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Seroepizootiology of *Helicobacter pylori* Gastric Infection in Nonhuman Primates Housed in Social Environments

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We determined the seroepizootiology of *Helicobacter pylori* infection in rhesus monkeys. Plasma was obtained from 196 animals (age range, 1 to 22 years) that were housed in social environments, either in indoor gang cages, in outdoor corrals, or in free-ranging forested conditions. Plasma immunoglobulin G levels were determined with a specific enzyme-linked immunosorbent assay, and the cutoff immunoglobulin G value for *H. pylori* seropositivity was determined from a study of 25 monkeys whose infection status was assessed by light microscopy and culture. One-year-old animals of both genders in all housing conditions had the lowest rate of positivity (60% in monkeys 1 year old versus 81% in monkeys 2 to 10 years old, $P = 0.026$). In addition, females tended to have higher rates of positivity than males. Seroconversion during a 1-year observation period occurred in 7 (28%) of 25 seronegative animals. Seroreversion occurred in 3 (4%) of the 78 positive animals; all 3 of these animals had received antimicrobial agents during the year. These observations demonstrate that the epizootiology of *H. pylori* infection in rhesus monkeys may serve as a model for human infection.

Helicobacter pylori is a spiral gram-negative bacterium that frequently infects the gastric mucosa of humans and causes chronic superficial gastritis (1). It now is clear that *H. pylori* infection is involved in the pathogenesis of peptic ulcer disease (22) as well as in adenocarcinoma of the distal stomach (17, 24, 26). The presence of *H. pylori* may be demonstrated by histology and culture of gastric mucosal biopsies or, noninvasively, by the urea breath test (29). In addition, determination of the presence of specific serum antibodies that consistently accompany the infection (2) has allowed the conduct of large epidemiologic studies (8, 28).

Ethical considerations make difficult the performance of studies attempting to clarify the pathophysiology of *H. pylori* infection in humans, and it is therefore important to develop and validate a relevant animal model. Although many animal species may be naturally or experimentally infected with various *Helicobacter* species that are similar to *H. pylori* isolated from patients (11, 13, 21), important phenotypic and genetic differences have been observed. In addition, the gastric pathology in these animal models is not identical to that present in humans.

Natural infection by *H. pylori*-like organisms has been reported to occur in several nonhuman primates (3–5, 10), and PCR amplification and partial 16S rRNA gene sequence analysis of spiral bacteria cultured from gastric biopsies of rhesus monkeys have indicated 98 to 100% homology with human strains of *H. pylori* (7, 15). Furthermore, this infection is persistent and is associated with acute as well as chronic gastritis and elevated specific immunoglobulin G (IgG) levels, all of which may be suppressed by therapy (9). Taken together, these data indicate that, as with humans, rhesus monkeys naturally have persistent *H. pylori* infection. Therefore, this animal appears to represent a good model to evaluate the role played by *H. pylori* in the production of gastritis and of the associated immune response. Furthermore, the model also permits the evaluation of novel therapies (9). However, the epizootiology of *H. pylori* in this species is at present unknown. The goal of our study, therefore, was to evaluate the seroepizootiology of *H. pylori* infection in rhesus monkeys of both genders across different age groups and different types of social housing conditions and habitat.

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MATERIALS AND METHODS

Animals. Domestic, colony-reared, male and female rhesus monkeys (*Macaca mulatta*) were randomly selected among different groups as indicated below. They were grouped as yearlings (0.5 to 1.5 years), juveniles/subadults (2 to 6 years), prime adults (7 to 10 years), and older adults (11 to 22 years). All experiments were conducted according to the principles set forth in reference 23a.

Housing conditions. Animals were bred in three types of housing conditions. The first type of housing was gang cages (10 by 11 by 10 ft [ca. 3.0 by 3.4 by 3.0 m]) with sealed cement floors, which contained an average of one adult male and five adult females. The total gang cage population consisted of approximately 319 animals. The second housing type was corrals (60 by 100 ft [ca. 18 by 30 m]) with a grass and dirt substrate and a large arboreal jungle gym. The average group was composed of 3 adult males and 23 adult females. The total corral population consisted of approximately 355 animals. The third environment was a 475-acre South Carolina sea island in which approximately 4,200 animals were free ranging in forested conditions. There were 34 groups on the island, each composed on average of 51 adults (range, 14 to 325 animals; means, 6 males and 45 females per group).

Sampling. At the time of the physical examination given annually to the rhesus monkeys, a total of 202 animals of both genders and various ages in the three housing conditions described above were randomly selected (gang-caged social groups, 36 males and 37 females; corralled social group, 30 males and 34 females; free-ranging social group, 23 males and 43 females). Five milliliters of EDTA-treated blood was obtained from each animal, and the plasma was frozen at −70°C. One year later, plasma was again obtained from 106 (77%) of the 137

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TABLE 1. Seropositivity for *H. pylori* in the three housing conditions

Housing condition and age range (yr)	Females			Males			Males and females		
	Total no.	No. positive	% Positive	Total no.	No. positive	% Positive	Total no.	No. positive	% Positive
Gang caged									
≤1.5	2	2	100	4	1	25.0	6	3	50.0
2–6	17	12	70.6	14	13	92.9	31	25	80.6
7–10	6	5	83.3	2	2	100	8	7	87.5
11–22	12	10	83.3	16	11	68.8	28	21	75.0
All	37	29	78.4	36	27	75.0	73	56	76.7
Corralled									
≤1.5	6	4	66.7	6	2	33.3	12	6	50.0
2–6	10	9	90.0	11	7	63.6	21	16	76.2
7–10	5	5	100	4	3	75.0	9	8	88.9
11–22	13	11	84.6	9	6	66.7	22	17	77.3
All	34	29	85.3	30	18	60.0	64	47	73.4
Free ranging									
≤1.5	6	4	66.7	6	5	83.3	12	9	75.0
2–6	14	11	78.6	11	9	81.8	25	20	80.0
7–10	12	10	83.3	2	2	100	14	12	85.7
11–22	10	10	100	4	3	75.0	14	13	92.9
All	43	35	81.4	23	19	82.6	65	54	83.1

monkeys from the gang cages and the corrals sampled previously (53 monkeys each from gang cages and corrals).

Measurement of *H. pylori*-specific plasma IgG. Plasma IgG levels were determined blindly by using a previously described enzyme-linked immunosorbent assay (ELISA) (8, 28). The *H. pylori* antigen used in the ELISA was prepared from five *H. pylori* strains and represents a pool of sonicated whole cells composed of a mixture of protein and lipopolysaccharide antigens, as described previously (28). The sonicates from each strain were pooled and diluted in 0.05 M carbonate buffer (pH 9.6) to yield the optimal protein concentration of 10 μg/ml. A 0.1-ml aliquot of this solution was added to each well of a flat-bottom Immulon 2 plate (Dynatech Laboratories, Alexandria, Va.). The screening serum dilutions were 1:800, while peroxidase conjugates of goat anti-human IgG (Tago, Inc., Burlingame, Calif.) were diluted 1:2,000. Results were corrected for day-by-day variation of the ELISA and were expressed as ratios, i.e., the fraction of the mean plus three standard deviations of results for 40 healthy U.S. children. All assays were run in duplicate on two or more days (i.e., at least four times). Tests for possible cross-reactivity of *H. pylori* antibodies had been done by absorbing sera from *H. pylori*-infected persons that had high values in the IgG ELISA with cells of other enteropathogens (28). The assay was >95% sensitive and specific for human infection when an IgG ratio of >1.0 was considered indicative of the presence of anti-*H. pylori* antibodies. In monkeys, the ELISA was 83% specific and 69% sensitive with a ratio cutoff for positivity of 0.6 (9). In earlier studies, the correlation when anti-monkey and anti-human IgG conjugates were used was highly significant ($r = 0.80$; $P < 0.001$) (9).

Serological definition. Seropositivity and seronegativity were defined as ratios of ≥0.6 and <0.6, respectively, as previously described (9). Seroconversion was defined as a >100% increase in the IgG ratio to >0.6, and seroreversion was defined as a >50% decrease of IgG to a ratio of ≤0.6.

Statistical analysis. Results were expressed as means ± 1 standard error of the mean. Fisher's exact test and the Mantel-Haenszel corrected chi-square test were performed to test differences between groups.

RESULTS

Seroprevalence. The overall frequency of seropositivity increased with age, and there were no statistically significant differences between the three housing conditions (Table 1). Animals of both genders under 2 years old in all housing conditions tended to have the lowest rates of positivity (60% in monkeys under 2 years old versus 81% in monkeys 2 to 10 years old; $P = 0.026$). However, this difference was entirely due to the difference among the males (50 versus 82%; $P = 0.021$); the difference among the females (71 versus 81%) was not significant. Except for this difference, gender did not significantly affect the rate of *H. pylori* infection, although males of all age classes tended to have lower rates of positivity than females (73 versus 82%; not significant), with the difference being particularly clear in the younger (50 versus 71%; not significant) and in the older (69 versus 88.5%; not significant) age groups (Fig. 1). Finally, 11- to 22-year-old males had a significantly lower rate of infection than did 4- to 10-year-old males (64 versus 91%; $P = 0.01$).

Follow-up at 1 year. At the 1-year follow-up, seroconversion was observed in 7 of 24 previously seronegative animals; 7 of 12 of those monkeys that were ≤6 years old seroconverted, versus 0 of 12 of those that were >6 years old (Fig. 2; $P < 0.001$). The mean (± standard error of the mean) age of animals that seroconverted was 3.3 ± 0.8 years, versus 11.3 ± 1.5 years for those that did not seroconvert (Fig. 2; $P < 0.001$). Five of six seronegative males among corralled animals seroconverted, while two of five seronegative females among gang-caged monkeys seroconverted. The number of seronegative animals in each gender and type of housing was too small to allow evaluation of statistical differentiation of the rate of seroconversion among these subgroups. Of 78 animals that were seropositive

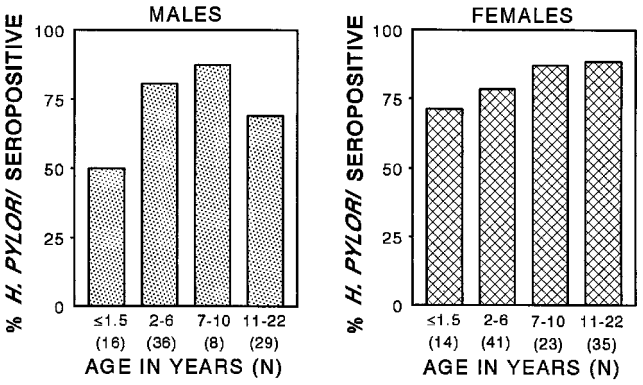

FIG. 1. Mean prevalence of seropositivity by age and gender among the 202 monkeys studied. The bars illustrate the percentage of *H. pylori*-positive animals in each age group in each gender. The numbers in parentheses represent the number of animals in each group.

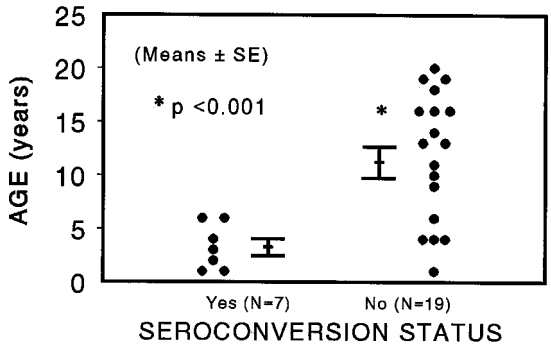

FIG. 2. Ages of previously seronegative monkeys that seroconverted (left) and did not seroconvert (right) at the 1-year follow-up. Each point illustrates the age of one animal, and the bar represents the mean age (± standard error of the mean) in each group. Two of the 26 seronegative animals were not tested at 1 year and do not appear on the figure.

at the first sampling, 3 (4%) had seroreverted at resampling 1 year later. Two were corralled females (ages 6 [odds ratio, 1.872 to 0.552] and 7 [odds ratio, 2.558 to 0.538] years), and one was a 20-year-old gang-caged male (odds ratio, 2.395 to 0.188). All three of these animals had received antimicrobial agents (erythromycin and/or Peptobismol) during the previous year, versus 27 of the other 78 persistently seropositive animals ($P = 0.047$).

DISCUSSION

The present studies demonstrate a high prevalence of seropositivity for *H. pylori* in the monkeys in each of the three housing conditions. This high seroprevalence is entirely consistent with our knowledge of the endemicity of *H. pylori* infection among humans in developing countries (23, 27). In addition, as often occurs in human populations in developing countries (23, 27), *H. pylori* infection was acquired before the age of 2 years by >50% of the rhesus monkeys housed in social environments. Although it was high from an early age, the seroprevalence of *H. pylori* in these animals further increased with age, as has been reported for humans (8, 14), and this effect may be due to a continuous risk of infection (32). However, there was little variation in the high (50 to 93%) positivity rate, similar to what is observed in developing countries in persons between the ages of 10 and 60 years. The lower rate of infection in older males may reflect a cohort effect or a decline in prevalence, possibly due to gastric atrophy (18). This latter possibility is supported by our recent longitudinal study of patients who developed severe atrophic gastritis with pernicious anemia and in whom serum antibody responses to *H. pylori* diminished and disappeared (4a).

Females tended to become infected at younger ages, and this could be related to their having more social interactions than males. In humans, no consistent difference between males and females has been reported (14, 23). *H. pylori* infection occurred as frequently in housing conditions in which there was little contact with humans (that is, in free-ranging animals) as in gang-caged and corralled monkeys, which were in closer contact with humans. Thus, as expected, the environment or the proximity to humans does not appear to play a substantial role in the epizootiology of the infection, and *H. pylori* is enzootic among the monkeys.

Only 4% of *H. pylori*-positive animals seroreverted during a 1-year period, and these three animals had received antimicrobial agents. This finding is consistent with our previous observation that, as it does in humans, *H. pylori* persists in naturally infected monkeys (9). In humans, the spontaneous rate at which infection is cleared in the absence of antibiotics also appeared to be <1%/year (6, 20, 25, 30).

In contrast, seroconversion occurred at a very high rate (28%) among seronegative animals. Furthermore, all of those monkeys that seroconverted were less than 6 years old, which means that the rate of seroconversion was >50% among the 1- to 6-year-old monkeys. This high rate is entirely consistent with the progressive age-related change in seropositivity until all the animals that are susceptible to the infection are infected, possibly by the age of 6 years under these conditions. Thus, the young monkeys were susceptible but had not yet been exposed, while the older monkeys no longer were susceptible as a consequence of *H. pylori*-related antral gastritis (9, 18). The small size of each subgroup of seronegative animals precluded analysis of the effect of gender or housing conditions on the rate of seroconversion. However, the observation that the seroprevalence remained higher among the older females suggests that this group may be continually more exposed to transmission of *H. pylori* or that atrophic changes progressed more slowly in females. Biopsies of older monkeys will be needed to address this question. Technical considerations prevented our sampling free-ranging animals at 1 year. However, the observation that the type of housing condition did not influence the seroprevalence suggests that similar conversion rates may be present in this latter group.

Like that in humans, the route of transmission of *H. pylori* infection in rhesus monkeys is unknown. Contamination of the water supply may play a role in certain developing countries (19), but this possibility appears to be excluded for our study because the water supply to the monkeys housed in gang cages and corrals was the chlorinated municipal water supply. Since the frequency of contacts with humans did not appear to substantially modify the seroprevalence of infection, it is likely that *H. pylori* is transmitted from monkey to monkey, possibly via the fecal-oral or the oral-oral route (12, 31). Consumption of feed contaminated by saliva (12) or feces (31) could be the vehicle of this transmission, as was recently demonstrated for vegetables (16).

In conclusion, our observations demonstrate that the epizootiology of *H. pylori* in rhesus monkeys is similar to the epidemiologic characteristics reported for human infection, especially in developing countries and in groups at low socioeconomic levels. Thus, this animal model may provide important and relevant information by allowing modifications of the environment that are not possible in studies involving humans. Correlation with gastric pathology should permit investigations and interventions under way that are relevant to both peptic ulcer disease and gastric cancer.

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