Seroprevalence of Hepatitis A Virus, Hepatitis E Virus, and *Helicobacter pylori* in Rural Communities of the Bolivian Chaco, 2013

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Abstract. In the Bolivian Chaco, south-east of Bolivia, studies conducted over the past three decades reported hepatitis A virus (HAV) and *Helicobacter pylori* seroprevalences above 90% and 60%, respectively. Hepatitis E virus (HEV) prevalence was previously found to be 6–7% but is probably an underestimate because of the poor sensitivity of the assays used. In November 2013, we conducted a cross-sectional study of 263 healthy volunteers from two rural communities of the Bolivian Chaco, aiming to reassess HAV, HEV, and *H. pylori* seroprevalence 10–20 years following the previous surveys. Hepatitis A virus seroprevalence was 95%, with universal exposure after the first decade of life; HEV seroprevalence was considerably higher (31–35%) than that previously reported; *H. pylori* seroprevalence was 59%, with an age-dependent distribution. The high prevalence of these infections suggests that major efforts are still needed to reduce fecal–oral transmission and to improve human health in the Bolivian Chaco.

INTRODUCTION

Hepatitis A is a common form of acute viral hepatitis worldwide. Hepatitis A virus (HAV) is transmitted by the fecal–oral route because of ingestion of contaminated food or water or through direct contact with an infectious individual. The spread of the virus is strongly correlated with poor socioeconomic and hygienic conditions. In low-income countries, where access to safe water and sanitation standards are inadequate, HAV is highly endemic, and infections occur almost universally in early childhood.^{1,2} The risk of disease after acute HAV infection varies by age, and the clinical course in children is usually asymptomatic or mild. By contrast, infected adolescents and adults more frequently develop classic symptoms of hepatitis, including jaundice. The infection induces lifelong protection, with detectable anti-HAV immunoglobulins (Ig)G.

Hepatitis E virus (HEV) has an extensive global distribution and causes epidemics and sporadic cases in many low-income countries. In endemic areas, HEV genotypes (gt) 1 and 2 are transmitted by the fecal–oral route, primarily through contaminated drinking water, but person-to-person transmission is uncommon.³ Unlike other hepatitis viruses, large reservoirs of HEV gt 3 and 4 have been recognized in various animal species, such as pigs, rabbits, boar, and deer. These observations suggest zoonotic transmission, which has been well documented in high-income countries, mainly in Europe. Hepatitis E virus infection by gt 1 and 2 causes an acute, self-limiting hepatitis, predominantly in young adults. Although the symptoms are generally mild, fulminant infection may occur, especially in pregnant women. In many developed countries, gt 3 and 4 are the dominant circulating HEV and cause acute

hepatitis usually in older males and chronic infection in the immunosuppressed. In contrast to HAV, global HEV seroprevalence is less than 10% in children younger than 10 years, and the peak of incidence occurs in young adults aged between 15 and 40 years in many areas endemic for HEV gt 1 and 2.^{3,4}

Helicobacter pylori is a common bacterium that infects the gastric mucosa of nearly half of the human population. Prevalence is higher in developing than in developed countries, and it seems to be related to inadequate sanitation practices, low social class, and overcrowded or high-density living conditions.⁵ Although the infection is likely to spread from person to person, the precise route of transmission is controversial, as data supporting fecal–oral, oral–oral, gastric–oral, waterborne, and zoonotic transmission have been reported.^{5,6} Most of the infections occur in early childhood and, if not treated, persist lifelong. Chronic infection with *H. pylori* is associated with gastrointestinal tract disorders, ranging from chronic gastritis to gastric adenocarcinoma, gastric lymphoma, and peptic ulcer.⁵

In the Bolivian Chaco, a tropical region in the south-east of Bolivia, previous studies showed a high prevalence of HAV, above 90% in the general population. However, a significant decrease in the HAV seroprevalence, from 86.9% to 64.7%, was observed among children aged 1–5 years, during the period 1987–1997.^{7,8} In the second survey, HEV seroprevalence was initially assessed in two areas of the Bolivian Chaco, reporting an overall prevalence of 7.3%, with significantly lower levels in individuals ≤ 30 years of age.⁸ In 2006, a further study in the same area showed a similar seroprevalence (6%). However, both these previous results are likely to be significant underestimates of HEV seroprevalence because of the poor sensitivity of the assays used.⁹ As far as *H. pylori* is concerned, in the same population surveyed for HAV and HEV in 1997, the prevalence of specific antibodies was 60.7% (A. Bartoloni, unpublished data).

In this study, the populations of two rural communities in the Bolivian Chaco were studied to 1) evaluate the trend of HAV seroprevalence 16 years after the last assessment; 2)

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reassess the HEV seroprevalence using more accurate assays; 3) evaluate *H. pylori* seroprevalence, its distribution by age, and potential association with the other two fecal–oral transmitted infections in that area.

MATERIALS AND METHODS

Study design and population. A population-based cross-sectional study was conducted in November 2013, in two rural communities of the Bolivian Chaco. The Chaco region is a semi-arid, homogeneous ecological zone, situated between the latitudes 17°59'–22°21' South and the longitudes 64°31'–58°51' West, and is approximately 127,755 km² in size. The region is sparsely populated and includes three departments (Santa Cruz, Chuquisaca, and Tarija) and five provinces (Cordillera, Luis Calvo, Hernando Siles, Gran Chaco, and O'Connor). The surveyed communities were Bartolo (16°66'S; 64°04'W; municipality of Monteagudo, Hernando Siles Province, Department of Chuquisaca) and Ivamirapinta (19°45'S; 63°47'W; municipality of Gutierrez, Cordillera Province, Department of Santa Cruz). In each community, the study was first explained during a preparatory meeting, involving local health care providers and community leaders of the Guaraní political organization (Asamblea del Pueblo Guaraní). With their collaboration, information about the total number of inhabitants and household locations was collected. Moreover, they helped to disseminate the invitation to participate, which was directed to all individuals of the communities during public meetings. The sample size was determined based on an expected prevalence of HAV, *H. pylori*, and HEV antibodies of 90%, 60%, and 20%, respectively, with a worst acceptable error of 5% and a confidence interval (CI) of 95%. A total of 263 healthy volunteers were consecutively enrolled, representing ≈50% and ≈25% of the populations of Bartolo and Ivamirapinta, respectively. Demographic data (sex and age) were recorded, and blood samples for testing were taken. The study population lives in poor dwellings with walls of sticks, straw and clay, and thatched roofs. The local economy is based on agriculture and animal farming. In the study area, domestic animals, especially dogs, poultry, and swine, are present in almost all the households. Inhabitants live in close contact with animals, which freely migrate from household to household, in the absence of adequate housing or fencing.

The study was devised and conducted in agreement with the Ministry of Health of Bolivia (within the Convenio Ministerio

de Salud y Deportes, Estado Plurinacional de Bolivia/Cátedra de Enfermedades Infecciosas, Universidad de Florencia, Italia) and with the support of the Guaraní political organization (Asamblea del Pueblo Guaraní). Ethical approval for the study was obtained from both the above-mentioned institutions.

Serologic assays. Sera, obtained from a sample of 5 mL venous blood, were stored at –20°C in Bolivia, transported to Italy, and stored at –70°C until tested. In July 2016, the anonymized samples were tested with 1) two commercial anti-HEV IgG enzyme-linked immunosorbent assay (ELISA) kits (DIA.PRO Srl, Milan, Italy; Wantai Biological Pharmacy Enterprise, Beijing, China); 2) a commercial chemiluminescence immunoassay for anti-HAV IgG (Architect HAVAb-IgG; Abbott Laboratories, Abbott Park, IL); and 3) a commercial enzyme immunoassay for detecting anti-*H. pylori* IgG (Premier® *H. pylori*; Meridian Bioscience, Milan, Italy). The assays were performed and interpreted according to the manufacturers' instructions.

Statistical analysis. Data were entered into a spreadsheet, using Microsoft Excel 2010 software (Microsoft Corp., Redmond, WA). Statistical analysis of the data was performed with STATA 11.0 (StataCorp, College Station, TX). Frequencies and percentages with 95% CIs for categorical variables, medians and interquartile ranges (IQRs) for continuous variables were calculated. The Mann–Whitney test was used to compare the median age. The Cochran–Mantel–Haenszel test stratified for age classes was used to compare results between communities. Concordance between the two commercial anti-HEV IgG ELISA was evaluated by computing the *k* statistics, with a *k*-value ≥ 0.75 representing excellent agreement. The χ^2 test (or Fisher's exact test, when appropriate) and logistic regression were used to investigate the association of the positive ELISA test with sex and age. Anti-HEV IgG borderline results were excluded from statistical analysis. Results were considered statistically significant when the *P* value was ≤ 0.05.

RESULTS

Demographic data. In total, 263 samples were collected, but six samples were excluded from further analysis because of absent or incomplete participant details. Of those 257 individuals, 113 were from Bartolo and 144 from Ivamirapinta; overall, 51% (131/257) were female, and the median age was 27 years (IQR: 12–46, ranging between 1 and 87 years), with no significant differences between the communities (*P* = 0.656). Thirteen samples were of low volume and were arbitrarily tested only for anti-HEV IgG.

TABLE 1
Prevalence of anti-HAV IgG stratified by age cohort and gender in two rural communities of the Bolivian Chaco

	Bartolo	Positive	%	Ivamirapinta	Positive	%	Total	Positive	%
Gender									
Female	48	40	83.3	77	77	100	125	117	93.6
Male	55	53	96.3	64	62	96.9	119	115	96.6
Age classes									
1–5	8	1	12.5	6	6	100	14	7	50
6–10	11	8	72.2	20	19	95	31	27	87.1
11–20	16	16	100	39	39	100	55	55	100
21–30	20	20	100	13	13	100	33	33	100
31–40	18	18	100	19	19	100	37	37	100
41–50	10	10	100	13	13	100	23	23	100
51–60	8	8	100	9	9	100	17	17	100
≥ 61	12	12	100	22	21	95.5	34	33	97.1
Total	103	93	90.3	141	139	98.6	244	232	95.1

HAV = hepatitis A virus; IgG = immunoglobulin G.

TABLE 2
Prevalence of anti-HEV IgG (Dia.pro kits) stratified by age cohort and gender in two rural communities of the Bolivian Chaco

	Bartolo	Positive	%	Ivamirapinta	Positive	%	Total	Positive	%
Gender									
Female	50	14	28	77	28	36.4	127	42	33.1
Male	59	27	45.8	64	18	28.1	123	45	36.6
Age classes									
1–5	11	0	0	7	0	0	18	0	0
6–10	13	1	7.7	21	0	0	34	1	2.9
11–20	16	2	12.5	39	7	17.9	55	9	16.4
21–30	21	8	38.1	13	5	38.5	34	13	38.2
31–40	16	8	50	18	7	38.9	34	15	44.1
41–50	12	8	66.7	12	6	50	24	14	58.3
51–60	8	5	62.5	9	6	66.7	17	11	64.7
≥ 61	12	9	75	22	15	68.2	34	24	70.5
Total	109	41	37.6	141	46	32.6	250	87	34.8

HEV = hepatitis E virus; IgG = immunoglobulin G.

Hepatitis A virus seroprevalence. Of the 244 serum samples tested for anti-HAV IgG, 232 (95.1%, 95% CI: 92.4–97.8) were positive. Seroprevalence was significantly higher in Ivamirapinta than that in Bartolo (98.6% versus 90.3%, $P = 0.003$). The imbalance of seroprevalence observed between both communities was accounted for by differences in young children aged 1–5 years (12.5% in Bartolo versus 100% in Ivamirapinta, $P = 0.005$), whereas in both communities, it was found to be $\geq 95\%$ in individuals aged > 10 years (Table 1). Anti-HAV positivity was strongly associated with increasing age (odds ratio [OR]: 1.14 for each 1-year increase, 95% CI: 1.05–1.26, $P = 0.003$). Stratification by age cohort showed that exposure occurred predominantly in subjects aged up to 10 years, reaching 100% in the second decade of life. No difference was observed in the gender distribution of seropositives ($P = 0.377$).

Hepatitis E virus seroprevalence. When using the HEV IgG Dia.pro kit, 7/257 samples gave a borderline result and were excluded from further statistical analysis. Overall, 87/250 of individuals studied were anti-HEV IgG positive (34.8%, 95% CI: 28.9–40.7). There was no difference in HEV seroprevalence in males and females ($P = 0.560$) or the two communities tested ($P = 0.741$). The seroprevalence of HEV steadily increased with age (OR: 1.05 for each 1-year increase, 95% CI: 1.04–1.07, $P < 0.001$), exceeding 50% by the fifth decade of life (Table 2).

Using the Wantai anti-HEV IgG kit, the results were similar with 77 positives of 251 individuals tested. After exclusion of

six borderline results, the seroprevalence was 30.7% (95% CI: 25.0–36.4). Concordance between both assays was excellent ($k = 0.95$).

***Helicobacter pylori* seroprevalence.** Among 244 samples tested for anti-*H. pylori* IgG, 143 were positive (58.6%, 95% CI: 52.4–64.8). No statistical differences were found in distribution between genders ($P = 0.172$) or communities ($P = 0.592$). In common with the other infections, *H. pylori* seroprevalence increased with age (OR: 1.02 for each 1-year increase, 95% CI: 1.01–1.03, $P = 0.003$) in subjects up to the age of 40 years, after which it ‘plateaued’ (Table 3).

Coinfections. Among 237 individuals with interpretable results, coinfection was found in 166 (70%), including 82 (34.6%) exposed to both HAV and HEV and 53 (22.4%) exposed to all three pathogens. No significant age-corrected associations were found between *H. pylori* infection and HAV (OR: 1.61, 95% CI: 0.37–6.95, $P = 0.516$), HEV (OR: 0.82, 95% CI: 0.43–1.56, $P = 0.546$), or HAV/HEV (OR: 0.88, 95% CI: 0.46–1.68, $P = 0.706$) exposure. Similarly, no association was found between HAV and HEV exposure ($P = 0.748$).

DISCUSSION

In this study, the seroprevalence of HAV, HEV, and *H. pylori* in the Bolivian Chaco was surveyed, 10 to 20 years after the last assessment in the same area. Hepatitis A virus seroprevalence was 95.1%, with universal exposure after the first decade of life (Figure 1A). This finding is unchanged from

TABLE 3
Prevalence of anti-*Helicobacter pylori* IgG stratified by age cohort and gender in two rural communities of the Bolivian Chaco

	Bartolo	Positive	%	Ivamirapinta	Positive	%	Total	Positive	%
Gender									
Female	48	23	47.9	77	45	58.4	125	68	54.4
Male	55	39	70.9	64	36	56.3	119	75	63
Age classes									
1–5	8	1	12.5	6	2	33.3	14	3	21.4
6–10	11	6	54.5	20	7	35	31	13	41.9
11–20	16	9	56.3	39	23	59	55	32	58.2
21–30	20	12	60	13	7	53.8	33	19	57.6
31–40	18	14	77.8	19	12	63.2	37	26	70.3
41–50	10	7	70	13	9	69.2	23	16	69.6
51–60	8	5	62.5	9	6	66.7	17	11	64.7
≥ 61	12	8	66.7	22	15	68.2	34	23	67.6
Total	103	62	60.2	141	81	57.4	244	143	58.6

IgG = immunoglobulin G.

FIGURE 1. (A–C) Seroprevalence trends of hepatitis A virus (HAV) (A), hepatitis E virus (HEV) (B), and *Helicobacter pylori* (C) in the human population of rural communities of the Bolivian Chaco: 1987–2013.

previous observations in Bolivia and Peru and is in line with World Health Organization (WHO) estimates for Andean Latin American countries, both in rural and urban areas.^{10–12} Previously, a significant decrease in HAV seroprevalence among children aged 1–5 was years observed from 1987 to 1997 (from 86.9% to 64.7%). However, in the current study, a decrease in HAV seroprevalence was seen only in the community of Bartolo (1/8, 12.5%) but not in Ivamirapinta (6/6, 100%). This observed difference in prevalence in such very young children between Bartolo and Ivamirapinta does not reflect differences in sanitation between both communities, and it

cannot be explained by family or household clustering (data not shown). These results need to be interpreted with caution, given the low number of children studied in this age cohort.

A decline in circulating HAV among children is consistent with reports from many parts of the world, where anti-HAV epidemiology is changing, probably because of improvements in socioeconomic conditions and local health education.¹⁰ Since the end of the last century, the prevalence of anti-HAV antibodies has decreased in several Latin American countries, including Argentina, Brazil, Venezuela, Chile, and Uruguay.^{13,14} As a consequence of the reduction in viral

exposure during early childhood, the peak age of infections is shifting to middle childhood or later, resulting in more clinical cases in adolescents and adults and an increased potential for clinically overt outbreaks. In these countries, monitoring of HAV epidemiology, especially in younger age cohorts, is important, as such data will inform preventive intervention strategies, such as vaccination campaigns.

The HEV seroprevalence was found to be an order of magnitude higher, using both newer Dia.pro (34.8%) and the well-validated Wantai kits (30.7%), compared with that reported in previous surveys from Bolivia and Latin America, including our studies in the Bolivian Chaco^{7-9,15} (Figure 1B). The finding is not surprising, considering that commercial assays for anti-HEV IgG detection show highly variable performance.¹⁶ The use of more sensitive assays has led to a three-times or four-times increase in estimates of HEV seroprevalence, including countries in Asia, where HEV gt 1 is the dominant circulating gt, and Europe, where gt 3 and 4 zoonotic HEV is endemic.¹⁷⁻¹⁹ Of note, in the community of Bartolo, previously surveyed in 2006 using an older version of the commercial ELISA kit Dia.pro, a five to six-times higher prevalence was observed (38% versus 7%).⁹ Because no HEV or jaundice outbreaks have been reported in recent years in this area, these discrepancies are likely due to the improved performance of the newer tests, whose reliability is corroborated by the excellent interassay agreement.²⁰ The finding of high HEV seroprevalence in the areas tested, the lack of relationship regarding coinfection with other common fecal-oral pathogens, together with the lack of outbreaks of jaundice/deaths in pregnant females, and our previous observations of HEV gt 3 in both pigs and humans from the same community suggest that the dominant mode of infection in the areas studied is likely to be zoonotic. This is congruent with recent studies from several countries in South America, which shows that HEV gt 3 is the dominant circulating gt and that the epidemiology is similar to that seen in locally acquired zoonotic infections in Europe.²¹⁻²⁴ However, previously reported sequencing data on porcine and human strains, detected in the Bartolo community, suggest that the source of human HEV infection is unlikely to be from the local pig population, as there was poor sequence homology between porcine HEV (gt 3i) and human HEV (gt 3e).²⁵

The seroprevalence of *H. pylori* was 58.6%, with an age-dependent distribution, reaching a plateau around 70% after the fourth decade of life. Unpublished data (A. Bartoloni) from the same area reported similar results in 1997 (61%) (Figure 1C). Previously, a high *H. pylori* seroprevalence (44%) was reported among children aged 6 months to 9 years from 17 rural communities in the Santa Cruz Department, Bolivia. This rose to 64% when just considering individuals aged 7-9 years.²⁶ Two cross-sectional surveys on *H. pylori* infections in Bolivia, conducted in the city of Sucre and in two villages of the eastern territories using the urea breath test (UBT), reported a prevalence of 74% and 80%, respectively.^{27,28} The higher prevalence found in the latter studies might be attributed to the higher sensitivity of UBT in comparison with serology. Serological assays for *H. pylori* cannot distinguish between ongoing and resolved infections because specific IgG persist for months or years after a successful eradication of the bacterium. Likewise, seronegativity does not entirely exclude the possibility of a previous infection.²⁹ However, it seems likely that the observed prevalence in the current study is a

reasonable estimate of the cumulative exposure burden over time, as local access to diagnostic tests and treatment of *H. pylori* infection are extremely limited. In Bolivia, stomach cancer, which is the main clinical sequela of *H. pylori* infection, was one of the five most frequently diagnosed cancers, as in many other countries of Latin America and, in 21% of cases it affects people younger than 50 years.³⁰

In the population we studied, no association was found between HAV, HEV, and *H. pylori* seropositivity when corrected for age. These findings are consistent with the conclusion of a recent systematic review.⁶ Although serostatus may not be an accurate marker for this association, our findings suggest that these three infections do not share the same route of transmission. This would fit with current notions of source and routes of infection: HAV is transmitted through the fecal-oral route, with humans as the main reservoir; gt 3 HEV is a porcine zoonosis, most likely due to either consumption of infected pork meat or close contact with infected animals; *H. pylori* seems to spread through multiple routes, depending on cultural and environmental conditions.⁶

The main limitation of the study is that the survey involved only two communities with a relatively small number of study participants. Moreover, it was not possible to weight the results back to the overall village population because there was no accurate census of the two communities. Thus, our findings may not be completely representative of the entire Chaco region. However, given that the Bolivian Chaco is a homogeneous ecological zone and the rural populations share the same hygienic and sanitary living conditions, it seems unlikely that there are major geographical differences in the distribution of such infections within the region. Indeed, there seems to be a strong relationship between poor socioeconomic conditions/poor hygiene standards and the seroprevalence of HAV, HEV (at least for gt 1), and *H. pylori* infections in many locations across the world. The high prevalence of these infections found in the Bolivian Chaco suggests that significant efforts are still needed to reduce transmission and to improve health/sanitary conditions in this area, a notion that is highlighted by the high prevalence of intestinal parasitic infections in the same area.^{31,32} Interventions should be inspired by the WASH principles, endorsed by the WHO, and focused on access to safe drinking water, improvement of sanitation facilities and hygiene promotion by health education.³³ As far as HEV is concerned, further studies are needed to improve our understanding of HEV epidemiology, especially regarding the main source of human infection in South America in general and Bolivia in particular.

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