High Rates of Exposures to Waterborne Pathogens in Indigenous Communities in the Amazon Region of Ecuador

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Abstract. Waterborne pathogens, associated with poverty and poor sanitary conditions, are a major cause of morbidity and mortality worldwide. There are limited data on the epidemiology of waterborne pathogens in indigenous populations living in the Amazon region. We did a cross-sectional survey in two indigenous Shuar communities in the Amazon region of Ecuador in which we documented the presence of pathogens representing different sources of environmental contamination of water. We detected protozoa and soil-transmitted helminths by microscopy of fecal samples and the presence of IgG antibodies to hepatitis A and *Leptospira* spp. in blood samples from individuals older than 2 years and collected data by questionnaire on sociodemographic factors and knowledge of infectious diseases. Seroprevalence for hepatitis A and *Leptospira* spp. were 98.1% (95% CI: 97.0–99.8) and 50.0% (95% CI: 43.3–56.6), respectively, whereas 62.6% (95% CI: 55.8–69.4) had enteric parasites in stool samples. In participants older than 6 years, eight of 10 had evidence of infection with or exposure to at least one of the pathogens studied. Although prevalence of pathogens varied by age, it did not vary significantly by gender, temporal migration, illiteracy, perceived morbidity, receipt of conditional cash transfers, water boiling practices, poor housing conditions, and anthropometric status. These findings indicate a high level of contamination of drinking water by human pathogens in these indigenous communities and the need for interventions to improve access to and use of clean drinking water in these marginalized communities.

INTRODUCTION

Waterborne pathogens constitute a group of bacteria, viruses, and parasites estimated to cause more than 2.2 million deaths per year worldwide of which 1.4 million are among children.¹

Approximately 1,400 pathogen species are estimated to infect humans through contaminated water including Hepatitis A virus (HAV), *Leptospira* spp., spirochetes, and enteric protozoa (e.g., *Giardia intestinalis* and *Entamoeba histolytica*) recognized as emerging global public health problems.² Soil-transmitted helminths (STH) infections such as *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm spp., and *Strongyloides stercoralis*^{3,4} are transmitted through contacts with a fecally contaminated environment including through water sources. Transmission of waterborne pathogens may be through human or animal contamination of water used for drinking, bathing, or other domestic uses^{5,6} and is associated with significant morbidity and mortality worldwide.^{7–9}

In the case of exposures to HAV and *Leptospira* spp., seroconversion occurs after the first infection, with or without symptoms, and convalescent individuals develop permanent immunity measured by the presence of specific IgG antibodies.^{10–12} In human population with high levels of exposure to HAV, the risk of infection may be greater than 15%.¹³

Leptospirosis is a widespread zoonotic infection often acquired through mucous membranes or skin lacerations while bathing in water contaminated with animal urine, primarily from rodents¹⁴ but also other mammals.^{15,16} Most reported outbreaks of *Leptospira* spp. between 1910 and 2010 have been

associated with extreme weather events and flooding in North America or Asia,⁴ but infections have been associated also with exposures to pristine water sources.¹⁷ Endemic transmission of *Leptospira* is related to the lack of clean water and sanitation in populations vulnerable to seasonal flooding.¹⁸

Enteric parasitic infections are a marker of poverty and the level of environmental contamination^{19,20} with human feces including contamination of drinking water sources.^{5,21,22}

In the Americas, more than 100 million people live without improved sanitation and greater than 35 million lack access to improved drinking water.¹ Prevalence rates for acute diarrhea and current infections with hepatitis A and *Leptospira* spp. in 2015 were reported to be 3.5, 32.3, and 0.8 per 100,000 population, respectively, in Ecuador²³; however, there are few data on enteric parasites.

In the present study, we surveyed two indigenous communities in the southern Amazon region of Ecuador to estimate seroprevalence rates for Hepatitis A and *Leptospira* spp. and prevalence of current infections with enteric parasites including STH, used as exposure to pathogen groups representing different forms of environmental contamination of water for human use, and evaluated associations with relevant sociodemographic determinants. We considered these three groups of neglected tropical pathogens together to obtain a broader view of the potential public health consequences of exposures to contaminated water, shared risk factors, and some overlap in symptomatology. Previously, we reported prevalence of STH infections in this study population of 46.9%.²⁴

MATERIAL AND METHODS

Study population and sampling. A cross-sectional study was carried out in two indigenous communities of Shuar ethnic group in the southern Amazon region of Ecuador

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(Figure 1). Traditionally, the Shuar live in sparsely populated communities (i.e., ~ 4 habitations/km²) in small houses made of traditional materials,²⁴ subsisting on a diet of low and declining quality based on horticulture, foraging, hunting, and fishing.^{25,26} A process of acculturation began through the establishment of evangelical missions during the 1940s that led to the establishment of more densely populated settlements. Presently, few Shuar households have access to clean water or basic sanitation, most live in close contact with domestic animals,²⁷ and almost all live in communities over 3 hours distance by road or river from the nearest health facility.

Participants were inhabitants of the two communities and older than 2 years, who provided informed consent (individually or through legal guardians for minors).

Participants were asked to provide data through an interviewer-administered questionnaire, and blood and stool samples and duplicate measures of height and weight were obtained. The questionnaire collected data on gender, age, temporary migrations (defined by 7–30 days absence from the community for work, health, or family reasons), illiteracy, receipt of conditional cash transfers (Bono de Desarrollo Humano, a cash transfer program launched by the Ecuadorian government in 2003, the receipt of which is not dependent on having children in the household), boiling water practices, housing condition, use of firewood for cooking, and overcrowding. In addition, questions (yes/no) were asked about perceived morbidity (both at some time in their life and in the year before the survey), knowledge regarding the transmission pathways of the infections under study, if they had received any treatments and source (i.e., “Western” doctors or native healers such as shamans), and if there had ever been any deaths in the family possibly related to the infections under study.²⁴

Water for human consumption in these communities was sourced from a stream and piped to untreated storage tanks and accessed through shared community taps. The fieldwork was carried out in June 2015 by a multidisciplinary team of doctors, epidemiologists, microbiologists, technicians, and students after several meetings with community leaders and the population to obtain community consent. Stool samples were examined by microscopy as described in the following paragraphs. Blood was collected and serum separated by centrifugation, aliquoted, and stored at -20°C until analysis.

Laboratory assays. Serum samples were analyzed for IgG antibodies to HAV (anti-HAV) and *Leptospira* spp. (anti-*Leptospira*) using commercial enzyme-linked immunosorbent assays (Diagnostic Bioprobes HAV Ab, Dia-Pro, Milan, Italy; AccuDiag™ ELISA *Leptospira* IgG, Cortez Diagnostics, Woodland Hills, CA) according to the manufacturer’s instructions. Stool samples were examined fresh by direct saline smears and the modified Kato-Katz method.²⁸

Remaining stool samples were preserved in 10% formaldehyde and examined later using the formol ethyl acetate concentration method.²⁸ The presence of any trophozoites, cysts, eggs, or larvae of enteric parasites or worms (EP/W) detected by any of the three microscopic techniques were considered positive.

Data analysis. Positivity for any of anti-HAV, anti-*Leptospira*, and the presence of enteric parasites (anti-HAV/anti-Lep/EP) was defined as the primary variable of interest. Age was stratified into three groups: 2–5, 6–19, and ≥ 20 years. Housing condition was classified as good when all of the following were present: concrete floor, wooden walls, doors, any windows, and iron roof. Body mass index (BMI) below the 15th centile (P_{15}) (using WHO references for 2–5 and 5–18 years, by

FIGURE 1. Map of Ecuador emphasizing the Morona Santiago region. Detailed representation of the study area (Santiago) in the Ecuador-Peru border.

age and gender)²⁹ or BMI under 18.5 for adults was defined as undernutrition. Comparisons of frequencies between categorical variables were done using the two-sided Fisher's exact test. Linear associations between pathogen positivity and age groups were analyzed by the linear-by-linear association test. To compare prevalence groups, we used prevalence ratios (PR). Data were analyzed using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY).

RESULTS

Social and demographic characterization. Blood samples were obtained from 216 participants and stool samples from 192 with 179 individuals providing both stool and blood sample. For the 216 individuals with blood samples, mean age was 22 years (interquartile range 8–30 years, range 2–78) of whom 34 (15.7%) were aged 2–5 years, 99 (45.8%) 6–19 years, and 83 (38.4%) 20 or more years; 91 (42.1%) were men; 70 (34.5%) had experienced temporary migrations; 38 (40.9%) were illiterate; 169 (79.3%) were in families receiving cash transfers; 67 (31.2%) boiled domestic water for drinking; 73 (34%) used firewood for cooking; 107 lived in overcrowding conditions (50.2%); 103 (48.8%) lived in housing of good condition; and 13 (7.3%) had undernutrition.

Hepatitis A infections and anti-HAV antibodies. Anti-HAV antibodies were detected in 212/216 (98.1%, 95% CI: 96.3–99.5) participants and seroprevalence increased with age (Fisher's exact test, $P = 0.03$, linear association test $P < 0.001$) (Table 1). No significant differences were found between HAV seropositivity and other variables. Eighteen (8.4%) participants reported being aware of the transmission pathway for HAV, whereas 27 (12.6%) declared having suffered HA in their lifetime of which for eight, this was within the previous year. Twenty (9.3%) participants had received treatment for Hepatitis A of which seven had received treatment in the previous year, all from traditional healers. Nobody was reported to have died from complications of HAV infection.

Leptospirosis and Anti-*Leptospira* IgG antibody. Anti-*Leptospira* IgG antibody was detected in 108/216 (50.0% 95% CI: 43.2–56.8) participants and prevalence increased with age (Fisher's exact test, $P < 0.001$) (Table 1). Seropositivity increased linearly with age (linear association test, $P < 0.001$). Among those not receiving cash transfers, seropositivity was 78.8% (PR 1.63, 95% CI: 1.24–2.14). There were no other associations between seropositivity and the other study variables. Only one participant reported having suffered leptospirosis previously, although three community members were thought to have died of the disease. No one had suffered from the disease in the previous year, knew the transmission pathway for the infection, or reported having received treatment for symptoms associated with the infection.

Enteric parasites. Of 192 participants providing a stool sample, 112 (62.6%, 95% CI: 55.5–69.7) had enteric parasitic infections: *Giardia lamblia* was observed in nine (4.7%), *Entamoeba histolytica* in 63 (32.8%), *A. lumbricoides* in 53 (27.6%), *Trichuris trichura* in 59 (30.7%), hookworm in seven (3.6%), and *Strongyloides stercoralis* in one (0.5%). The presence of enteric parasites decreased with age (Fisher's exact test, $P < 0.001$) (Table 1). Enteric parasites were more common among participants whose families did not boil water (83/119 or 48.3%, PR 1.44, 95% CI: 1.08–1.92) but no significant associations were observed for other variables.

Single and multiple exposures to study pathogens. Data for seroprevalence and enteric parasites were available for 179 participants; 59 (33.0%) were positive for all three pathogen groups (anti-HAV/L/EP/W) of whom 30 (50.8%) were younger than 19 years; 52 (29.1%) were positive for all three groups except anti-*Leptospira* of whom 43 (82.7%) were younger than 19 years; one (age 3 years) was positive for all three groups except anti-HAV; one was negative for all pathogens; and none were positive only for anti-*Leptospira*. Among participants older than 6 years, 8/10 had been in contact with two or more of the three pathogen groups studied (123/151, 81.5%). Figure 2 shows the joint distribution of these different exposure groups stratified by age. Evidence of exposures to all pathogens (anti-HAV/anti-Lep/EP) increased with age (Fisher's exact test, $P = 0.001$; linear-by-linear association test, $P < 0.001$) and was greatest among families that received cash transfers (24.4% versus 17.2%; Fisher's exact test, $P = 0.003$), although the association disappeared after adjusting for age.

All 60 registered families participated. All families had at least one, and 58.3% had three or more participating children younger than 5 years. Family participation rate for at least one clinical sample was 75.5% (range 11–100%) with numbers of participating family members ranging one to nine persons. Fifty-five (91.2%) families were included in the analysis of pathogen exposures. Families with at least one member exposed to or infected with HAV, *Leptospira*, enteric parasites, and anti-HAV/anti-Lep/EP were 100.0%, 85.0%, 70.9%, and 43.6%, respectively. On average, there were 1.6 (range 1–5) people per family exposed to the three groups of pathogens.

DISCUSSION

The present study provides the first published report of the epidemiology and risk factors for waterborne pathogens among indigenous communities in the Ecuadorian Amazonia. Previous reports have studied the presence of enteric parasites in Shuar or other indigenous populations living in this region.^{19,30–32}

A third (33.0%) of inhabitants in the study communities from the southeast Amazon region had serological memory for HAV and *Leptospira* spp., and active infections with enteric parasites and a high proportion were positive (62.6%) for more than one of the pathogen groups. These data indicate a high level of contamination of drinking or bathing water, putting these populations at risk of epidemic outbreaks, likely to be accentuated by the high rainfall in this tropical equatorial forest setting,^{33,34} lack of access to health-care services, and general distrust toward “Western” health-care providers.^{35,36}

The risk of infections with all pathogens and with anti-HAV and anti-*Leptospira* spp. increased with age indicating time-dependent accumulation of risk. A high level of endemicity and

TABLE 1
Distribution of anti-hepatitis A, IgG anti-*Leptospira* and active infections with enteric parasites and EP/W by age groups

Age groups	Anti-HAV		IgG anti- <i>Leptospira</i>		EP/W	
	n	%	n	%	n	%
2–5	31/34	91.2	9/34	26.5	22/28	78.6
6–19	98/99	99.0	42/99	42.4	52/112	66.7
≥ 20	83/83	100.0	57/83	68.7	38/73	52.1

Anti-HAV = anti-HAV antibodies; EP/W = enteric parasites and worms; HAV = Hepatitis A virus; IgG anti-*Leptospira* = anti-*Leptospira* IgG antibody.

FIGURE 2. Distribution of the rates of exposures to waterborne pathogens studied stratified by age.

transmission of HAV was likely in these communities given that all subjects were seropositive by the age of 6 years. Moreover, although HAV infection in early childhood is generally considered to be asymptomatic or associated with a mild illness, all the inhabitants who were declared having the disease were assisted by a traditional healer or shaman, illustrating either a lack of access to health-care services or a general mistrust toward them.

Studies of age of first infection with HAV have shown a shift from childhood to older age groups,³⁷ largely because of the introduction of the hepatitis A vaccine.³⁸ Data from the Andes region of Latin America between 1990 and 2005 showed a shift in the median age of HAV infection from infancy to mid-childhood.³⁹ In this study, we observed a seroprevalence of HAV for of 25.9%, 19.2%, and 17.8% for 2–5, 6–19, and 20 or more years old, respectively. In areas of high endemicity with a lifetime risk of infection above 90%, most HAV infections occur in early childhood and clinical symptoms of hepatitis A are observed infrequently.^{40,41}

The Ecuadorian public health system neither has a vaccination program for Hepatitis A nor carried out cost-effectiveness studies for Hepatitis A vaccine.²³ In addition, the annual infection risk for HAV, an indicator of the probability of exposure to HAV, in Ecuador is unknown. In this regard, the only other data available were from an unpublished study among 80 children aged 2 to 4 years from a subtropical region in rural Pichincha Province, which estimated a seroprevalence of 50.0% (P. Cooper, personal communication).

In connection to seropositivity to *Leptospira* spp. used as a marker for zoonotic contamination of drinking water, approximately half the population showed evidence of past exposure, many of whom were exposed also to other pathogen groups. Of the 59 participants who had exposure to all pathogen groups, seven (11.9%) were young children (2–5 years of age). This high seroprevalence indicates not only widespread contamination of

drinking water by animals but also a high level of misdiagnosis and underreporting of leptospirosis, which presents as febrile illness,^{2,12} likely associated with poor access and barriers to accessing health-care services. A previous study observed that among febrile patients in coastal Ecuador, 64% were *Leptospira* DNA-positive in rural areas compared with 25% in semi-urban and 21% from urban areas, indicating potentially high levels of transmission in rural regions of Ecuador.⁴²

Interestingly, seroprevalence for leptospirosis was lower among those receiving cash transfers from the government which might indicate under-registration of the poorest or a potentially beneficial health effect for those in receipt of cash transfers.

The study population, defined using data from the Ecuadorian census as a population with a high percentage of unsatisfied basic needs,⁴³ had limited knowledge of transmission or clinical presentation of the infections we were studying, and few participants treated water before consumption. Water sources were located outside participants' housing and taps were close to the ground, potentially exposing water sources to contamination by domestic animals (especially dogs and rodents) and contact with sewage.

Notifications of the diseases caused by these infections will be important for monitoring trends and detecting outbreaks,⁴⁴ but this will require improved surveillance. Better surveillance, in turn, will require strategies to improve data collection and the recognition of these illnesses within the health system, improved access of marginalized populations to health-care services, and improved education to ensure that such populations access and use such services appropriately.

This study has several limitations: 1) the communities who participated were accessible by road, a situation that does not reflect the reality of most indigenous communities located beyond road access; 2) exposure risk measured through active infection (enteric pathogens) versus serological memory

(HAV and *Leptospira* spp.) do not necessarily measure the same risks—the former measures were recent, whereas the latter measures were cumulative exposures; 3) this study did not use molecular techniques for the detection of enteric pathogens, which is likely to underestimate prevalence;³ 4) participation rates among adult males, who often work at considerable distances or who migrate temporarily for work, was low; and 5) the measurement of IgG antibodies for HAV and leptospirosis tends to reflect past exposures rather than current infections.

In conclusion, the findings of this study show high levels of exposure to groups of pathogens associated with waterborne transmission of infectious diseases in two highly marginalized indigenous communities in the Amazon region of Ecuador. High seroprevalence rates were observed for both HAV and *Leptospira* spp., as well as a high prevalence of infections with enteric parasites. Our findings emphasize the importance of prioritizing a multidisciplinary and inter-institutional approach to the control of waterborne pathogens to ensure that interventions are not only sustainable but modify the human behavior that maintains transmission pathways.

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REFERENCES

1. UN Water, World Health Organization, 2016. *Investing in Water and Sanitation: Increasing Access, Reducing Inequalities: GLAAS 2014 Findings—Highlights for the Region of the Americas*. Geneva, Switzerland: WHO Press.
2. Vijayachari P, Sugunan AP, Shriram AN, 2008. Leptospirosis: an emerging global public health problem. *J Biosci* 33: 557–569.
3. Ramírez-Castillo FY, Loera-Muro A, Jacques M, Gameau P, Avelar-González FJ, Harel J, Guerrero-Barrera AL, 2015. Waterborne pathogens: detection methods and challenges. *Pathogens* 4: 307–334.
4. Cann KF, Thomas DR, Salmon RL, Wyn-Jones AP, Kay D, 2013. Extreme water-related weather events and waterborne disease. *Epidemiol Infect* 141: 671–686.
5. Khan K et al., 2018. Prevalent fecal contamination in drinking water resources and potential health risks in Swat, Pakistan. *J Environ Sci* 72: 1–12.
6. Neumann NF, Smith DW, Belosevic M, 2005. Waterborne disease: an old foe re-emerging? *J Environ Eng Sci* 4: 155–171.
7. Gibney KB, O'Toole J, Sinclair M, Leder K, 2017. Burden of disease attributed to waterborne transmission of selected enteric pathogens, Australia, 2010. *Am J Trop Med Hyg* 96: 1400–1403.
8. Gall AM, Mariñas BJ, Lu Y, Shisler JL, 2015. Waterborne viruses: a barrier to safe drinking water. *PLoS Pathog* 11: e1004867.
9. Paiva RFDPS, Souza MFDP, 2018. Association between socio-economic, health, and primary care conditions and hospital morbidity due to waterborne diseases in Brazil [article in Portuguese]. *Cad Saude Publica* 34: e00017316.
10. Scheifele DW, De Serres G, Gilca V, Duval B, Milner R, Ho M, Ochnio JJ, 2010. A nationwide survey of past hepatitis A infections among Canadian adults. *Vaccine* 28: 5174–5178.
11. García-Fulgueiras A, Rodríguez T, Tormo MJ, Pérez-Flores D, Chirlaque D, Navarro C, 1997. Prevalence of hepatitis A antibodies in southeastern Spain: a population-based study. *Eur J Epidemiol* 13: 481–483.
12. Ismail TF et al., 2006. Retrospective serosurvey of leptospirosis among patients with acute febrile illness and hepatitis in Egypt. *Am J Trop Med Hyg* 75: 1085–1089.
13. Mayorga O, Bühler S, Jaeger VK, Bally S, Hatz C, Frösner G, Protzer U, Van Damme P, Egger M, Herzog C, 2016. Single-dose hepatitis A immunization: 7.5-year observational pilot study in Nicaraguan children to assess protective effectiveness and humoral immune memory response. *J Infect Dis* 214: 1498–1506.
14. Barragan V, Olivas S, Keim P, Pearson T, 2017. Critical knowledge gaps in our understanding of environmental cycling and transmission of *Leptospira* spp. *Appl Environ Microbiol* 83: e01190-17.
15. Mesquita GSS, Rocha KS, Monteiro TRM, Rosário MKSD, Baia IWM, Pereira HS, Cerqueira VD, Moraes CCG, 2018. Detection of antibodies against *Leptospira* spp in free-living marsupials caught in the eastern Amazon. *Rev Soc Bras Med Trop* 51: 368–371.
16. Guedes IB, Araújo SAA, de Souza GO, de Souza Silva SO, Taniwaki SA, Cortez A, Brandão PE, Heinemann MB, 2019. Circulating *Leptospira* species identified in cattle of the Brazilian Amazon. *Acta Trop* 191: 212–216.
17. Casanovas-Massana A et al., 2018. Spatial and temporal dynamics of pathogenic *Leptospira* in surface waters from the urban slum environment. *Water Res* 130: 176–184.
18. Ribeiro P et al., 2017. Seroepidemiology of leptospirosis among febrile patients in a rapidly growing suburban slum and a flood-vulnerable rural district in Mozambique, 2012–2014: implications for the management of fever. *Int J Infect Dis* 64: 50–57.
19. Moncayo AL, Lovato R, Cooper PJ, 2018. Soil-transmitted helminth infections and nutritional status in Ecuador: findings from a national survey and implications for control strategies. *BMJ Open* 8: e021319.
20. Speare R, Durrheim DN, 2004. Strongyloides serology—useful for diagnosis and management of strongyloidiasis in rural indigenous populations, but important gaps in knowledge remain. *Rural Remote Health* 4: 264.
21. Bain R et al., 2014. Global assessment of exposure to faecal contamination through drinking water based on a systematic review. *Trop Med Int Health* 19: 917–927.
22. Schriever A, Odagiri M, Wuertz S, Misra PR, Panigrahi P, Clasen T, Jenkins MW, 2015. Human and animal fecal contamination of community water sources, stored drinking water and hands in

- rural India measured with validated microbial source tracking assays. *Am J Trop Med Hyg* 93: 509–516.
23. Ministerio de Salud Pública de Ecuador, 2016. Enfermedades Zoonóticas 1994–2017 - Dirección Nacional de Vigilancia Epidemiológica_MSP | Tableau Public. Available at: https://public.tableau.com/profile/vvicentee80#!/vizhome/cronicas_2016/ANUARIO. Accessed October 9, 2018.
 24. Romero-Sandoval N, Ortiz-Rico C, Sánchez-Pérez HJ, Valdivieso D, Sandoval C, Pástor J, Martín M, 2017. Soil transmitted helminthiasis in indigenous groups. A community cross sectional study in the Amazonian southern border region of Ecuador. *BMJ Open* 7: e013626.
 25. Rubenstein S, 2001. Colonialism, the Shuar federation, and the Ecuadorian state. *Environ Plan D* 19: 263–293.
 26. Liebert MA, Snodgrass JJ, Madimenos FC, Cepon TJ, Blackwell AD, Sugiyama LS, 2013. Implications of market integration for cardiovascular and metabolic health among an indigenous Amazonian Ecuadorian population. *Ann Human Biology* 40: 228–242.
 27. Jokisch BD, McSweeney K, 2011. Assessing the potential of indigenous-run demographic/health surveys: the 2005 Shuar survey, Ecuador. *Hum Ecol* 39: 683–698.
 28. Montresor A, Crompton DW, Hall A, Bundy DA, Savioli L; World Health Organization, 1998. *Guidelines for the Evaluation of Soil-Transmitted Helminthiasis and Schistosomiasis at Community Level: A Guide for Managers of Control Programmes*. Geneva, Switzerland: WHO.
 29. Onis M de, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J, 2007. Development of a WHO growth reference for school-aged children and adolescents. *Bull World Health Organ* 85: 660–667.
 30. Blackwell AD, Snodgrass JJ, Madimenos FC, Sugiyama LS, 2010. Life history, immune function, and intestinal helminths: trade-offs among immunoglobulin E, C-reactive protein, and growth in an Amazonian population. *Am J Hum Biol* 22: 836–848.
 31. Gildner TE, Cepon-Robins TJ, Liebert MA, Urlacher SS, Madimenos FC, Snodgrass JJ, Sugiyama LS, 2016. Regional variation in *Ascaris lumbricoides* and *Trichuris trichiura* infections by age cohort and sex: effects of market integration among the indigenous Shuar of Amazonian Ecuador. *J Physiol Anthropol* 35: 28.
 32. Cepon-Robins TJ, Liebert MA, Gildner TE, Urlacher SS, Colehour AM, Snodgrass JJ, Madimenos FC, Sugiyama LS, 2014. Soil-transmitted helminth prevalence and infection intensity among geographically and economically distinct Shuar communities in the Ecuadorian Amazon. *J Parasitol* 100: 598–607.
 33. Berendes D et al., 2017. Household sanitation is associated with lower risk of bacterial and protozoal enteric infections, but not viral infections and diarrhoea, in a cohort study in a low-income urban neighbourhood in Vellore, India. *Trop Med Int Health* 22: 1119–1129.
 34. Vieira AS, Pinto PS, Lilenbaum W, 2018. A systematic review of leptospirosis on wild animals in Latin America. *Trop Anim Health Prod* 50: 229–238.
 35. López-Cevallos DF, Chi C, 2010. Health care utilization in Ecuador: a multilevel analysis of socio-economic determinants and inequality issues. *Health Policy Plan* 25: 209–218.
 36. Anderson I et al., 2016. Indigenous and tribal peoples' health (The Lancet-Lowitja Institute Global Collaboration): a population study. *Lancet* 388: 131–157.
 37. Kim YJ, Lee H-S, 2010. Increasing incidence of hepatitis A in Korean adults. *Intervirology* 53: 10–14.
 38. Jayasundara D, Hui BB, Regan DG, Heywood AE, MacIntyre CR, Wood JG, 2019. Modelling the decline and future of hepatitis A transmission in Australia. *J Viral Hepat* 26: 199–207.
 39. Jacobsen KH, Wiersma ST, 2010. Hepatitis A virus seroprevalence by age and world region, 1990 and 2005. *Vaccine* 28: 6653–6657.
 40. World Health Organization, 2016. *Investing in Water and Sanitation: Increasing Access, Reducing Inequalities: GLAAS 2014 Findings-Highlights for the Region of the Americas*, WHO/FWC/WSH/16.41, World Health Organization. Available at: http://apps.who.int/iris/bitstream/10665/204597/1/WHO_FWC_WSH_16.41_eng.pdf?ua=1. Accessed February 28, 2018.
 41. Mayorga Perez O, Brinkhof MWG, Egger M, Frösner G, Herzog C, Zwahlen M, 2014. Decreasing risk of hepatitis a infection in León, Nicaragua: evidence from cross-sectional and longitudinal seroepidemiology studies. *PLoS One* 9: e87643.
 42. Chiriboga J et al., 2015. High prevalence of intermediate *Leptospira* spp. DNA in febrile humans from urban and rural Ecuador. *Emerging Infect Dis* 21: 2141–2147.
 43. Instituto Nacional de Estadística y Censos, 2011. *Pobreza por Necesidades Básicas Insatisfechas*. Quito, Ecuador: Instituto Nacional de Estadística y Censos. Available at: <http://www.ecuadorencifras.gob.ec/pobreza-por-necesidades-basicas-insatisfechas/>. Accessed March 4, 2018.
 44. Wilson SE, Deeks SL, Hatchette TF, Crowcroft NS, 2012. The role of seroepidemiology in the comprehensive surveillance of vaccine-preventable diseases. *CMAJ* 184: E70–E76.