**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. This organism has been linked to increased risk of diarrhea, but results of other studies are contradictory, finding no association or even a protective effect.

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 *Anthostoma duodenale* and *Necator americanus* can cause blood loss and anemia, and *Entamoeba histolytica* can cause intestinal ulceration, bloody diarrhea, and systemic complications. Because of their relationship with disease, intestinal parasites have been extensively eradicated in industrialized human societies, in which *H. pylori* also is disappearing.

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 morphology. 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 immunity; and 3) by affecting hormonal modulation of appetite, body composition, and reproduction. 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 individuals who are traditionally nomadic hunters and gatherers. 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 subgroup), 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 subject to the highest incidence of diarrheal diseases and infant mortality.

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...
TABLE 1
Prevalence of Helicobacter pylori by age in 90 asymptomatic Amerindian subjects

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>2–11</th>
<th>12–20</th>
<th>&gt; 20</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects examined</td>
<td>41</td>
<td>15</td>
<td>34</td>
<td>90</td>
</tr>
<tr>
<td>Number H. pylori-positive</td>
<td>19</td>
<td>7</td>
<td>28</td>
<td>54</td>
</tr>
<tr>
<td>%</td>
<td>46</td>
<td>47</td>
<td>82</td>
<td>60</td>
</tr>
</tbody>
</table>

Results

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](image-url)
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* = 0.008) and decreased with higher number of siblings (*P* = 0.02) and with higher household size (*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-

![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.](image-url)
trointestinal 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 helmhinx infections do not contribute significantly to the poor growth of rural Bangladeshi children.\(^{50}\) 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 perireproductive 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 deleterious might exert a protective effect under some specific circumstances. For example, in areas in which malaria is holoendemic, selection of adaptive immune responses against malaria has occurred in populations concomitantly infected by intestinal helmhinx.\(^{51}\) By inducing anemia, helmhinx and nematodes may enhance resistance to malaria-induced mortality.\(^{52}\) *H. pylori* and other iron-scavenging bacteria also might confer protection.\(^{53}\) 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) domain 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 imbalance 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 children carrying *H. pylori*. These results challenge traditional definitions of gastrointestinal pathogens in relation to nutritional status.

Received June 3, 2006. Accepted for publication October 31, 2006.

Acknowledgments: We thank the Amerindian communities for participating in the study.

Financial support: The study was partially supported by the Regione Autonoma Sardegna contributions (L.R. 11.4.1996 N.19), Italy, and the Belfer Program in Human Microbial Ecology in Health and Disease. The American Society of Tropical Medicine and Hygiene (ASTMH) assisted with publication expenses.

Authors’ addresses: Elisabetta Marini, Roberto Buffa, and Giovanni Floris. Department of Experimental Biology. University of Cagliari, Cagliari, Italy. Telephone: 39-7-675-4156, 39-7-675-4164, and 39-7-675-4130, Fax: 39-7-675-4032, E-mails: emarini@unica.it, rbuffa@unica.it, and floris@unica.it. Ana L. Maldonado-Contreras, Venezuelan Institute of Scientific Research, Caracas, Venezuela. Telephone: 787-764-0000 extension 7798; Fax: 787-764-2610. E-mail: almaldon@gmail.com. Stefano Cabras and Walter Racugno, Department of Mathematics, University of Cagliari, Cagliari, Italy, Telephone: 39-7-675-8536 and 39-7-675-8532, Fax: 39-7-675-8504. E-mails: s.cabras@unicia.it and racugno@unicia.it. Gilda Hidalgo and Aura Marin, Amazonic Center for Research and Control of Tropical Diseases, Centro Amazónico para Investigación y Control de Enfermedades Tropicales, Puerto Ayacucho, Venezuela. Telephone: 58-248-521-2223, Fax: 58-248-521-3319. E-mails: ghlidalgo@ivic.ve and auramarin@hotmail.com. Luis R. Pericchi, Department of Mathematics of University of Puerto Rico, San Juan, PR, 00931, Telephone: 787-528-1235, Fax: 787-281-0651, E-mail: lpericchi@email.uprrp.edu. Maria E. Castellanos, Department of Statistics, Rey Juan Carlos University, Madrid, Spain, Telephone: 34-91-488-8244, Fax: 34-91-488-7626, E-mail: maria.castellanos@urjc.es. Michael Gröschl, Laboratory of Endocrinology, Klinik mit Poliklinik für Kinder und Jugendliche, Erlangen, Germany, Telephone: 49-9131-835-3745, Fax: 49-9131-835-3714, E-mail: Michael.Groeschl@kinder.med.uni-erlangen.de. Martin J. Blaser, Departments of Medicine and Microbiology, New York University School of Medicine, New York, NY 10016, Telephone: 212-263-6394, Fax: 212-263-7700, E-mail: martin.blaser@med.nyu.edu. Maria G. Domínguez-Bello, Department of Biology, University of Puerto Rico, San Juan, PR, 00931, Telephone: 787-764-0000, Extension 4884, Fax: 787-764-2610, E-mails: mgdbello@uprr.pr and mgbello@gmail.com.

Reprint requests: Maria G. Domínguez-Bello, Department of Biology, University of Puerto Rico, Av. Ponce Leon, N CN 343, San Juan, PR 00931.

REFERENCES