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ORIGINAL ARTICLE

Prevalence of *Helicobacter pylori* infection and intestinal parasitosis in children of the Xingu Indian Reservation

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Abstract

Objective: To evaluate the prevalence of *Helicobacter pylori* infection and its association with intestinal parasitoses in children from indigenous communities of the Xingu Indian Reservation, in Brazil.

Methods: A total of 245 Native Brazilian children between 2 and 9 years of age, from six villages of the Xingu River region, a tributary of the Amazon River, were assessed. *H. pylori* was detected using the ^{13}C-urea breath test. Breath samples were collected at baseline and 30 minutes after ingestion of 50 mg of ^{13}C-urea diluted with 100 mL of water flavored with passion fruit juice and sweetener. Stool samples were collected for the stool ova and parasites exam for 202/245 (82.4%) children.

Results: The overall prevalence of *H. pylori* was 73.5%. A significant association of *H. pylori* with increased age was observed among the different villages and ethnic groups. Positive results for the presence of parasites – 97.5% (198/202) – from the stool samples collected showed no association with *H. pylori*. Giardia showed an association with *H. pylori* in the multivariate analysis. Risk factors for *H. pylori* infection were observed in Kisêjê and Kaibi ethnic groups (OR [odds ratio] = 3.36 and 4.00, respectively), as well as in Tuiararé, Ngojwere, Capivara, Diauarum, and Pavuru villages (OR = 8.10, 4.10, 4.88, 1.85, and 1.40, respectively).

Conclusions: *H. pylori* infection is highly prevalent in these communities, as well as intestinal parasitoses. However, there were significant differences in the prevalence of *H. pylori* among the diverse villages studied. Giardia was closely associated with *H. pylori* infection.

J Pediatr (Rio J). 2011;87(5):393-8: Epidemiology, prevalence, *Helicobacter pylori*, giardia.

Introduction

Helicobacter pylori (*H. pylori*) is a ubiquitous bacterium that colonizes the gastric mucosa of more than half of the world's population, with a clear predominance among developing countries' population. The infection is mainly contracted during childhood, primarily in high-risk populations such as socially deprived families, some ethnic groups, and health professionals.[1,2] In Brazil, the different levels of prevalence of *H. pylori* infection observed are possibly due to the great social, cultural and economic diversity found in our environment, and perhaps also as a result of different ethnic groups in the Brazilian population. However, a clear trend towards reducing the prevalence of *H. pylori* infection in children was found in a metropolitan area of our country.[3]

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The specific mechanism of transmission remains unclear at present. In urban societies, it is believed that *H. pylori* is spread by people who live in the same household through intimate contact, particularly from an infected mother or other relative within the household.[4-6] However, horizontal transmission may be the preferential means of spreading the infection among individuals living in rural areas.[7]

The fecal-oral route has been considered an important form of transmission, especially in developing countries, in which poor sanitation and lack of hygiene appear to play a fundamental role in the dissemination of the bacteria.[8] Although the microorganism has been detected in the stools of children with diarrhea, the isolation of this type of specimen is not common, due to the large quantity of contaminating microorganisms, which hinders the growth of *H. pylori*. Transmission may also occur directly through contaminated hands, inadequate water supply, or ingestion of contaminated raw vegetables. Intestinal helminthiasis, just like *H. pylori* infection, is endemic in certain regions of developing countries where children are constantly exposed to a series of enteropathogens.[9]

Few prevalence studies on *H. pylori* have been carried out with a large enough sample size and locally validated noninvasive diagnostic methods in children from indigenous communities.[10,11] Therefore, it is imperative that we obtain a better understanding of the epidemiology of *H. pylori* infection in children from the so-called primitive communities.

The objective of the present study was to evaluate the prevalence of infection by *H. pylori* and its association with intestinal parasitoses in children from 2 to 9 years of age who live in villages of the Xingu Indian Reservation (Parque Indígena do Xingu, PIX), in the state of Mato Grosso, Brazil.

Methods

According to a 2005 Census, the total number of children from 2 to 9 years old in the studied villages of the PIX was estimated to be 365. We evaluated approximately 2/3 of them (67%, 245/365). Children with chronic incapacitating diseases were excluded from the study. Children received no antihelminthic, antibiotic or proton pump inhibitor treatment at least in the 2 months previous to the study. The study was held from August 7 to 17, 2007, a month of low rainfall, at which time river navigation between the villages is feasible.

Study population

The communities of the PIX are isolated and supposedly stationary, maintaining their traditions. The PIX, created in 1961, is situated in the Brazilian Midwest, along the Xingu River, which flows into the Amazon River.[12] The area within its borders measures 2,900,000 hectares, and it hosts a variety of indigenous groups, which differ in ethnicity, language, and culture. There are 14 different people, distributed over 67 villages. Outside the reservation, where the headwaters of the Xingu River and its tributaries are, there are small cities with inadequate basic sanitation that contaminate the water. Additionally, residues from pesticides used intensively in the soybean farms surrounding the reservation could make the water of the river unsafe to drink. As a result, the water quality of the river has been deteriorating for many years, and, in 2003, wells of up to 50 meters in depth were dug in the villages and solar powered hydraulic pumps were installed. The water pumped from the system feeds a reservoir that, in turn, distributes water to the indigenous communities through faucets placed near their huts. Some traditional habits of this population also increase the overall contamination of the environment. There is no appropriate destination for domestic garbage. Furthermore, Native Brazilians traditionally evacuate in the forest nearby the village, in the dirt. The Universidade Federal de São Paulo – Escola Paulista de Medicina (UNIFESP-EPM) has been providing medical care to this community since 1965.[12]

Selection of villages studied

Six villages located along the middle and lower Xingu River course were selected: Pavuru, Moygu, Tuiararé, Diauarum, Capivara, and Ngojwere. The selection criteria were: 1. Villages that receive medical care from UNIFESP-EPM; 2. Villages with the largest number of inhabitants; 3. The sixth village (Ngojwere), located on the Suyá-Missue River, a tributary of the Xingu River, was chosen not only for its large number of inhabitants, but also because it belongs to a different ethnic group, and because it lays along the easiest path for exiting the reservation at the end of the sample collection. The Pavuru and Moygu villages belong to the Ikpeng ethnic group; the Tuiararé, Diauarum, and Capivara villages belong to the Kaiabi ethnic group; and the Ngojwere village belongs to the Kisêjê ethnic group. Children were identified (name, village, and ID number) using UNIFESP-EPM records. All children aged 2 to 9 years old in the selected villages were asked to participate in the study.

Sample collection

The day before the collection of the samples, a meeting was held with local health workers in each village, when information on the project was provided. Later, the health workers visited the huts and provided explanations to the mothers about the collection method, using local language; then, collection flasks were distributed and instructions were given for collecting the stool samples. Furthermore, the breath sample procedures were explained and the test was scheduled to the following morning. On the next day, the mothers helped their children to carry out the breath test and returned the collected feces.

^{13}C-urea breath test

Two breath samples were collected, at baseline and 30 minutes after the intake of ^{13}C-urea diluted in 100 mL of water flavored with passion fruit juice and sweetener. To collect the breath samples, 650 mL aluminized bags connected to one-way valves were employed; for children unable to blow voluntarily, a mask was connected to the valve. Measurement of the $^{13}CO_2/^{12}CO_2$ ratio was carried out using an infrared spectrophotometer manufactured by IRIS (Infrared Isotope Analyzer, Wagner Analysen Technik, Bremen, Germany), with a cut-off value of delta over baseline (DOB) = 4‰.[13]

The samples were collected simultaneously in groups of 10 children; none of the children rejected the solution. One of the adults entertained the children with drawings to help keeping them relaxed and together. After the breath samples were collected, they were shipped to the nearest city, from where they were sent by post to our laboratory.

Stool ova and parasites exam

A collection kit with preservatives was used to conserve the fecal samples. They were shipped to our laboratory at the same time as the breath samples. The helminth and protozoan examinations were performed using the Hoffman method, with subsequent study of eggs and cysts by optical microscopy.

Statistical methods

The age of the individuals from each village was compared using analysis of variance. The association between each co-variable and the binary response variable (infection by *H. pylori*) was conducted according to the univariate binary logistic regression method. The assumption of linearity of continuous covariates on the logit scale was tested using fractional polynomial analysis. The multiple binary logistic regression model was then determined from variables that presented significance probability (p-value) of less than 0.25 in the univariate analysis. The fit of the multiple regression model was evaluated according to the Hosmer-Lemeshow statistic, whose p-value above 0.05 indicates a good fit. All the significance levels presented are two-tailed, and values below 0.05 were considered statistically significant. Both the statistical packages SPSS 10 (SPSS Inc., Chicago, IL, USA) and Stata 9.0 (Stata Corporation, College Station, TX, USA) were used for this analysis.

Ethical considerations

The research project was approved beforehand by the PIX community and subsequently by the EPM/UNIFESP Medical Research Ethics Committee.

Results

A total of 245 children were evaluated, representing a percentage from 41 to 90% of all the children from each village: Pavuru 26/40 (65%), Moygu 61/79 (77%), Tuiararé 46/51 (90%), Diauarum 40/97 (41%), Capivara 35/55 (63.6%), and Ngojwere 37/43 (86%). The mean age of the 245 children was 5.3±2.2 years, and 50% of them were under 5.1 years of age. There were no significant differences between the mean age of the children from the various studied villages ($p = 0.599$).

The overall prevalence of *H. pylori* infection was 73.5%, with no difference regarding gender. There was an association of the infection with increased age, villages, and ethnic groups (Table 1).

Parasitological exam

Stool samples were collected from 202/245 (82.45%) children. The result for the parasitological exam was positive in 197/202 (97.5%), with no statistical association with age ($p = 0.233$). The exam found: hookworm (*Ancilostoma duodenalis*) (3.5%), roundworm (*Ascaris lumbricoides*) (8.4%), *Entamoeba coli* (48.5%), *Giardia duodenalis* (30.7%), *Endolimax nana* (50.9%), *Hymenolepis nana* (21.8%), *Schistossoma mansoni* (0.5%), *Entamoeba histolytica* (0.5%), and *Iodamoeba butschlii* (6.4%). There was no association between the presence of parasites (helminths and protozoans) and the *H. pylori* status.

Multiple logistic regression

The Hosmer-Lemeshow test was used under the hypothesis that the multiple logistic regression model presents a well fit value ($p = 0.8705$). Since there is a close association between ethnic group and village — variables that are not independent from each other — only the variable village was included in the model, and, additionally, *Giardia sp.* was analyzed ($p < 0.2$) (Table 2). In the multivariate analysis, *Giardia sp.* infestation, age, and villages (Capivara, Diauarum, Ngojwere, and Tuiararé) were significantly associated with *H. pylori* infection (Table 3).

Discussion

In this study, we obtained a sample achieving almost 70% of the children population. We found that the infection rates are high from the earliest ages, affecting 60% of children in the first 3 years of life and rising to 85.3% between 8 and 9 years of age. The choice of a noninvasive test for epidemiological study in children is of fundamental importance. Two locally validated noninvasive tests were available: the ^{13}C-urea breath test and the fecal antigen test, using monoclonal antibodies.[14] In our local validation study, the sensitivity was 93.3% (95%CI [95% confidence interval] 86.9-99.8%), and the specificity was 96.2% (95%CI 93.6-98.8%).[13]

Table 1 - Prevalence of *H. pylori* according to sex, age, village, and ethnic group

	H. pylori			
Variable	**Negative, n (%)**	**Positive, n (%)**	**Total**	**p***
Sex				
Female	32 (27.8)	83 (72.2)	115	0.666
Male	33 (25.4)	97 (74.6)	130	
Age bracket				0.002†
2-3 years	34 (40.0)	51 (60.0)	85	0.005
4-5 years	13 (19.4)	54 (80.6)	67	
6-7 years	13 (22.0)	46 (78.0)	59	
8-9 years	5 (14.7)	29 (85.3)	34	
Village				
Pavuru	10 (38.5)	16 (61.5)	26	< 0.001
Moygu	27 (44.3)	34 (55.7)	61	
Tuiararé	5 (10.9)	41 (89.1)	46	
Diauarum	12 (30.0)	28 (70.0)	40	
Capivara	5 (14.3)	30 (85.7)	35	
Ngojwere	6 (16.2)	31 (83.8)	37	
Ethnic group				
Ikpeng	36 (42.4)	49 (57.6)	85	< 0.001
Juruna	3 (75.0)	1 (25.0)	4	
Kaiabi		18 (15.5)	98 (84.5)	116
Kisêjê	7 (17.9)	32 (82.1)	39	
Wauralkpe	1 (100)	0 (0.0)	1	

* Pearson chi-square test of association.
† p-value trend.

Table 2 - Univariate logistic regression of infection by *H. pylori* and the studied characteristics

Characteristics	**OR**	**P**	**95%CI for OR**
Sex (ref: female)			
Male	1.12	0.692	0.64-1.98
Age (in years)	1.26	0.001	1.09-1.45
Age bracket (ref: 2-3 years)			
4-5 years	2.76	0.007	1.31-5.83
6-7 years	2.36	0.026	1.11-5.01
8-9 years	3.87	0.011	1.36-10.98
Village (ref: Moygu)			
Capivara	4.76	0.004	1.63-13.93
Diauarum	1.85	0.152	0.80-4.31
Ngojwere	4.10	0.006	1.50-11.26
Pavuru	1.27	0.617	0.50-3.25
Tuiararé	6.51	0.001	2.26-18.74
Ethnic group (ref: Ikpeng)			
Juruna	0.24	0.231	0.02-2.45
Kaiabi	4.00	< 0.001	2.06-7.75
Kisêjê	3.36	0.010	1.33-8.46
Feces samples (ref: no)	0.48	0.099	0.20-1.15
Positive parasitological exam (ref: no)	0.61	0.666	0.07-5.61
Positive for pathogenic parasites (ref: no)	1.04	0.899	0.56-1.95
Protozoans (ref: no)	1.14	0.815	0.38-3.44
Helminths (ref: no)	0.90	0.760	0.47-1.73
Amoebas	0.79	0.539	0.37-1.69
Giardia duodenalis	1.78	0.108	0.88-3.62
Entamoeba coli	1.11	0.723	0.61-2.06
Hymenolepis nana	1.75	0.175	0.78-3.92
Iodamoeba butschlii	2.31	0.285	0.50-10.79
Ancilostoma duodenalis	0.52	0.407	0.11-2.42
Ascaris lumbricoides	0.53	0.241	0.20-1.51

95%CI = 95% confidence interval; OR = odds ratio.

Table 3 - Logistic regression model for the prevalence of *H. pylori* in children of the Xingu Indian Reservation

Characteristics	OR_{crude}	$OR_{adjusted}$	p	95%CI for $OR_{adjusted}$
Giardia sp. (ref: no)	1.78	2.57	0.017	1.18-5.6
Age	1.26	1.25	0.006	1.07-1.47
Village (ref: Moygu)				
Capivara	4.76	4.88	0.006	1.56-15.31
Diauarum	1.85	3.13	0.023	1.17-8.39
Ngojwere	4.10	7.46	0.001	2.17-25.65
Pavuru	1.27	1.43	0.487	0.52-3.96
Tuiararé	6.51	8.10	0.001	2.38-27.56

95%CI = 95% confidence interval; OR = odds ratio; $OR_{adjusted}$ = adjusted odds ratio; OR_{crude} = crude odds ratio.
Hosmer-Lemeshow chi-square test = 10.8, p = 0.213.

The risk factors associated with a high prevalence of *H. pylori*, such as overcrowding, lack of basic sanitation, ingestion of untreated water, and certain cultural habits, like eating with one's hands, defecating in the environment, and lack of food preservation methods, are common variables in the PIX and other indigenous communities.[15,16] Preventative measures will facilitate the development of strategies to reduce the prevalence of this infection in these populations.[17]

Despite sharing a similar habitat and similar social and cultural background, the difference between the villages could be justified by cultural differences (food processing), genetics,[18] or even the habitat (location of the village along the river). The Kaiabi ethnic group presented the highest prevalence of *H. pylori*. Kaiabi Native Brazilians normally ferment some types of food in their mouths; pre-mastication is still common in some traditional communities, and is linked to a higher risk of *H. pylori* transmission.[19] Evidence indicates that *H. pylori* may be present both temporarily and permanently in the oral cavity of human beings, with the formation of microaerophilic niches in plaques in the gum and the teeth that would serve as an additional gastric source of transmission for *H. pylori*. Efforts to cultivate *H. pylori* from oral samples, however, have not been successful, and there are few studies that report success in isolating *H. pylori* from dental plaque and saliva.

We observed that the prevalence of *H. pylori* in the villages studied was lower in the upper stream of the river (Pavuru and Moygu). It would be interesting to evaluate the prevalence in the population farthest upstream (Springs) and farthest downstream, to evaluate the influence of greater contamination in lower regions.[20] *H. pylori* bacteria have the capacity to form biofilms in water, which protect them against environmental threats[21] and may enhance the transmission.[22]

The six villages that we studied have wells, but the consumption of river water continues, especially by the children, when they play in the river, while fishing, and even when they travel to other locations in their canoes. Since well water has been consumed in these villages for 5 years, we could expect a lower prevalence of *H. pylori* in children under 5 years of age, due to the cohort effect, but the incidence remains high.

Polymerase chain reaction-based methods can overestimate *H. pylori* in water, while culture methods can underestimate it.[23] *H. pylori* can survive over 7 days in water,[24] according to a study that reported successfully cultivating the bacterium from drinking water.[25] Drinking contaminated water has to be considered a potential source of transmission of *H. pylori*.[26] The dissemination of *H. pylori* could occur by water in regions where water treatment is suboptimal, or by the ingestion of well[26] or river water.[20]

Giardia infection was an independent factor associated with infection by *H. pylori*, and this finding was not observed with other intestinal parasites detected in the stools of the Native Brazilian children. This observation was similar to that reported by Moreira et al.,[9] suggesting a common mode of transmission of these two microorganisms.

The high prevalence of parasitic infestation and hepatitis A, found in PIX communities, is an indirect evidence of the fecal-oral route for *H. pylori* infection.[27,28] Moreover, the concept that the transmission of *H. pylori* is due to the fecal-oral route is accepted by several different authors.[29,30]

Regardless of the means of transmission, the population of the indigenous community presents high risk for *H. pylori* infection at any time during childhood, and the fecal-oral transmission should be considered the most likely route.

In conclusion, the present study showed a high prevalence of *H. pylori* infection in these indigenous communities. However, there were significant differences in the prevalence of *H. pylori* among the diverse villages studied. Giardia was closely associated with *H. pylori* infection.

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