Short Report: *Helicobacter pylori* Seroprevalence in Amerindians from Isolated Locations

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**Abstract.** *Helicobacter pylori* seems universally distributed in all human populations, with high prevalence in the third world. Because *H. pylori* is an ancestral indigenous microbe of the human stomach, we hypothesized that its prevalence in isolated Amerindians would be high. A serologic study was performed on 19 Guahibo-Piaroa and 17 Warao in Venezuela, using *H. pylori* whole cell (WC) and CagA antigens from US strains. For Guahibo-Piaroa Amerindians, CagA seropositivity was 95%, but WC seropositivity was only 74%. For Warao, both CagA and WC seropositive proportions were low (65% and 76%, respectively). Because all CagA-seropositive individuals carry *H. pylori*, the results suggest that there has been bacterial antigen divergence, probably caused by genetic drift/natural selection, on humans and their microbes in isolated human groups.

Humans are universally colonized with *Helicobacter pylori*. Prevalence is universally high in all human groups, until recently; *H. pylori* is now disappearing from modern societies.1 *H. pylori* have a geographic genetic structure, the Amerindian strains having Asian characteristics2 consistent with the East Asian ancestry of Amerindians. Today’s Amerindians descend from Asians that arrived in America > 20,000 years ago.3 Divergence between the Central and South American populations fall between 13,000 and 19,000 years.4 In contrast to Andean Amerindians, some groups east of the Andes have remained in relatively high isolation and have diverged from the former, showing lower levels of intra-population genetic variability.4 In Venezuela, Amerindian groups form small-sized populations, some remaining highly isolated over the years. Warao (Chibchan-Paezan linguistic affiliation) are found in the Orinoco Delta, and Piaroa/Guahibo (equatorial) are in the Amazonas (south). Evidence for this isolation is supported by human microbes. First is viral evidence by Asian type hepatitis GBV-C virus (HGV) circulating among Amerindians groups.5 The lack of common viruses widely found in some Amerindian populations also reflects this isolation. Bari and Yukpa Amerindians from Sierra Perijá, for example, are not infected with hepatitis C virus (HCV).6

Although *H. pylori* have been found in the stomach of all human populations examined, isolated human groups have barely been studied. We hypothesized that Amerindian groups relatively isolated in Amazonas and in Orinoco Delta of Venezuela would have high prevalence of *H. pylori* colonization, consistent with their poor hygienic and rural conditions.

Sera were from 18 males and 18 female Amerindians, 10-55 years of age, 19 from the Amazonas Guahibo/Piaroa (average age, 30 ± 13 years) and 17 from Delta Amacuro Warao Amerindians (average age, 23 ± 12 years; Figure 1). The 36 serum samples included in this study were part of an effort pursued in 1991 to study hepatitis viruses.7 Samples were kept frozen at ultralow temperatures, under a code that precludes patient’s identification, and therefore, there was no treatment of any kind offered to the patients as part of this study. The protocol was approved by the IVIC Ethical Committee.

Serum IgG was detected by ELISA, using *H. pylori* whole cell (WC) and CagA protein as antigens. WC antigens were derived from a characterized pool of sonicates from five US strains. Sensitivity and specificity of this serologic test on serum from US persons was 96% and 93.5%, respectively.8 For the WC antigen, an optical density ratio ≥ 1.0 in 1:800 sera dilutions was considered positive. To assess serologic response to CagA, a 66-kDa CagA fragment that had been cloned in *Escherichia coli* as pORV220,9 was used as antigen. For this assay, sera diluted 1:100 were considered positive if the optical density was ≥ 0.35.

χ² analysis allowed comparisons of proportions of values between groups. In each case, *P* < 0.05 was defined as significant.

This study examined serum IgG responses of two isolated Amerindian peoples to *H. pylori* WC and CagA antigens. Only 3 of the 36 subjects were negative to both antigens, and these subjects were young (age, 12, 14, and 19 years). There was a significant association of *H. pylori* with age (P = 10⁻¹⁴).

Serologic response to either or both antigens seemed to be a better indicator of *H. pylori* positivity than either antigen alone (Figure 1). There was a low response to *H. pylori* WC antigens in relation to what was expected according to recognition of any of the *H. pylori* antigens (95% in Guahibo-Piaroa and 88% in Warao; Figure 1). A proportion of Guahibo (4/19) and Warao subjects (2/17) were seropositive to CagA but not to WC (Table 1).

Guahibo and Piaroa populations in Venezuela were traditionally nomadic hunters and gatherers, but since the 1970s, many live in government houses in small villages led by a political “captain.” Present day Guahibo and Piaroa people maintain their respective languages: Amerindian, Equatorial-Tucanoan stock, Equatorial group, subgroups Macro-Arawakan the Guahibo and Piaroa, respectively.10 They still maintain high levels of endogamy and a relatively sedentary life. Guahibo/Piaroa people inhabit the Northwestern Amazonas region in Venezuela, whereas Warao people are constrained to narrow coastal and riverside fringes along the central Orinoco Delta. Warao Amerindians are poor, because

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some of the Orinoco affluents have been blocked, impairing fishing and agricultural activities in a severely affected environment. Warao people represent a cluster of Chibchan speakers of Paleo-Mongoloid descent, distinct from the Guahibo-Piaroa of more recent arrival to the continent. Based on their mythology and ethno-ecologic studies, it has been proposed that the Warao people represent littoral populations that split from their ancestors in Central America between 7,000 and 9,000 years ago (W. Wilbert, personal communication).

Our Amerindian groups seem to have a high prevalence of H. pylori, unlike in a few isolated human coastal groups in Asia, who have unusually low H. pylori prevalence. Serology allowed detection of H. pylori in 95% of the Piaroa population. This is consistent with previous results obtained in many other parts of the third world. Because, in Piaroa, sera recognition of CagA antigens was better than that of WC antigens, the CagA antigen test improved the chances of detecting H. pylori. However, the Warao people, in which a high prevalence of H. pylori was expected, showed low seropositivity to both CagA and WC antigens, with 36% of sera recognizing only one of the two H. pylori antigens. The results could respond to three possible scenarios: 1) an impaired Th1 immune response caused by multiple parasitic infections; 2) low H. pylori prevalence; and 3) poor recognition of H. pylori antigens circulating in the United States. Amerindian populations often have a high prevalence of viruses (eg, hepatitis B and G viruses), associated with an impaired Th1 response, with low numbers of T and B lymphocytes in the peripheral blood. However, a poor Th1 response is not consistent with a high response to the CagA antigen found in the Guahibo/Piaroa population. Because H. pylori is universally present in all human groups—only recently disappearing in industrialized countries—and because the antigenic response against H. pylori has been broadly shown to occur in many human groups, we favor the interpretation of distinct H. pylori strains circulating among Amerindians.

Poor antigenic recognition would mean that strains of H. pylori circulating in the subjects are different to the strains from which the antigens were prepared. Antigens used in our serology were prepared from a mix of five US strains. Serologic responses to H. pylori in Thailand, China, and India have led to suggest that antigen differences have a geographic component. In isolated human populations, an accurate serology is better achieved by using local antigens. In a previous work with US strain antigens, serology failed to detect as much as 20% of the H. pylori–positive persons in Ladakh.

_Helicobacter pylori_ adapts to its hosts, selecting treats that increase fitness and losing those that do not confer competitive advantages. A good example of host adaptation in _H. pylori_ is given by the function of the _H. pylori_ BabA adhesin, which recognizes ABO blood group antigens in the stomach of the human host. Most human societies have people with A, B, and O blood groups, and _H. pylori_ strains from these humans recognize all three blood group antigens. Amerindians, however, have the O blood group, and their strains preferably bind O group antigens. Therefore, _H. pylori_ circulating among different human groups are different.

We hypothesize that genetic drift/natural selection within the Amerindian host may have been a source of genetic and antigen divergence. It has recently been reported that some Amerindians have deletions in the _cag_ pathogenicity island, which could certainly affect antigenic recognition if antigens are prepared from other _H. pylori_ populations. This preliminary study stresses the importance of pursuing other studies to confirm the hypothesis of antigenic variability among isolated populations, because thus far, commercial serologic tests for _H. pylori_ based on local antigens may fail and lead to false-negative results. An important study should be testing commercial and local serologic tests in Amerindians with a positive _H. pylori_ breath test. In addition, studies performed today in the same locations, 15 years after the sera for this study was collected, will also show if the trend of _H. pylori_ to disappear is confirmed also for these communities.

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