HELIcobacter pylori AND intestinal parasites are NOT detrimental to the nutritional status of Amerindians

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Abstract. Gastrointestinal parasites have evolved with humans and colonize many asymptomatic subjects. We investigated the influence of microbial gastrointestinal colonization on the nutritional status of rural Amerindians (40 males and 61 females). Helicobacter pylori was detected by 13C-breath test, and intestinal parasites were detected in fecal specimens. Body morphometry and bioelectrical impedance measurements were measured. Although Amerindians showed low height and weight for age, they had an adequate body mass index, morphometric parameters, and cell mass. Intestinal parasites were detected in 99% of the subjects, with no detrimental effect on nutritional parameters. Helicobacter pylori was present in 82% of adults and half the children, and was positively correlated with improved nutritional status. Despite the high prevalence of gastrointestinal microbes often associated with disease, the studied population of Amerindians had a body morphometry and composition indicative of good nutritional status, and improved in children positive for gastric H. pylori.

INTRODUCTION

Helicobacter pylori is a well-known gastric bacterium because of its causative role in the development of chronic gastric inflammation, which predisposes to peptic ulceration and gastric cancer.1 This organism has been linked to increased risk of diarrhea,2 but results of other studies are contradictory, finding no association3–5 or even a protective effect.6

Intestinal protozoa and helminths are widely known as parasites because they can cause a variety of diseases. Giardia lamblia can cause vomiting and diarrhea, the hookworms Ankylostoma duodenale and Necator americanus can cause blood loss and anemia, and Entamoeba histolytica can cause intestinal ulceration, bloody diarrhea, and systemic complications.7 Because of their relationship with disease, intestinal parasites have been extensively eradicated in industrialized human societies, in which H. pylori also is disappearing.8

It is widely believed that individuals carrying gastrointestinal parasites have a poorer nutritional status than those without these microbes. However, despite the clear linkage of gastrointestinal microbes with disease, most individuals in developing countries remain persistently colonized but asymptomatic. Are these individuals adversely affected by these microbes, despite having no disease?

If eradication using antimicrobial therapy is not pursued, both intestinal parasites and H. pylori frequently and persistently colonize individuals in developing countries. Despite the well-known health risks posed by certain microbes (e.g., gastric H. pylori, intestinal protozoa and helminths), carriage of indigenous organisms that have coevolved with humans might also confer some benefits. According to the hygiene hypothesis, modern lifestyle has eliminated helminths and protozoa and is substantially affecting our indigenous bacteria. Exposure to microbes is a critical environmental determinant in the development of human infant immunity and body morphometry. Even in the absence of pathology, colonization by indigenous microbes affects the host in at least three ways: 1) by altering host responses to non-indigenous parasites, a property known as colonization resistance; 2) by affecting host immunity9 and 3) by affecting hormonal modulation of appetite, body composition,10 immunity,11,12 and reproduction.13 The purpose of this study was to investigate the extent of H. pylori and parasite colonization in humans living under rural conditions and to understand their influence on nutritional status.

SUBJECTS AND METHODS

Subjects. The study included 101 volunteers (40 male and 61 female Guahibo Amerindians 2–70 years of age) from two villages (Platanillal and Coromoto) near Puerto Ayacucho, the capital city of the Venezuelan Amazonas State. The Guahibo population in Venezuela consists of approximately 11,000 individuals14 who are traditionally nomadic hunters and gatherers.15,16 Since the 1970s, many live in government-built houses in small villages led by a political captain. Present-day Guahibos maintain their language (Amerind, Equatorial-Tucanoan stock, Equatorial group, and Macro-Arawakan subgroup17), high levels of endogamy, and a relatively sedentary life except for hunting periods involving groups of men. They generally earn their living by fishing and hunting, preparing manioc, and selling crafts, made mostly by women. Sanitary conditions are deficient, and according to local medical statistics, small children 2–5 years of age (after breast-feeding) are subject to the highest incidence of diarrheal diseases and infant mortality.18

Volunteers from each town were sampled. We attempted to enroll approximately 20 individuals from each of the four age groups less than 40 years of age (Supplemental Table 1). For each individual, the following data were collected: age, sex, family unit, number of siblings, cohort position, and
household size. Written informed consent was obtained from all participants and their parents, in the case of children, as well as from the community chief and local medical doctor. The experimental protocol was reviewed and approved by the Venezuelan Institute of Scientific Research and the Centro Amazónico para Investigación y Control de Enfermedades Tropicales bioethical commissions, and had the support of the community. Informed consent was obtained from subjects or their guardians according to human experimentation guidelines of the Helsinki Declaration of 1975, as revised in 1983. Results of the project were presented to the communities, together with recommendations on hygienic habits and water chlorination. Cases in children with low nutritional status were reported to the family and local doctor and antiparasitic drugs were provided to the rural medicalambulatory to be used in symptomatic cases.

Methods. Detection of *H. pylori* was performed in 90 of the 101 subjects by the $^{13}$C-urea breath test (UBT) with $^{13}$C detected by mass spectrometry (Isomed, Madrid, Spain). According to the manufacturer’s information, the Isomed UBT has a sensitivity of 94.3% and a specificity of 96.3% (www.isomed.com). The UBTs were performed in the morning with the subject fasting for at least two hours. Subjects swallowed $^{13}$C urea (100 mg) with a citric acid drink after basal breath samples were taken. After 30 minutes, breath samples were retaken. All samples were analyzed in duplicate.

Fecal samples were collected from 72 of the subjects; samples were obtained using a regular feces collector, and transported to the medical center in each village, where they were kept on ice until they were brought to the laboratory for microscopic examination within 24 hours of sampling. Simple microscopic examination of fecal parasites enabled the estimation of parasitic diversity, which was the number of different protozoa or helminths found per person without quantification of parasite density or parasitic load.

Body weight, height, subscapular and triceps skinfolds were determined according to standard procedures, and body mass index (BMI, kg/m$^2$) was calculated and compared with World Health Organization standard curves. All anthropometric measurements were taken by an experienced and well-trained observer, whose technical error was low: 0.18 (height, cm), 0.04 (weight, kg), 0.18 (triceps skinfold, mm), and 0.12 (subscapular skinfold, mm). Bioelectrical parameters of resistance (R) and reactance (Xc) were measured with a single-frequency impedance analyzer (BIA 101, Akern, Firenze, Italy) with an operating frequency of 50 kHz at 800 $\mu$A.

Body composition was assessed by bioelectrical impedance vector analysis (BIVA) because it has high sensitivity, is increasingly used in clinical applications, and is easy to use in the field. Edefonti and others found that the sensitivity of this technique was greater than that of anthropometry, classic bioimpedance analysis, or dual-energy x-ray absorptiometry in the classification of malnutrition. Furthermore, BIVA distinguished between clinically assessed edematous subjects and obese individuals with 100% sensitivity and high specificity (90.4% in men, 92.5% in women). BIVA detects excess body water in patients with edema that total body weight estimates from anthropometry equations fail to detect. Individual impedance vectors were plotted within the tolerance ellipses (RXc-score graph), in which the major axis indicates hydration status and the minor axis indicates cell mass. The individual impedance vectors were translated into nutritional scores, depending on their location in the tolerance ellipse. Scores were assigned as follows: 1 = tendency to deficient nutritional status, or dehydration (Z vectors outside the 50% ellipse toward the right, or upper pole); 2 = normal nutritional status (Z vectors inside the 50% ellipse); or 3 = good nutritional status (Z vectors outside the 50% ellipse towards the left side, corresponding to high cell mass and adequate hydration).

To evaluate the effect of *H. pylori* and parasite carriage on anthropometric parameters (Y), regression analyses were performed using generalized linear models (GLIM) with random response variables, BMI, triceps skinfold, subscapular skinfold, and nutritional score assuming a gamma distribution for Y. The significance of the final set of regressor variables (age, sex, and *H. pylori* or parasite diversity) was obtained by means of the single regression coefficients. Maximum likelihood allowed estimation of column vector of coefficients, $\beta$, in the function $f(\mu_{Y}) = \chi \beta$, where $f()$ is the link function. We chose the logarithmic link ($\log(\mu_{Y}) = \chi \beta$), based on the optimization of both the Akaike Information Criterion and the Bayesian Information Criterion. Analyses were performed using R with the library MASS. Because *H. pylori*-negative subjects were children, we sought to confirm the robustness of the results of a hypothetical age-balanced case-control study. For this purpose, we used non-parametric bootstrapping, restricted to a sub-sample of young subjects (age = 4–9 years). Using the original dataset, we formed 129 groups each with 4 subjects (male/female with *H. pylori* and male/female without *H. pylori*) with age differences < 1.0 year. We re-sampled 1,000 times with replacement of 10 of 129 groups at each iteration, and obtained 1,000 P values.

## RESULTS

The two villages studied each had populations of approximately 200 individuals. Several families were related, with the same surnames. Average family size was seven, mean age of mothers was 29 years, and individuals recruited in the study were between 2 and 70 years of age. Demographic information by age group, sex, and the number of samples included in each analysis is shown in Supplementary Table 1.

We examined feces for parasites in 72 (36%) of 200 individuals. This ensures with 95% confidence that the true prevalence lies within a maximum distance of 9.4% from the true proportion. Furthermore, in our GLIM analyses, we performed data augmentation through bootstrap sampling, i.e.,
the same results that previously relied on large samples are now, by bootstrapping, valid for smaller samples. Bootstrapped results are quite consistent with the original analyses, a further indication that our original sample is representative of the population sampled, for the purposes intended.

The results show that of the young subjects (2–20 years of age) in this study, 74% had stature for age below the 10th percentile and 89% had weight for age below the 50th percentile. However, BMI was distributed more centrally around the 50th percentile (Figure 1 and Supplementary

**Figure 1.** Distribution of weight, stature, and body mass index (BMI) of Amerindian subjects less than 20 years of age in comparison with World Health Organization standard curves (bold lines) by age (25). Hp = Helicobacter pylori.
Table 2). Bioimpedance results indicated an adequate general nutritional status, with individual bioelectrical Z vectors centered or shifted toward the left quadrant of the ellipse, which is associated with higher cell mass in relation to the reference population (Figure 2).

The proportion of *H. pylori*–positive individuals increased with age (*P* = 0.03), from 47% in children to 82% in adults (Table 1) with no effect of sex, number of siblings, cohort position, household size, or family unit (*P* > 0.05). *Helicobacter pylori*–positive individuals had greater soft tissue mass, which is indicative of better nutritional status than *H. pylori*-negative individuals (Figure 2). When analyzed by general linear models, the presence of *H. pylori* was significantly associated with increased subscapular skinfold and nutritional scores (Table 2), and the other variables did not change. Approximate and bootstrapping analyses of the regression by sex and age confirmed higher nutritional scores and subscapular skinfold in *H. pylori*-positive subjects compared with *H. pylori*-negative subjects (Supplementary Figure 1).

Parasites were observed in 99% of examined subjects (Table 3), with most having 2–4 different species (Figure 3). Parasite diversity (defined as the number of diverse fecal protozoa or helminths observed) was age-dependent, with the greatest diversity detected in children 2–11 years of age (*P* = 0.007). There was no effect of sex (*P* = 0.67), but parasite diversity varied among family units (*P* = 0.006). Parasite diversity had no effect on BMI (*P* = 0.59), triceps skinfold (*P* = 0.31), subscapular skinfold (*P* = 0.59), or nutritional score (*P* = 0.18).

### DISCUSSION

Nutritional status and human growth are affected by both intrinsic (genetic, endocrine, immune) and environmental factors (Figure 4). That environment influences nutritional status is shown by the rapid secular trend toward increased height and weight in many developed countries. In societies with large socioeconomic differences, increased height is associated with higher socioeconomic status. A trend towards taller and heavier individuals appears as societies adopt modern lifestyles. This trend is not always beneficial because high birth weight, rapid growth, and high energy intake may be associated with development of cancer, diabetes, hypertension, and stroke. In this study, we have shown that the nutritional status of Amerindians was adequate, despite their low weight and height, and despite their burden of gas-

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**Table 2**

<table>
<thead>
<tr>
<th>Nutritional measurement</th>
<th>Age</th>
<th>Sex</th>
<th>Age/sex</th>
<th><em>Helicobacter pylori</em> status</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.000</td>
<td>0.081</td>
<td>0.038</td>
<td>0.060</td>
</tr>
<tr>
<td>Triceps skinfold</td>
<td>0.000</td>
<td>0.000</td>
<td>0.001</td>
<td>0.298</td>
</tr>
<tr>
<td>Subscapular skinfold</td>
<td>0.000</td>
<td>0.005</td>
<td>0.066</td>
<td>0.034</td>
</tr>
<tr>
<td>Nutritional score*</td>
<td>0.209</td>
<td>0.758</td>
<td>0.065</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*a* BMI = body mass index.

*b* Determined by *t*-tests; *p* values < 0.1 for the effects of *H. pylori* are shown in bold.

*c* Based on body composition, as measured by impedance vectors and their location on the tolerance ellipse of Figure 2.

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**Figure 2.** Sample distribution of impedance vectors on tolerance ellipses, according to *Helicobacter pylori* status (*H. pylori* positive, *n* = 43; *H. pylori* negative; *n* = 29). The long axis of the ellipse indicates hydration, and the short axis indicates soft tissue (cell) mass. The ellipses represent 95%, 75%, and 50% inclusivity, based on a reference population of Italian standards.
intestinal parasites. Lower energy intake, slower childhood growth rates, and shorter stature do not necessarily imply impairment in childhood development, and possibly might improve health at later stages of adult development.

Secular variation in height may be related not only to diet and socioeconomic conditions, but also to reduced microbial transmission because of improved hygiene and extensive use of antibiotics. Sub-therapeutic dosages of antibiotics have been widely used by the livestock industry as growth promoters. Possible mechanisms for the effect of intestinal microbes on body morphometry include the energetic costs of having such organisms, the effects on gut function, and the induction of illness with catabolic states. Colonization with *H. pylori* in children has been associated with shorter stature, and most persons in the third world who in general have shorter stature than individuals in developed countries, also have high intestinal parasitism. However, as shown here, height or weight alone is not a good nutritional indicator in Amerindian populations.

*Helicobacter pylori* up-regulates gastric leptin, the anorexigenic hormone that affects energy homeostasis and body composition. Eradication of *H. pylori* in humans decreases gastric leptin production but does not appear to affect serum leptin levels. Ghrelin, a hormone also related to satiety and energy homeostasis, is produced mainly in the stomach and appears to be down-regulated by *H. pylori*. In adults, lack of *H. pylori* has been linked to increased gastric and plasma ghrelin, although the effect of *H. pylori* eradication on body weight is not clear.

Despite their roles in disease, *H. pylori* and intestinal parasites have evolved with their natural human hosts. *Helicobacter pylori* is an ancient human colonizer and other members of the genus are widely found in other mammalian stom-
ach. The results of the present work on parasites, although
descriptive and relatively preliminary, provide a good basis
for more quantitative and mechanistic studies in the future.
Little is known about the natural evolution of the intestinal
eukaryotes because studies are heavily biased towards clinical
aspects. That most subjects in our study have good nutritional
status with asymptomatic colonization by intestinal parasites
and that H. pylori is associated with better nutritional status
in children supports the hypothesis that these gastrointestinal
microbes may be mutualistic with humans, at least early in
life. It has been shown that low-intensity helminth infections
do not contribute significantly to the poor growth of rural
Bangladeshi children. In disadvantaged societies threatened
by malnutrition and highly pathogenic microbes, indigenous
gastrointestinal organisms could prime more robust immune
responses in children, e.g., by the effect of leptin on endocrine
and immune function. Because pre-reproductive and peri-
reproductive life is subjected to natural selection, benefits to
the host during early life that result in enhanced metabolic
disease risk later in life, after the reproductive years, may be
beneficial.

Other microbial effects that are generally considered del-
erious might exert a protective effect under some specific
circumstances. For example, in areas in which malaria is ho-
loendemic, selection of adaptive immune responses against
malaria has occurred in populations concomitantly infected
by intestinal helminths. By inducing anemia, helminths and
nematodes may enhance resistance to malaria-induced mor-
tality. H. pylori and other iron-scavenging bacteria also
might confer protection. Microbes therefore may induce a
range of antagonist and synergistic interactions, depending on
hosts and environmental circumstances. It is paradoxical that
relatively primitive societies in Latin America may provide
novel insights for improving human health and nutrition.
Concepts deeply anchored in the scientific (and public) do-
main about the role of particular common microbes in health
and disease may need revision. Diseases related to indigenous
human parasites might be a consequence of an ecologic im-
balance when one of the parasite groups overgrows, rather
than by the simple presence of these microbes in their natural
habitat.

In conclusion, our study in this particular human group
shows no deleterious effect of intestinal parasite diversity on
human morphometry, as well as improved nutrition in chil-
dren carrying H. pylori. These results challenge traditional
definitions of gastrointestinal pathogens in relation to nutri-
tional status.

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