisms that were Nal^s and either hippurate positive or negative were consistently isolated from each of the 18 infants. Because of reports that some isolates of *C. jejuni* are hippurate negative (18, 28, 36, 46), dot blot DNA hybridization was conducted on isolates from infants 1 to 4 and 15 to 18 for species identification of *C. jejuni* and *C. coli*. One strain of *C. jejuni* was hippurate negative. All other hippurate-negative strains were *C. coli*. All of the hippurate-positive isolates were *C. jejuni* by dot blot hybridization. Approximately half of the strains were *C. jejuni* and half were *C. coli* (Table 1). Of the 18 infants, 13 were also infected with nalidixic acid-resistant (Nal^r) *Campylobacter* sp. (these organisms had small pinpoint colonies at 48 to 96 h of culture, and they were negative in the hippurate hydrolysis test). A total of 281 *C. jejuni* or *C. coli* and 25 Nal^r *Campylobacter* sp. isolates were stored during the entire prospective study (i.e., 12 to 25 isolates per infant).

Five infants initially became infected at 8 to 11 weeks of age. The other two-thirds were positive at 1 to 4 weeks of age. The mean age of initial infection was 7.3 ± 3.5 weeks.

TABLE 1. Identification of *C. jejuni* and *C. coli* strains by dot blot DNA hybridization and PAGE protein profile[a]

Infant no.	No. of isolates	No. of page categories	DNA hybridization of PAGE categories	
			C. jejuni	*C. coli*
1	13	6	2	4
2	15	11	5	6
3	13	5	3	2
4	11	10	5	5
15	15	9	4	5
16	15	7	2	5
17	15	8	4	4
18	14	10	6	4
Mean ± SD	13.9 ± 1.4	8.3 ± 2.0	3.9 ± 1.4	4.4 ± 1.1

[a] One hundred and eleven isolates were characterized from eight infant macaques that were cultured weekly from birth to 1 year of age.

Nine infants were positive at 1 to 2 weeks of age (seven of them were born at a PFS and were positive on arrival at the IPRL).

Once an infant was positive, there were only occasional periods of negative culture (mean, 4.3 ± 1.9 per infant) and they were brief (mean, 2.0 ± 1.5 weeks). The 18 infants were persistently infected with *C. jejuni* or *C. coli* and, less commonly, Nal^r *Campylobacter* sp. This is illustrated in Fig. 1 for infant 18.

The characterization of 111 *C. jejuni* or *C. coli* isolates from eight infants (infants 1 to 4 and 15 to 18) by serotyping, PAGE protein profiles, Nal^s, and DNA hybridization showed a mean of 8.3 ± 2.0 strains per infant (Table 1). Sixty-nine isolates were classified into 10 serotypes (Table 2). The remaining isolates were untypeable with the antisera available. PAGE analysis enabled identification of strains within the same serotype and also among isolates that were not typeable. As an example, the data from infant 18 are summarized in Fig. 1 and 2. This infant was infected with 10 strains of *C. jejuni* or *C. coli* with PAGE protein profiles A to J and also two strains of Nal^r *Campylobacter* sp. (Fig. 1). The PAGE profiles are presented in Fig. 2 to demonstrate the different banding patterns among 10 strains of *C. jejuni* and *C. coli* from infant 18. Similar results were observed in infants 1 to 4 and 15 to 17. The PAGE protein profile of each strain was distinct because of differences in the migration of a particular protein (e.g., the porin protein at approximately 45,000) and/or the presence of unique bands that were not present in other strains. The differences between the PAGE profiles of strains D and I from infant 18 (serotypes 38 and 34, respectively), which were less distinctive with a 10% gel (Fig. 2), became clearly evident when the two strains were run in an 8.5% gel (Fig. 3). On the basis of identification of individual strains, the mean duration of infection with a particular strain was 3.0 ± 2.3 weeks. The longest duration for isolation of a particular strain was 11 weeks. Seven strains were detected for 6 to 7 weeks, and another strain was isolated for 10 weeks. In general, each of the infants had different patterns of infection in regard to the number of strains per infant (Table 1) and the chronology of infection with various strains.

Isolates of *C. jejuni* or *C. coli* with similar PAGE patterns and serotypes were recultured on a subsequent occasion for five of the eight infants. This is illustrated in Fig. 1 for infant 18 (a strain with PAGE protein profile C and serotype 30/34 was isolated at 14 to 17 weeks of age and again at 31 weeks of age, and PAGE profile G-serotype 5 isolates were cultured

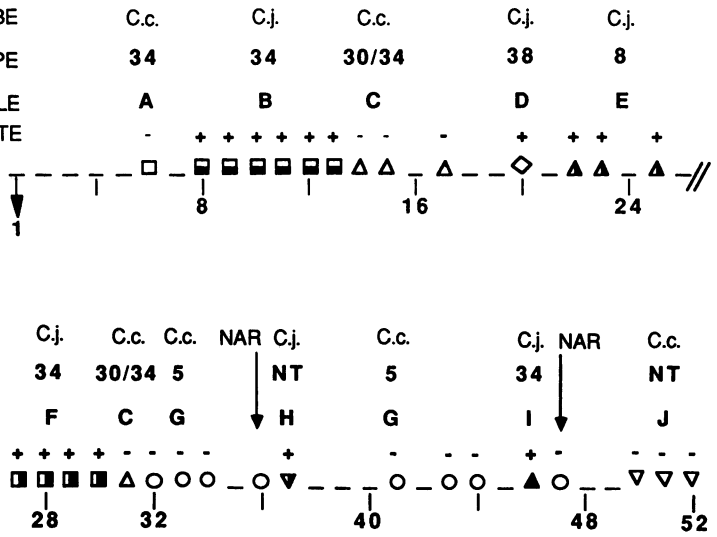

FIG. 1. Chronological infection of infant 18 with different strains of *C. jejuni*, *C. coli*, and Nal^r (NAR) *Campylobacter* sp. The infant was cultured weekly in a prospective study from birth to 1 year of age. Strains of *C. jejuni* and *C. coli* were identified by serotype and PAGE profile. The PAGE characterization of different strains is designated by letters A to J for individual strains. Each symbol represents a distinct strain of *C. jejuni* or *C. coli*. The PAGE profiles are shown in Fig. 2. The hippurate hydrolysis test (positive [+] or negative [−]) of Nal^s *Campylobacter* sp. correlates with *C. jejuni* and *C. coli*, respectively, with dot blot hybridization. NT, Strains that could not be serotyped with the available antisera.

at 32 to 34 weeks and again 7 weeks later). The initial isolation and reisolation observations for infants 1, 3, 15, 17, and 18 are summarized in Table 3.

The duration of infection with Nal^r *Campylobacter* sp. in 13 infants was 1 to 6 (mean, 1.6 ± 1.4) weeks. For 6 of the 13 infants, Nal^r *Campylobacter* sp. organisms were reisolated on one or two subsequent occasions.

Fluctuations in the semiquantitative scores of the eight infants (infants 1 to 4 and 15 to 18) generally corresponded to declining numbers during consecutive weeks of isolation of the same strain and an increase in the score correlating with the isolation of a different strain. Sometimes there was no change in the semiquantitative score when different strains were isolated chronologically. For example, from 31 to 46 weeks of age, infant 18 was infected with four strains (PAGE profiles C, G, H, and I) (Fig. 1). The semiquantitative score remained consistently low (1+) throughout this period, until the score increased to 3+ when a PAGE profile J strain was isolated at 50 weeks of age.

TABLE 2. Serotypes and nontypeable strains of *C. jejuni* and *C. coli* identified in eight infant macaques studied from birth to 1 year of age

Infant	Serotypes identified	No. of strains identified by PAGE but not serotyped
1	5, 30/34	4
2	5, 19, 30, 34, 30/34	5
3	8, 19, 30, 34	1
4	5, 34, 30/34	7
15	5, 8, 24, 30, 34, 30/34, 46	2
16	4, 5, 8, 24, 34, 46	0
17	5, 8, 24, 46, 34	3
18	5, 8, 34, 38, 30/34	2

Concurrent mixed infections were observed in seven infants. Dual infections with *C. jejuni* or *C. coli* and Nal^r *Campylobacter* sp. were detected in five infants (at 10 to 49 weeks of age). Mixed infection with *C. jejuni* and *C. coli* was observed in infants 2 (at 13 weeks of age) and 17 (at 36 weeks of age).

All infants had multiple episodes of diarrhea during the study (mean, 5.8 ± 2.0 episodes for 16 infants, i.e., excluding infants 8 and 14, which died during the study). The initial infection correlated with an episode of watery diarrhea in

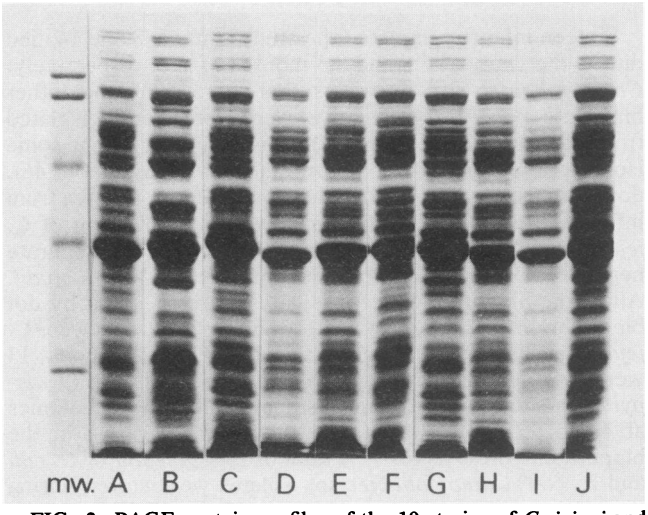

FIG. 2. PAGE protein profiles of the 10 strains of *C. jejuni* and *C. coli* from infant 18. The age and duration of infection with these strains are shown in Fig. 1. Lane mw. contained molecular weight markers (carbonic anhydrase, 29000; egg albumin, 45000; bovine albumin, 66000; phosphorylase *b*, 97.4000; β-galactosidase, 116,000).

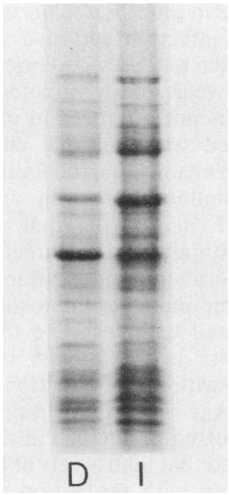

FIG. 3. PAGE protein profiles of strains D (serotype 38) and E (serotype 34) from Fig. 1 after being run in an 8.5% gel. This illustrates the differences between the protein banding patterns of these two strains that were present but less readily discerned in the 10% gel shown in Fig. 1. All other strains in Fig. 1 were readily distinguished in 10% gels.

four infants (Table 4). In two of the infants, the infection was with *C. jejuni*. *C. coli* was isolated from the other two. Also, infant 12 had diarrhea correlating with a mixed infection of *C. jejuni* and cryptosporidia at 5 weeks of age. Diarrheal episodes correlating with *Campylobacter* reinfection was observed in six infants at 1 to 4 months of age (Table 4).

In a previous study of medical records (40), we reported that the incidence of diarrhea in *M. nemestrina* in this facility was highest (18.6 per 1,000 animal days) in neonates less than 1 month old compared with 2.0 per 1,000 animal days in infants 1 to 6 months old. In this prospective study, the incidences of diarrhea in these age groups were 27.7 and 5.4 per 1,000 animal days, respectively. The incidence of these diarrheal episodes which correlated with *Campylobacter* sp. infection was 3.7 per 1,000 animal days in neonates initially infected at <1 month of age. In infants 1 to 6 months old, the incidence of *Campylobacter* sp. infection that correlated with diarrheal episodes was 3.7 per 1,000 animal days. However, *Campylobacter* sp. infection correlated with diarrhea only in neonates and infants <4 months old, with an overall incidence of 5.5 per 1,000 animal days, and in infants 1 to 4 months old (i.e., excluding neonates <1 month old), the incidence of initial and recurrent *Campylobacter*-associated diarrhea was 6.2 per 1,000 animal days.

TABLE 3. Reisolation of the same strain of *C. jejuni* or *C. coli* in individual macaques studied from birth to 1 year of age[a]

Infant	Strain[b]	Age (wk) at:		Interval (wk) between isolations
		Initial isolation	Reisolation	
1	1F	39–42	50–52	8
3	3C	18–22	36–46	14
15	15B	15–20	47	27
	15E	28	34	6
17	17C	22–26	31, 36	5, 5
	17D	27–28	48–52	20
	17F	36	42	8
18	18C	14–15	31	16
	18G	32–34	41	7

[a] During the intervening period before reisolation of the same strain, either cultures were negative or different strains were isolated.
[b] Each strain was designated by the animal number and a letter for the PAGE protein profile.

TABLE 4. Correlation of episodes of diarrhea with *C. jejuni* or *C. coli* infection

Diarrhea cause and infant no.	Age (wk)	Culture result		Age (wk) when previously positive by culture[a]
		Isolate	Score	
Initial infection				
1	9	*C. coli*	4+	
7	10	*C. jejuni*	4+	
16	2	*C. jejuni*	4+	
17	2	*C. coli*	3+	
Recurrent infection				
1	12	*C. jejuni*	4+	9
6	8	*C. jejuni*	4+	2
11	13	*C. coli*	4+[b]	8
8	4	*C. coli*	4+	1
	8	*C. jejuni*	4+	4
14	13	Nal[r] *Campylobacter* sp.	NR[c]	2
16	10	*C. jejuni*	4+	2
	16	*C. jejuni*	1+	10

[a] All infants except no. 6 were treated with antibiotics (erythromycin-estolate; 75 mg/kg of body weight twice daily; Dista Products Co., Carolina, P.R.) for 10 days during episodes of diarrhea.
[b] Mixed infection with cryptosporidia.
[c] NR, Not recorded.

DISCUSSION

Infants were infected with *Campylobacter* sp. at an early age. The seven neonates from the PFS that were positive on arrival at the IPRL had been nursed by their mothers, suggesting that this may have been the source of infection. Five of the six infants born at the IPRL did not become infected until removed from isolettes into single cages in the holding room. Finger contact between animals in adjacent cages and during socialization provides methods for fecal-oral transmission. These observations indicate that there may be greater opportunity for infection at a younger age in infants reared by their mothers at the PFS than in those in the IPRL nursery. This requires further study. The long-term positive cultures in each of the 18 infants supports previous data indicating that *Campylobacter* sp. is hyperendemic in the IPRL (39, 40).

Multiple procedures have been used to distinguish between isolates of *C. jejuni* and *C. coli* by serotype (8, 14, 15, 18, 19, 22–24, 29, 43), biotype (7, 37), auxotype (44, 45), lectin agglutination, (9), enzymatic profile (12), plasmid profile (45), or chromosomal restriction pattern (8). In this study, we elected to distinguish among the isolates from each of the eight infants by PAGE, serotyping, DNA hybridization, and hippurate hydrolysis. We selected these criteria because serotyping using the Penner scheme is based on the bacterial lipopolysaccharide (27, 33) and has been useful in previous epidemiologic studies for discriminating strains. Also, because Coomassie blue stains the bacterial proteins, these two analyses provided unrelated parameters for strain differentiation. Our results appear to substantiate the con-

clusion that these criteria enabled differentiation between strains among all of the isolates collected from an individual infant.

The data indicate that different strains were cultured continually from each infant during the chronological study (Fig. 1). (i) The five different serotypes from infant 18 had different PAGE protein profiles, consistent with their classification as different strains. (ii) For isolates within the same serotype category (e.g., serotype 34) that were different species (*C. jejuni* or *C. coli*), the corresponding PAGE profiles were also different (e.g., in Fig. 1, serotype 34-A was *C. coli* whereas serotype 34-B was *C. jejuni*). These observations validate the use of PAGE for distinguishing strains.

PAGE profile patterns that distinguished strains were retained when the isolates were regrown from stock cultures and when repeatedly rerun in gels. Other investigators have also reported that PAGE profiles are reproducible (4). For some strains, analysis of PAGE profiles was facilitated by rerunning the isolates side by side in duplicate lanes in 10% gels and/or by using 8.5% gels. Further evidence supporting the use of PAGE protein profiles was that multiple isolates with identical PAGE profiles were also the same serotype and species. Other workers have reported different PAGE protein profile patterns among isolates of *C. jejuni* and *C. coli* when either whole-cell lysates (13) or outer membrane proteins (4, 11) were used. We found no evidence that variation in protein profiles could be attributed to antigenic drift. (i) Strains isolated chronologically from infants were distinguished by serotyping and DNA hybridization, as well as by PAGE. (ii) Many of the proteins accounting for differences in PAGE protein profiles do not correspond to proteins that have been identified as outer membrane proteins (4, 8).

Particular strains of *C. jejuni* or *C. coli* were reisolated from some infants at different times, e.g., infant 18 (Fig. 1). This could be explained by reinfection with the same strain. The evidence for this conclusion is strongest when the reisolations were at long intervals (14 to 27 weeks apart) and with negative cultures in the intervening period, such as in infants 3, 15, 17, and 18. Reisolation at shorter intervals of 4 to 8 weeks (Table 3) may also have been due to reinfection. There is also the possibility of failure to isolate the organism among mixed infections or because the numbers of organisms were fewer than the minimum of 10^2/g to 10^3/g required for detection by rectal swab (38) or for technical reasons. On the basis of these considerations, the duration of infection with an individual strain was recalculated by using the reisolation data in Table 3 (for ≤8-week intervals only) as the estimate of duration of infection with particular strains. The mean duration per strain was recalculated as 3.7 ± 3.3 (compared with the previous calculation of 3.0 ± 2.3). In conclusion, then, the 3- to 4-week duration of infection with a particular strain is similar to the course of infection in susceptible humans (5, 6) and experimentally challenged *M. nemestrina* (38). The data, therefore, favor the interpretation that long-term infection under endemic conditions may be attributed to continual reinfection, usually with different strains, and in most infants is probably not caused by a prolonged carrier state.

Mixed infections were detected occasionally. Sampling of multiple colonies on a culture plate was not undertaken to measure the extent of mixed infections. Mixed infections have also been reported in humans (14, 22).

Most of the episodes of diarrhea in neonates were not attributable to *C. jejuni*, *C. coli* or Nal^r *Campylobacter* sp. The data suggest than age-related susceptibility to *C. jejuni* or *C. coli*-associated-diarrhea occurs in infants between 1 and 4 months of age in the IPRL. This was observed in some infants with initial infection and also with recurrent infections (in infants which had previous brief periods of infection lasting less than 1 week and terminated by antibiotic treatment). Diarrhea was not observed in infants over 4 months old when reinfected with multiple strains. This suggests acquired protection against diarrheal disease but not against recolonization. Immune protection against diarrhea was demonstrated in *M. nemestrina* that were experimentally rechallenged 1 month after initial infection (38). The infants had elevated humoral immunity after initial challenge as measured by enzyme-linked immunosorbent assay. Further studies are warranted to investigate the course of passive and active immunity in the infants in this study and to correlate immunity with age of infection and susceptibility to diarrheal disease. Age-related elevations in humoral immunity have been reported in humans in developing countries; it has been suggested that high levels of immunoglobulin A in serum may correlate with protection under these circumstances (2).

Despite apparent protection from diarrhea afforded by age and previous infection, reinfection and recolonization occurred repeatedly. Because semiquantitative scores correlate approximately with titers of organisms in the feces, our results indicate that colonization with high numbers of *C. jejuni* or *C. coli* (with estimated titers of 10^8/g to 10^{10}/g) was not inhibited by increasing age. However, reinfection with low numbers of organisms suggests that various factors, e.g., the antigenic relationship between strains and the dose of infection, may also contribute to fluctuations in the numbers of organisms. We reported previously that after rechallenge of *M. nemestrina* infants, the homologous strain reached high titers but was cleared quickly (within 7 days), whereas a heterologous strain remained at higher titers for the 15 days of observation after rechallenge (38).

In conclusion, both the data reported in this study and also those from previous reports of multiple serotypes cultured from children during a 12-week study (43) support the hypothesis that long-term positive cultures observed under endemic conditions are caused by reinfection with different strains. This indicates that numerous strains are continually available for reinfection and transient colonization. Further studies of immune protection and cross-immunity between strains is needed to better understand the epidemiology and the immune basis of protection against disease.

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