Indian J Gastroenterol (March–April 2016) 35(2):106–112
DOI 10.1007/s12664-016-0641-4

ORIGINAL ARTICLE

Association between *Helicobacter pylori* and intestinal parasites in an Añu indigenous community of Venezuela

Alisbeth D. Fuenmayor-Boscán [1] · Ileana M. Hernández [2] · Kutchynskaya J. Valero [1] · América M. Paz [1] · Lisette B. Sandrea [1] · Zulbey Rivero [1]

Received: 15 December 2015 / Accepted: 13 March 2016 / Published online: 2 May 2016

Abstract
Background *Helicobacter pylori* (*Hp*) and enteroparasite infections are highly prevalent in populations with poor living conditions, like the Amerindian communities. Identifying associations between both types of infectious agents could help to detect shared risk factors or transmission routes in these minority ethnic groups. Therefore, the prevalence and association between *Hp* and enteroparasites were investigated in an indigenous community whose living conditions favor such infectious diseases.
Methods Seropositivity (anti-*Hp*-specific IgG) and active infection (stool antigen test), intestinal parasitosis (direct and concentrated coproparasitological test, methylene blue, and Kinyoun stains), and risk factors for fecal-oral transmission were determined in 167 children and 151 adults of the Añu indigenous community living at the Sinamaica Lagoon, in Venezuela.
Results A high rate of *Hp* infection (seropositivity and active infection) and enteroparasitosis was evidenced, as expected. Some significant associations were detected: direct associations between *Hp* and polyparasitic infection, helminths, and protozoan (particularly in children); inverse association between *Hp* and *Giardia lamblia*. No shared epidemiological factors were identified for *Hp* and the detected intestinal parasites, probably due to overlapping factors.
Conclusion Direct associations detected support the participation of the fecal-oral route in the transmission of the involved infectious agents. Inverse relationship (*Hp*) and *G. lamblia* may suggest the existence of antagonistic interactions between them. Further research is required to elucidate the mechanisms underlying these associations.

Keywords Añu · *Helicobacter pylori* · Indigenous community · Intestinal parasites · Transmission

✉ Alisbeth D. Fuenmayor-Boscán
alisbethdfb@gmail.com

[1] Facultad de Medicina, Departamento de Microbiología. Escuela de Bioanálisis, Universidad del Zulia, Maracaibo, Venezuela

[2] Facultad de Medicina Instituto de Investigaciones Biológicas, Universidad del Zulia, Maracaibo, Venezuela

Introduction

Helicobacter pylori (*Hp*) is the most common chronic bacterial infection in humans worldwide. Its prevalence is higher in developing countries (80 % to 90 %) than in industrialized nations [1, 2]. Poverty, poor drinking-water quality, infected parents, and ethnicity are included among the risk factors associated to its high prevalence [3–6]. Such factors have been also widely involved in the epidemiology of intestinal parasite diseases.

Co-infections by *Hp* and punctual intestinal parasites have been studied in previous investigations with different purposes and controversial outcomes [7–15]. Some of these studies have suggested that antagonist and/or synergistic interactions between these agents could trigger significant consequences for human health, probably depending on hosts and environmental circumstances [12, 13, 15]. In this respect, it has been proposed that certain intestinal parasites could reduce the probability of infection by *Hp*. In fact, a minor prevalence of infection by this microorganism has been described in subjects with *Ascaris lumbricoides* and *Entamoeba histolytica* [15, 16]. Nevertheless, the nature of such interactions has not been enough clarified up to date. From the epidemiological point of view, it could be assumed that if *Hp* and intestinal parasites have similar transmission routes or share environmental risk factors for the acquisition and retention of infection, prevalence and coexistence of both types of agents should be very high in populations with poor living conditions, such as those predominant in indigenous communities of Latin America. On the other hand, if indeed intestinal parasites exert some type of protection against *Hp*, then the prevalence of this infection should be lower in communities exhibiting chronic intestinal parasitic infections. Elucidation of this matter could contribute both to the knowledge regarding the effects that these infectious agents have on the human gastrointestinal (GI) tract and to the formulation of new prevention and control strategies against *Hp* infection in high risk populations.

Therefore, the purpose of this study was to investigate *Hp* prevalence and its possible association with a wider variety of intestinal parasitic infections. Its study was realized in a Venezuelan indigenous community whose living conditions appear to favor the prevalence of both types of infectious agents, further contributing to characterize these infections in this minority ethnic group of Latin America.

Methods

Population

This investigation was performed on an "Añu" native community located on the Sinamaica Lagoon, Zulia State, Northwest Venezuela. The Añu, whose name means "water people," live on rudimentary houses built with mangrove branch and thatch, supported on stilts over the water or on the shore's humid grounds. Their houses lack potable water and of an adequate excreta and waste disposal. Served water, feces, and waste drain directly into the lagoon, which is also used for consumption and domestic activities. Potable water is delivered in tank trucks to the nearest town and then taken by boat to the houses where it is stored in improvised barrels. Additionally, a wide variety of animals walk around and inside the houses. Rudimentary fishing and handcrafts are their principal economic activities.

Sixty-two Añu family groups were randomly selected, representing approximately 10 % of all families living in the lagoon to the time of the study. The selection was made using a sketch with the distribution of housing, prepared by the Culture Department of the Universidad del Zulia. All participants were informed about the characteristics of this study obtaining their previous written consent. Parental authorization was requested for children participation. The survey was performed in 318 individuals with or without GI symptoms: 167 children (age range 3 months to 15 years old; age average 7.4 ± 4.1 years old) and 151 adults (age range 16 to 84 years old; age average: 39.1 ± 17.1 years old).

Biological samples and data collection

A structured verbal interview was administered to the mothers or grandmothers of the Añu community in order to obtain information regarding the family members including age, sex, socioeconomic status (Graffar scale) [17], the presence or absence of humid soil around the houses, the presence of children under 14 years old and number of children at home, overcrowding, and markers of exposure to fecal-oral transmission route: access to potable water, treatment applied to drinkable water (chlorine vs. non-chlorine), and keeping animals at home or surroundings.

Biological samples were collected in every home. Venous blood (5 to 10 mL) was aseptically collected from each participating individual. Serum aliquots were preserved at −20 °C. A fresh stool sample was obtained from each individual, analyzed the same day (coproparasitological test), and then preserved at −20 °C for subsequent detection of *Hp* antigens.

Detection of seropositivity and active infection by *H. pylori*

Detection of the IgG serum antibodies, specific for *Hp* antigens, was performed using a commercial enzymatic immunoassay (Pyloriset® EIA-G III, Orion Diagnostic, Espoo, Finland) following the manufacturer's instructions. High sensitivity and specificity have been previously reported for this test in other countries [18, 19], and its high sensitivity (100 %) has been also proven in an ethnically mixed population of the State of Zulia, Venezuela, using a combination of microbiologic culture, rapid urease test, and the polymerase chain reaction in biopsies, as a gold standard [20].

As an indicator of active infection, fecal excretion of *Hp* specific antigens was determined in 163 seropositive individuals randomly selected, using a commercial enzymatic immunoassay (Premier Platinum HpSA®, Meridian Diagnostics, Inc., Cincinnati, USA) following the manufacturer's instructions. This trial presented a sensitivity of 100 % and a specificity of 79 % in a sample of ethnically mixed population of Caracas, Venezuela [21].

Coproparasitological diagnosis

Fresh stool samples were analyzed to detect parasites through a direct microscopic examination using saline solution, temporary staining for protozoa (Lugol and buffered methylene blue), and the modified Ritchie concentration technique (formalin/ethyl acetate). In this study, no specific diagnosis method was applied for *Enterobius vermicularis*. Parasitic forms similar to *E. histolytica* were reported as "*Entamoeba histolytica/E-dispar* complex." All stool preparations were independently examined by two expert parasitologists.

Statistical analysis

The statistical significance of the differences, dependence between variables, and linear tendencies were determined by the χ^2 test ($p < 0.05$). Risk factor analysis was based on the odds ratio (OR) with 95 % confidence intervals.

Results

H. pylori prevalence

Seropositivity for *Hp* was detected in 76.4 % of the population sample, being higher in adults than in children (Table 1). The specific fecal antigen excretion of the microorganism was positive in 93.9 % of the seropositive individuals studied, with no significant difference between children and adults (data not shown). Since this test was not practiced on seronegative individuals and taking into consideration that active infection was found in the majority of the seropositive individuals, seropositivity was the parameter used for the subsequent analysis.

Intestinal parasites prevalence

The presence of intestinal parasites was evidenced in 89.6 % of the individuals, with predominance of polyparasitism (more than one parasite species in one individual) and protozoan infections, both children and adults. The prevalence of helminth infections resulted considerably lower compared to the protozoan infections even in children (Table 1).

H. pylori and intestinal parasite infections by age group

Upon analyzing seropositivity for *Hp* and intestinal parasite infections by age group, a high prevalence of both types of infections was evidenced since early childhood. However, parasitic infections in general, and protozoan, outnumbered the *Hp* seroprevalence, even in adults (Fig. 1). During the first life decade, an ascending tendency in the prevalence of almost all the investigated infections was observed. *Hp* seropositivity reached its maximum level during adulthood (~90 %). In contrast, prevalence of *Giardia lamblia* and helminths tended to progressively diminish with age (Fig. 1).

Association between *H. pylori* and intestinal parasites

Seropositivity for *Hp* resulted significantly associated to polyparasitism and helminths in children. The presence of these forms of parasitism doubles the risk of *Hp* infection in this population group (Table 2). An ascending tendency for *Hp* seropositivity in relation to the number of infecting parasite species was observed in children, with a risk six times greater for seropositivity in the presence of simultaneous coinfection by four or more parasite species (Fig. 2). Additionally, *Hp* seropositivity was greater in individuals with protozoan (Table 2); however, these parasites behave as a risk factor for *Hp* in the total population, but not in children or adults separately. It is important to emphasize that, statistically, *G. lamblia* behaved as a protection factor against *Hp* infection in the total population sample, being the risk of seropositivity 0.4 times lower in individuals with this protozoan than in individuals free of it ($p < 0.01$) (Table 2).

Figure 3 shows the analysis of associations between intestinal parasites and *Hp* seropositivity in age groups less than 20 years. In individuals with polyparasitism, helminths, or protozoan, a higher seropositivity was evident in most of age groups, when they were compared with non-parasited individuals. Nevertheless, it was significant only in the 0–5-year-old group with helminths and in the 16–20-year-old group with protozoan infections. With respect to the negative association between *G. lamblia* and *Hp*, it was present in several age groups but only resulted significant in the 6–10-year-old age group. In other adult age groups, no significant associations were obtained (data not shown).

Risk factors for *H. pylori* and intestinal parasite

In an attempt to identify possible shared risk factors that could explain the positive associations between *Hp* and certain intestinal parasite, some environmental markers indicative of exposure to fecal-oral transmission route were researched. It is important to recognize that the living conditions of the

Table 1 Prevalence of *H. pylori* seropositivity and enteric parasitic infection in children and adults

Age		*H. pylori*+ (%)	Parasitic infection+ (%)			Parasite type (%)		
Group	*n*		Total	Monoparasitic	Polyparasitic	Helminths	Protozoan	*G. lamblia*
Children	167	65.9a	89.2a	19.2	70.1	47.3c	83.8	23.4d
Adults	151	88.1a	90.1	18.5	71.5	27.2c	85.4	7.9d
Total	318	76.4b	89.6b	18.9	70.8	37.7	84.6	16.0

Data which contain the same lowercase letters indicate differences between results which are statistically significant ($p < 0.01$)

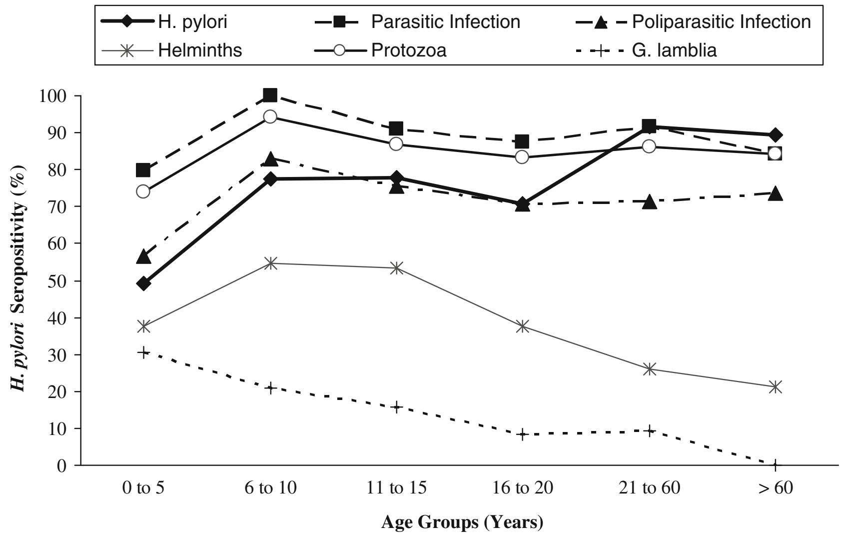

Fig. 1 Prevalence of *H. pylori* seropositivity and enteric parasitic infections, according to age groups. *p*-values for linear trends (χ^2 test): *H. pylori*, helminths, and *G. lamblia*: $p < 0.01$; parasitic infection: $p < 0.05$; poliparasitic infection and protozoan: $p > 0.05$

population hindered the independent and objective study of these factors. Variables such as overcrowding, non-chlorinated water, presence and number of children at home (more than three) were risk factors only for intestinal parasite infections. Humid soil surrounding the houses constituted a risk factor only for geo-helminths infections. None of the studied variables behaved as a shared risk factor for *Hp* and intestinal parasites (data not shown). Due to the fact that all participants belonged to the socioeconomic stratum IV or V (poverty or extreme poverty as per the Graffar scale), it was not possible to analyze the role of the socioeconomic stratification as a risk factor within this population.

Table 2 Enteric parasitic infections as risk factors for *H. pylori* seropositivity, in children and adults

Age group	*Hp*+/PI status (%)		OR	95 % CI
	Hp+/PPI+	*Hp*+/PPI–		
Children	84/117 (71.8)	26/50 (52.0)	2.35*	1.18–4.66
Adults	98/108 (90.7)	35/43 (81.4)	2.24	0.82–6.13
Total	182/225 (80.9)	61/93 (65.6)	2.22**	1.29–3.82
	Hp+/helminths+	*Hp*+/helminths–		
Children	61/79 (77.2)	49/88 (55.7)	2.70**	1.38–5.29
Adults	38/41 (92.7)	95/110 (86.4)	2.0	0.55–7.31
Total	99/120 (82.5)	144/198 (72.7)	1.77*	1.01–3.11
	Hp+/protozoan+	*Hp*+/protozoan–		
Children	96/140 (68.6)	14/27 (51.9)	2.03	0.88–4.67
Adults	116/129 (89.9)	17/22 (77.3)	2.62	0.83–8.29
Total	212/269 (78.8)	31/49 (63.3)	2.16*	1.13–4.14
	Hp+/*G. lamblia*+	*Hp*+/*G. lamblia*–		
Children	22/39 (56.4)	88/128 (68.8)	0.59	0.28–1.23
Adults	9/12 (75.0)	124/139 (89.2)	0.36	0.09–1.49
Total	31/51 (60.8)	212/267 (79.4)	0.40**	0.21–0.76

Parasitic status considered for the risk analysis were those significantly associated to *H. pylori* according to chi-square statistic (data not shown)

Hp H. pylori, *PI* parasitic infection, *PPI* poliparasitic infection, *OR* odds ratio

*$p < 0.05$; **$p < 0.01$, when both ratios were compared into the same age group

Discussion

Indigenous communities are among the poorest groups in Latin America. Geographical and socioeconomic segregation turns them into an easy target for acquisition of infectious agents. Therefore, microbiological research in these human groups could be very useful from an epidemiological perspective. For these reasons, an indigenous "Añu" community of Venezuela was chosen for this research. Although intestinal parasite infections has been frequently studied in this type of communities, the epidemiological characteristics of *Hp* infection and its possible association with the first one has been scarcely investigated.

The high *Hp* seropositivity detected in the Añu community, both in adults and children, is similar to the previously described in other Latin American ethnic minorities [10, 12, 22, 23]. Nevertheless, prevalence rates different from the ones reported herein have been documented in children from some Amazonian indigenous groups [12, 22, 23]. Such variability could be related to cultural or environmental differences. To support this argument, some investigations have attributed a significant influence to socioeconomic and seasons variations on the prevalence of *Hp* infection [24, 25]. In agreement to the high seropositivity for *Hp*, an elevated prevalence of active infection by this microorganism was also detected in Añu individuals; this suggests that microorganism eradication through spontaneous clearance or pharmacological treatment is rare in this population. A high re-infection rate could also explain these results.

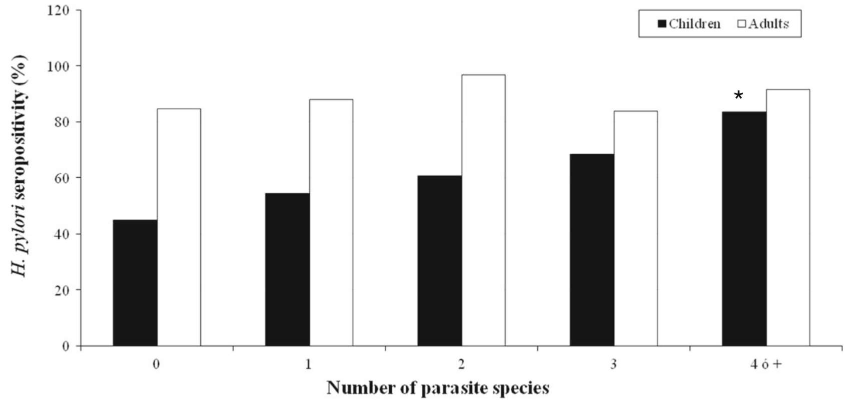

Fig. 2 Trend *H. pylori* seropositivity in children and adults depending on the number of parasite species detected. χ^2 14.28 in children ($p < 0.01$); 3.54 in adults ($p > 0.05$). Asterisk indicates OR to four or more parasites in children: 6.24; 95 % CI: 2.01–19.41 ($p < 0.01$)

Regarding the observed increase in *Hp* seroprevalence with age, this could obey to the continuous risk of infection during lifetime, as it has been proposed for other communities with deficient life conditions [26, 27]. The high frequency of parasite infections in Añu individuals of all age groups seems to support this argument. Although some authors have attributed similar results to a possible "cohort effect" based on the improvement in the quality of life of the communities through the years [28–30], there is scarce evidence in favor of an economical or environmental evolution in the Añu community. In fact, both children and adults continue to consume and use the lagoon's water in spite of its increasing fecal contamination [31].

The high intestinal parasite prevalence, the degree of polyparasitism and the predominance of protozoan infections, confirms that the life style of Añu community is favorable for the permanent contact with infection sources and re-infection by GI microorganisms. On the other hand, helminth infections could be the consequence of periodic exposures of Añu to humid soils contaminated with human and animal stool residues, whenever the lagoon's water level descend during the dry season. It has been previously described that the type of soil, temperature, and environment humidity of the Sinamaica Lagoon favor the preservation of the infecting power of helminth's eggs [32].

In the present work, *Hp* and intestinal parasite infections resulted directly and significantly associated, as deduced from the similar prevalence of both types of infections regarding age, especially during the first two life decades. This suggests the presence of shared epidemiological elements for both types of infections, one of which could be the fecal-oral transmission route. Other authors have used this argument to explain some associations between *Hp* and intestinal protozoan, as *G. lamblia* and *Blastocystis hominis* [14, 33]. However, the environment exposure markers to the fecal-oral transmission route investigated herein did not associate to the *Hp* seropositivity or any parasite species in particular. It is necessary to recognize that the elevated frequency of both groups of infectious diseases (*Hp* and intestinal parasites), their endemic character, the high frequency of coinfection, and the homogenous life style of this population may have masked any possible relation between environmental factors and such infections.

Some authors have proposed that infections by *Hp* and *G. lamblia* could be directly associated, particularly in children [14, 34, 35]. In contrast, there are no reports regarding inverse associations between these agents such as the one evidenced in the Añu community. This interesting finding could suggest the existence of an antagonistic interaction

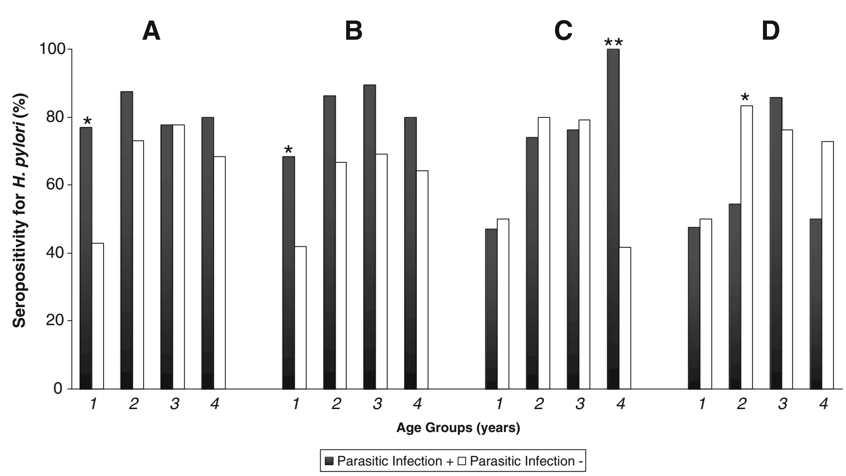

Fig. 3 Association between *H. pylori* seropositivity and parasitic species by age group *Capital letters* identify the species of parasites: *A Ascaris lumbricoides*, *B Entamoeba coli*, *C Endolimax nana*, *D Giardia lamblia*. The *numbers* identify age groups: *1* 0–5 years, *2* 6–10 years, *3* 11–15 years, *4* 16–20 years. *$p < 0.05$; ** $p < 0.01$

between *Hp* and some intestinal parasite. One possible explanation for these findings is that *Hp*, by means of excreting some products containing antimicrobial activity, may limit the colonization by exogenous intestinal pathogens [36]. Indeed, it has been described that children who are *Hp*-positive have less probabilities of suffering diarrheic diseases than children who are *Hp*-negative [36, 37]. Inverse associations between *Hp* and the parasites *E. histolytica* or *A. lumbricoides* have been previously attributed to an immunomodulatory effect induced by these parasites through of a Th2-predominant immune response [15, 16]. Previous investigations seem to support this argument. In certain endemic areas, *G. lamblia* infection seems to induce a persistent elevation of the secreting IgA [35], an antibody with certain protective effect against *Hp* [38, 39]. Therefore, the possibility that the inverse association between *Hp* and *G. lamblia* might be due to a protective mechanism of immune type triggered by the parasite should be considered. However, demonstration of such hypothesis is beyond the scope of this investigation.

In conclusion, the Añu indigenous population exhibited a high rate of seropositivity and active infection by *Hp* as well as an elevated prevalence for intestinal parasites. There are significant associations between certain enteroparasite and *Hp* seropositivity which are more evident in children. Parasite species involved in such associations and the risk factors for both types of infection are different to the ones previously reported; this might be due to a particular combination of factors inherent to the surveyed community. The results of this investigation contribute to support the important role of the fecal-oral route for the combined transmission of *Hp* and some intestinal parasites in the Añu population, while the inverse association between *H. pylori* and *G. lamblia* points out to the existence of a protective interaction between these microorganisms. Additional investigation is required to explain the nature of such interaction.

Acknowledgments The authors thank Professor Alí Fernández from the Culture Division of the Universidad del Zulia for the logistic support and information provided regarding the Añu population.

Source of funding This work was funded by the Scientific and Humanistic Development Council of the Universidad del Zulia, Venezuela (Subvention No. CC-0660-10).

Compliance with ethical standards

Conflict of interest ADFF-B, IMH, KJV, AMP, LBS, and ZR have no conflicts of interest to declare.

Ethics statement All procedures performed in this study were in accordance with the ethical standards of the Universidad del Zulia, the Code of Ethics for the Life of Venezuela, and ethical principles stated in the Declaration of Helsinki of 1975, as revised in 2000 and 2008 concerning Human Rights. And the authors followed the policy concerning Informed Consent as shown on Springer.com.

References

1. Go MF. Natural history and epidemiology of Helicobacter pylori infection. Aliment Pharmacol Ther. 2002;16 Suppl 1:3–15.
2. Tsongo L, Nakavuma J, Mugasa C, Kamalha E. Helicobacter pylori among patients with symptoms of gastroduodenal ulcer disease in rural Uganda. Infect Ecol Epidemiol. 2015;5:26785.
3. World Gastroenterology Organization. World gastroenterology organization global guideline: Helicobacter pylori in developing countries. J Clin Gastroenterol. 2011;45:383–8.
4. Khalifa MM, Sharaf RR, Aziz RK. Helicobacter pylori: a poor man's gut pathogen? Gut Pathog. 2010;2:2.
5. Salih BA. Helicobacter pylori infection in developing countries: the burden for how long? J Gastroenterol. 2009;15:201–7.
6. Coelho LG, León-Barúa R, Quigley EM. Latin-American consensus conference on Helicobacter pylori infection. Latin-American National Gastroenterological Societies affiliated with the Inter-American Association of Gastroenterology (AIGE). Am J Gastroenterol. 2000;95:2688–91.
7. Sabah AA, Gneidy MR, Saleh NM. Prevalence of Helicobacter pylori infection among adult patients with different gastrointestinal parasites in Tanta City district. J Egypt Soc Parasitol. 2015;45:101–6.
8. Ek C, Whary MT, Ihrig M, Bravo LE, Correa P, Fox JG. Serologic evidence that Ascaris and Toxoplasma infections impact inflammatory responses to Helicobacter pylori in Colombians. Helicobacter. 2012;17:107–15.
9. Escobar-Pardo ML, Ortiz de Godoy AP, Strehl Machado R, Rodrigues D, Fagundes Neto U, Kawakami E. Prevalence of Helicobacter pylori infection and intestinal parasitosis in children of the Xingu Indian reservation. J Pediatr (Rio J). 2011;87:393–8.
10. Rodriguez OL, Ortiz-Princz D, Cavazza ME, López E, Hagel I. Evaluation of the possible association between the presence of intestinal parasites and Helicobacter pylori in children of ethnic Warao Venezuela. Bol Mal Salud Amb. 2011;11:41–50.
11. Zeyrek D, Zeyrek F, Cakmak A, Cekin A. Association of Helicobacter pylori and giardiasis in children with recurrent abdominal pain. Turkiye Parazitol Derg. 2008;32:4–7.
12. Marini E, Maldonado-Contreras AL, Cabras S, et al. Helicobacter pylori and intestinal parasites are not detrimental to the nutritional status of Amerindians. Am J Trop Med Hyg. 2007;76:534–40.
13. Whary MT, Sundina N, Bravo LE, et al. Intestinal helminthiasis in Colombian children promotes a Th2 response to Helicobacter pylori: possible implications for gastric carcinogenesis. Cancer Epidemiol Biomarkers Prev. 2005;14:1464–9.
14. Moreira ED Jr, Nassri VB, Santos RS, et al. Association of Helicobacter pylori infection and giardiasis: results from a study of surrogate markers for fecal exposure among children. World J Gastroenterol. 2005;11:2759–63.
15. Torres J, Pérez-Pérez G, Ximenez C, et al. The association of intestinal parasitosis and *Helicobacter pylori* infection in children and adults from a Mexican community with high prevalence of parasitosis. Helicobacter. 2003;8:179–85.
16. Fernando N, Holton J, Zulu I, Vaira D, Mwaba P, Kelly P. Helicobacter pylori infection in an urban African population. J Clin Microbiol. 2001;39:1323–7.
17. Méndez-Castellano H. Estratificación social; método Graffar modificado. Arch Ven Pueric Ped. 1986;49:93–104.
18. Juncal AR, Garea MT, Cid A, et al. Assessment of 4 commercial immunoenzymatic assays for the serologic diagnosis of Helicobacter pylori infection. Enferm Infec Microbiol Clin. 1998;16:185–9.
19. Kindermann A, Konstantopoulos N, Lehn N, Demmelmair H, Koletzko S. Evaluation of two commercial enzyme immunoassays testing immunoglobulin G (IgG) and IgA responses for diagnosis of

Helicobacter pylori infection in children. J Clin Microbiol. 2001;39:3591–6.
20. Fuenmayor A, Avila M, Botero L, Cavazza ME. Diagnóstico Microbiológico y Serológico de Infección por Helicobacter pylori en Población Marabina. XXVI Jornadas Venezolanas de Microbiología "Dr. José Esparza". 01 al 04 de Noviembre de 1999. Valencia. Estado Carabobo. Venezuela.
21. Urrestarazu MI, Serrano N, Uzcátegui A, et al. Evaluation of an immunoenzymatic assay for the detection of Helicobacter pylori in stool samples. Rev Soc Ven Microbiol. 2001;21:23–5.
22. Ortiz D, Cavazza ME, Rodríguez O, Hagel I, Correnti M, Convit J. Prevalence of Helicobacter pylori infection in Warao lineage communities of Delta Amacuro State. Venezuela Mem Inst Oswaldo Cruz. 2003;98:721–5.
23. Robinson LG, Black FL, Lee FK, et al. Helicobacter pylori prevalence among indigenous peoples of South America. J Infect Dis. 2002;186:1131–7.
24. Domínguez-Bello MG, Beker B, Guelrud M, et al. Short report: socioeconomic and seasonal variations of *Helicobacter pylori* infection in patients in Venezuela. Am J Trop Med Hyg. 2002;66:49–51.
25. Camargo MC, Yepez MC, Ceron C, et al. Age at acquisition of Helicobacter pylori infection: comparison of two areas with contrasting risk of gastric cancer. Helicobacter. 2004;9:262–70.
26. Veldhuyzen van Zanten SJ, Pollak PT, Best LM, Bezanson GS, Marrie T. Increasing prevalence of *Helicobacter pylori* infection with age: continuous risk of infection in adults rather than cohort effect. J Infect Dis. 1994;169:434–7.
27. Dutra F, Fernandes C, Aguiar G, Rocha A, Nogueira E, Magalhaes M. Prevalence of *Helicobacter pylori* infection in a rural area of the state of Mato Grosso Brazil. Mem Inst Oswaldo Cruz. 1998;93:171–4.
28. Banatvala N, Mayo K, Megraud F, Jennings R, Deeks JJ, Feldman RA. The cohort effect and Helicobacter pylori. J Infect Dis. 1993;168:219–21.
29. Roosendaal R, Kuipers EJ, Buitenwerf J, et al. Helicobacter pylori and the birth cohort effect: evidence of a continuous decrease of infection rates in childhood. Am J Gastroenterol. 1997;92:1480–2.
30. Tkachenko MA, Zhannat NZ, Erman LV, et al. Dramatic changes in the prevalence of *Helicobacter pylori* infection during childhood: a 10-year follow-up study in Russia. J Pediatr Gastroenterol Nutr. 2007;45:428–32.
31. Montiel de Morales M, Zambrano JL, Castejón O, Oliveros C, Botero de Ledesma L. Bacterial indicators of fecal contamination and coliforms in the water of the Sinamaica's Lagoon, state of Zulia. Venezuela Ciencia. 2005;13:291–301.
32. Maldonado A, Rivero-Rodríguez Z, Chourio-Lozano G, et al. Prevalence of enteric parasites and related environmental factors in two native communities of Zulia State. Kasmera. 2008;36:53–66.
33. Chen TL, Chan CC, Chen HP, et al. Clinical characteristics and endoscopic findings associated with *Blastocystis hominis* in healthy adults. Am J Trop Med Hyg. 2003;69:213–16.
34. Júlio C, Vilares A, Oleastro M, et al. Prevalence and risk factors for *Giardia duodenalis* infection among children: a case study in Portugal. Parasit Vectors. 2012;5:22.
35. Janoff EN, Taylor DN, Echeverria P, Glode MP, Blaser MJ. Serum antibodies to *Giardia lamblia* by age in populations in Colorado and Thailand. West J Med. 1990;152:253–6.
36. Bylund J, Christophe T, Boulay F, Nyström T, Karlsson A, Dahlgren C. Proinflammatory activity of a cecropin-like antibacterial peptide from Helicobacter pylori. Antimicrob Agents Chemother. 2001;45:1700–4.
37. Rothenbacher D, Blaser MJ, Bode G, Brenner H. Inverse relationship between gastric colonization of Helicobacter pylori and diarrheal illnesses in children: results of a population-based cross-sectional study. J Infect Dis. 2000;182:1446–9.
38. Ikewaki J, Nishizono A, Goto T, Fujioka T, Mifune K. Therapeutic oral vaccination induces mucosal immune response sufficient to eliminate long-term Helicobacter pylori infection. Microbiol Immunol. 2000;44:29–39.
39. Nystrom J, Raqhavan S, Svennerholm AM. Mucosal immune responses are related to reduction of bacterial colonization in the stomach after therapeutic Helicobacter pylori immunization in mice. Microbes Infect. 2006;8:442–9.