

## *Helicobacter pylori* Infection in an Urban African Population

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**We have studied 221 adults drawn from an impoverished urban population with high human immunodeficiency virus (HIV) seroprevalence (35%) to determine the prevalence of gastroduodenal pathology and its relationship to serological markers of *Helicobacter pylori* virulence proteins and other potential environmental and immunological determinants of disease including HIV infection. Eighty-one percent were *H. pylori* seropositive, and 35% were HIV seropositive. Urban upbringing and low CD4 count were associated with a reduced likelihood of *H. pylori* seropositivity, as was current *Ascaris* infection, in keeping with recent evidence from an animal model. One hundred ninety-one adults underwent gastroduodenoscopy, and 14 had gastroduodenal pathology. Mucosal lesions were a major cause of abdominal pain in this population. While the majority of patients with gastroduodenal pathology (12 of 14) were seropositive for *H. pylori*, none were seropositive for HIV. Smoking was associated with increased risk of macroscopic pathology, and a history of *Mycobacterium bovis* BCG immunization was associated with reduced risk. Antibodies to *H. pylori* lipopolysaccharide were associated with pathology. HIV infection was associated with protection against mucosal lesions, suggesting that fully functional CD4 lymphocytes may be required for the genesis of gastroduodenal pathology.**

A central unanswered question in understanding the impact of *Helicobacter pylori* on the human host relates to pathogenesis: why does *Helicobacter* infection cause disease only in a small proportion of the infected population, and why are different diseases associated with *Helicobacter* in separate geographic locations? *Helicobacter* is one of the most common chronic bacterial infections of humans, affecting more than 50% of the world's population, but the majority of those infected remain asymptomatic throughout life. About 20% of infected adults manifest one of many different outcomes, such as duodenal ulcer, gastric ulcer disease, gastric cancers, or lymphoma (15, 22). Several studies have highlighted inconsistencies between the prevalence rates for *Helicobacter* and disease. In industrialized countries there is generally a low prevalence of *Helicobacter* and gastric cancer yet a relatively high prevalence of peptic ulcer disease. On the other hand, some countries with high *Helicobacter* prevalence rates, have high gastric cancer prevalence rates but low peptic ulcer disease prevalence rates (e.g., Peru), yet other nonindustrialized countries with similar high *Helicobacter* prevalence rates have a disease distribution similar to that in industrialized countries (e.g., Iran) (2).

This question is particularly intriguing in sub-Saharan Africa, where *H. pylori* infection is common but several studies have indicated a low incidence of peptic ulceration (14, 16). Seroepidemiological studies have shown an early age of acquisition in children (50% by 10 years) (16), and prevalence in

asymptomatic individuals is approximately equal to that in dyspeptic individuals.

A possible explanation for this "African enigma" (14) may be that other factors are involved; these could relate to specific bacterial virulence factors, to differences in the host response to *Helicobacter* antigens, to differences in the environment (e.g., levels of antioxidants in the diet, water contamination, opportunities for hand washing), or to a combination of these, which might alter the processes by which ulceration or cancer develop. Alternatively, the burden of comorbidity or coinfection may modify the outcome of colonization by *Helicobacter*.

A number of studies have addressed the question of whether *H. pylori* infection is more or less frequent in people also infected with human immunodeficiency virus (HIV). The results are contradictory, with some studies demonstrating no difference in prevalence compared to a control population, while others show a lower or a higher prevalence (1, 7, 17, 18). Most studies have investigated either North American, European, or Australian populations. In one study of African children a lower prevalence of *H. pylori* colonization was noted (3). In an Italian study the prevalences of both *H. pylori* colonization and peptic ulcer disease were noted, and these both correlated with CD4 count (4).

Several potential virulence factors or markers such as cytotoxin-associated protein (CagA), urease (12), lipopolysaccharide (LPS) (23), or vacuolating cytotoxin (VacA) have been proposed (29). However, the relative contributions of these factors are still debated. Additionally, host genetics may also be involved in determining the outcome of infection, as it has recently been demonstrated that polymorphism in the interleukin-1 (IL-1) gene may predispose to the development of gastric cancer (11). Laboratory evidence also suggests that there is an interaction between *Helicobacter* and viral or par-

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asitic infections which may modify the outcome of either or both infective processes (24).

The objective of the work described here was to assess the prevalence of *H. pylori* infection and gastroduodenal pathology in a population in sub-Saharan Africa with high HIV seroprevalence and to relate this to immune status, environmental factors, and bacterial pathogenicity factors.

#### MATERIALS AND METHODS

The data and sera used for this study were drawn from a longitudinal study of intestinal infectious disease in an unplanned residential area in the southern part of Lusaka, Zambia, which was begun in 1999. This population is impoverished, civic amenities are few, housing is of poor quality, and overcrowding is intense. Authorization to conduct a 3-year study in this residential area was obtained from the Lusaka Urban District Health Management Board, and approval was obtained from the Research Ethics committees of the University of Zambia (UNZA) and the London School of Hygiene and Tropical Medicine.

The study included unselected adults (18 years of age or older) with or without abdominal symptoms, and 52% of the adult residents in the study area agreed to participate. As part of the baseline data collection, all recruits were asked about current symptoms of ill health. The following information was also collected: age, gender, place of upbringing (urban versus rural), size of family, previous *Mycobacterium bovis* BCG vaccination, history of smoking, and educational level. Stool samples were screened for ova, cysts, and parasites using wet preparations of stool samples and formol-ether concentration. Only the most frequently detected infections (*Ascaris lumbricoides* and hookworm) are described in this paper, because infrequently detected organisms could not be used in the multivariate analysis. Participants were examined and nutritional was assessment performed using two anthropometric measures: height (in meters) and body mass index (BMI; in kilograms per square meter).

Blood was collected and serum samples were stored at  $-80^{\circ}\text{C}$  on the same day on which endoscopy was performed with an Olympus fiber-optic SIF-10 endoscope. The recruitment and consent process included information about HIV testing, and participants who consented to inclusion in the study were offered the option of HIV testing (together with CD4 count) with full pre- and posttest counselling, but they were also free not to have the test. This conforms with the policy of the UNZA Research Ethics Committee. HIV antibody testing was performed using a rapid test (Capillus; Trinity Biotech, Bray Co., Wicklow, Ireland) and an enzyme-linked immunosorbent assay (ELISA) (Recombigen HIV 1+2 EIA), and results declared were positive only if both were positive. Discrepant test results were resolved by Western blotting (New LAVBL0T1; Sanofi Diagnostics). Peripheral blood CD4 counts were carried out using a FACSCount (Becton Dickinson) flow cytometric assay according to the manufacturer's instructions.

**Host immune response to *H. pylori*.** Seroprevalence of immunoglobulin G (IgG) was assessed by a standard ELISA against a whole bacterial antigen preparation (SIGMA, Poole, Dorset, United Kingdom), Sigma, and antibodies to CagA protein were also assessed by ELISA (CTX Helori; Eurospital, Trieste, Italy). The plates were read as recommended, and optical density was recorded. The serological response to the urease-heat shock protein (hsp) complex and to LPS was determined by an in-house ELISA, using antigens extracted from a standard *H. pylori* isolate (NCTC 11368) according to published methods (6, 31). The presence of LPS and urease-hsp was confirmed by gel electrophoresis using 15% resolving gels stained with silver and Coomassie blue, respectively (27).

Each of the antigens (5  $\mu\text{g}$  of LPS and 10  $\mu\text{g}$  of urease-hsp respectively calibrated with a chequer board titration) was dissolved in 50 mM sodium bicarbonate (pH 9.8) and used to coat the ELISA plates overnight at  $4^{\circ}\text{C}$ . Serum was added at a 1/100 dilution in phosphate-buffered saline (PBS) with 0.05% Tween 20 and 2% bovine serum albumin and incubated for 2 h at  $37^{\circ}\text{C}$ . Human anti-IgG conjugated to horseradish peroxidase (HRP) (Sigma Diagnostics) was added at a 1/1,000 dilution, and the mixture was reincubated for 1 h. All steps were separated by three washings in PBS with Tween 20. ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] was added as a substrate, and plates were read at 630 nm.

Sera were also studied by immunoblotting as previously described (21). Briefly, 50  $\mu\text{g}$  of an *H. pylori* whole-cell lysate was separated on a 12% gel in a Mini-Protein II electrophoresis cell and blotted onto nitrocellulose (Hybond-C; Amersham Life Sciences, Little Chalfont, Buckinghamshire, United Kingdom) for 60 min at 350 mA. A 1/100 dilution of each serum sample was incubated with the nitrocellulose strips for 2 h after blocking with 5% skim milk overnight at  $4^{\circ}\text{C}$ . All steps were separated by thorough washing in PBS-Tween 20 buffer. Bands were

visualized with a 1/200 dilution of HRP-conjugated goat anti-human IgG and 4 chloro-1-naphthol solution as the substrate.

Six commonly detected bands were chosen because they signal antibody against potential virulence factors. The molecular masses of the bands were as follows: CagA, 120 to 116 kDa; vacuolating cytotoxin A (VacA), 89 kDa; two urease subunits, 66 and 26.5 kDa; and two outer membrane proteins, 35 and 19 kDa. Their presence or absence in serum was noted.

**Statistical methods.** Continuous variables were compared using a parametric (*t* test) or nonparametric (Kruskal-Wallis) test as appropriate. While BMI and height were apparently normally distributed, ELISA readings were not. Proportions were compared using a  $\chi^2$  test or Fisher's exact test, and for potential predictive factors the odds ratio (OR) or risk ratio (RR) was calculated, together with 95% confidence intervals (95% CI). Multivariate analysis was performed using unconditional logistic regression with a stepwise backwards elimination strategy beginning with the variables outlined above. For modelling gastroduodenal mucosal lesion as an outcome variable, results of immunoblotting for putative *H. pylori* pathogenicity antigens were added to the initial variables.

#### RESULTS

Clinical and serological data were obtained from 221 adults (79 men and 142 women), and 191 underwent complete endoscopic evaluation. Demographic and clinical data, as used in univariate and multivariate analysis, showed that men were older than women ( $P < 0.001$ ), were more likely to smoke tobacco ( $P < 0.001$ ), had somewhat higher educational achievement ( $P = 0.002$ ), and were less likely to have ova of *A. lumbricoides* in stool samples submitted ( $P = 0.04$ ).

Of 191 adults who underwent satisfactory endoscopy, 14 had gastroduodenal mucosal lesions (8 had macroscopic gastritis, 5 had duodenal ulceration, 1 had gastric ulceration, and 1 had pyloric erosions; 1 had both gastritis and duodenal ulceration). In addition, one had esophageal candidiasis, two had distal esophageal inflammation, and seven had nematode infections visible in the jejunum (three with *A. lumbricoides* and four with hookworm). One hundred seventy-six of these people consented to HIV testing, and 35% were seropositive. None of the 12 patients with gastroduodenal mucosal lesions who consented to HIV testing were HIV seropositive, but 49 of 140 adults without mucosal lesions were HIV seropositive (OR = 0.0;  $P = 0.01$ ).

Fifteen adults complained of abdominal pain when interviewed at their baseline examination. Six of 14 individuals with gastroduodenal mucosal lesions at endoscopy complained of abdominal pain compared to 9 of 177 without (OR, 14.0; 95% CI, 4.2 to 48;  $P < 0.001$ ), suggesting that these gastroduodenal mucosal lesions are a major cause of abdominal pain in this population.

***H. pylori* serology.** Serological responses to *H. pylori* tested by ELISA and by immunoblotting were closely related ( $P = 0.001$ ), and 81% of adults tested by ELISA were positive. *H. pylori* seropositivity was found by ELISA in 100 of 115 HIV-seronegative adults and in 46 of 61 HIV-seropositive adults ( $P = 0.05$ ). Serological responses to putative pathogenicity-related antigens differed in HIV-seropositive and HIV-seronegative individuals (Table 1). Multivariate analysis was performed using the variables indicated in Materials and Methods, by unconditional logistic regression. The final model ( $n = 163$ ) included only three variables, all associated with reduced likelihood of a positive ELISA result: adults who had been brought up in a city (OR, 0.29; 95% CI, 0.10 to 0.83;  $P = 0.021$ ), *Ascaris* ova in the stool (OR, 0.36; 95% CI, 0.14 to 0.94;  $P = 0.036$ ), and a CD4 count below  $200/\text{mm}^3$  (OR, 0.29; 95% CI, 0.09 to 0.93;  $P = 0.037$ ).

TABLE 1. Serological responses to *H. pylori* antigens identified by immunoblotting and ELISA according to HIV status

Immunoblot band (kDa) or antigen	No. responding to immunoblot band		<i>P</i>	Median response <sup>a</sup> to antigen		<i>P</i>
	HIV seropositive ( <i>n</i> = 61)	HIV seronegative ( <i>n</i> = 115)		HIV seropositive ( <i>n</i> = 61)	HIV seronegative ( <i>n</i> = 115)	
Immunoblot band						
120	27	68	0.06			
89	31	87	0.001			
66	36	73	0.56			
35	34	80	0.05			
26	28	75	0.01			
19	19	65	0.001			
Antigen						
Cag				0.45 (0.2–0.66)	0.60 (0.28–0.73)	0.07
Urease				0.71 (0.25–0.90)	0.86 (0.3–0.94)	0.03
LPS				0.36 (0.19–0.52)	0.39 (0.26–0.56)	0.12

<sup>a</sup> Readings are given in OD units; values in parentheses indicate the range. *n* = number of subjects.

**Gastroduodenal mucosal lesions—predictive factors.** *H. pylori* ELISA results were positive for 12 of 14 individuals with gastroduodenal mucosal lesions and for 143 of 177 individuals without lesions (*P* = 1.0). In univariate analysis, gastroduodenal mucosal lesions were less likely to be found in women (RR, 0.33; 95% CI, 0.11 to 0.94; *P* = 0.03) and in HIV-seropositive adults (as described above) but more likely in smokers (RR, 3.2; 95% CI, 1.2 to 8.4; *P* = 0.02). Putative pathogenicity-related antigens were not statistically significantly associated with gastroduodenal mucosal lesions, except for LPS, which was more frequently recognized by sera from individuals with mucosal lesions (Table 2).

Multivariate analysis was used to determine the contribution of potential risk factors to the development of mucosal lesions in individuals who were positive for *H. pylori* antibodies by ELISA. None of the adults with gastroduodenal mucosal lesions were HIV seropositive (see above) or had hookworm infection (*P* = 0.21), and so these individuals had to be removed from the model. In the final model (*n* = 133), two terms remained: smoking was associated with higher risk (OR, 3.9; 95% CI, 1.1 to 13.7; *P* = 0.035), and BCG immunization was

associated with lower risk (OR, 0.22; 95% CI, 0.06 to 0.78; *P* = 0.02).

## DISCUSSION

The data presented in this paper indicate that *H. pylori* seroprevalence is high (81%), consistent with other data from developing countries. The prevalences of gastroduodenal mucosal lesions (duodenal ulcer, 2.6%; gastric ulcer, 0.5%; gastritis, 4%) were comparable with estimates of population prevalences in industrialized countries. Most available information relating to the epidemiology of duodenal or gastric ulceration has been derived from hospital studies, but such population-based data as exist suggest that the prevalence of peptic ulceration is on the order of 1 to 2% in unselected adults (25). A high prevalence of gastroduodenal mucosal lesions has also been reported from Sudan (10) and Nigeria (19), but there is older evidence that prevalence may be low in some other countries, and there may be differences between rural and urban populations (26).

The adults examined were fairly representative of the pop-

TABLE 2. Serological responses to *H. pylori* antigens identified by immunoblotting and ELISA according to presence of gastroduodenal mucosal lesions

Immunoblot band (kDa) or antigen	No. responding to immunoblot		<i>P</i>	Median response <sup>a</sup> to antigen for individuals with:		<i>P</i>
	Mucosal lesion present ( <i>n</i> = 14)	No disease ( <i>n</i> = 177)		Mucosal lesion ( <i>n</i> = 14)	No disease ( <i>n</i> = 177)	
Immunoblot band						
120	9	98	0.59			
89	11	114	0.39			
66	9	103	0.78			
35	11	114	0.39			
26	9	100	0.78			
19	7	84	1.00			
Antigen						
Cag				0.64 (0.45–0.78)	0.53 (0.22–0.72)	0.33
Urease				0.72 (0.28–0.9)	0.82 (0.28–0.92)	0.66
LPS				0.64 (0.45–0.76)	0.37 (0.25–0.51)	0.004

<sup>a</sup> Reading are given in OD units; values in parentheses indicate the range. *n* = number of subjects.

ulation from which they were drawn, but our analysis may be limited by two other factors. First, gastric biopsies were not taken, as this was not the purpose for which the endoscopies were carried out. Second, the ELISA cutoff used (0.3 optical density [OD] units as in the manufacturer's instructions) has not been validated in a sub-Saharan African population. However, the good correlation between the ELISA results and the Western blotting results encourages us to believe that any overdiagnosis is modest, probably no more than five to six cases at most. A large prospective study carried out by Vaira et al. (28) has shown that serology is a reliable marker of *H. pylori* infection in HIV-positive patients, including those with advanced disease.

Multivariate analysis indicated that environmental and immunological factors may influence *H. pylori* infection. *Ascaris* infection was associated with reduced *H. pylori* seropositivity, as was a childhood spent in an urban environment. Whether the effect of urban upbringing is related to water chlorination or another unidentified environmental variable is unclear. There was a strong negative association between gastroduodenal mucosal lesions and HIV infection; HIV-infected adults were also less likely to have a positive ELISA result, and their serum samples less frequently recognized *H. pylori* antigens on immunoblots. A low CD4 count, more than HIV infection itself, was associated with less-frequent detection of *H. pylori* antibodies. This could either signify a failure of recognition of *H. pylori* antigens or reduced colonization in patients with HIV infection. Since mucosal lesions were also less likely in patients with HIV infection or a low CD4 count, we postulate that as CD4 cells play a role in inducing gastritis, this gastritis may be a mechanism by which *H. pylori* colonization is enhanced by increasing transexudation of serum components. Adults with HIV infection and/or a low CD4 count would then lose this tropic mechanism by which *H. pylori* colonization is sustained, and infection intensity would diminish. Gastroduodenal pathology may sometimes be related to opportunistic infections in AIDS patients with low CD4 counts rather than to *H. pylori* (30).

We found no evidence that either of the putative virulence antigens CagA and VacA was associated with the development of gastroduodenal mucosal pathology, in keeping with other work from Japan. On the other hand, antibody to LPS was associated with the development of mucosal lesions in univariate but not multivariate analysis.

These data suggest complex interactions between host immunology and *Helicobacter*-related mucosal pathology, and two observations in particular merit further study. First, there was a protective effect of infection with *A. lumbricoides*, the common intestinal roundworm with which millions of human beings are infected, against *H. pylori* infection as assessed serologically. This might be explained by work which demonstrates the effect of intestinal nematodes in reducing TH1-mediated gastric pathology in mice (13), probably by induction of TH2-mediated responses. It has been shown in rhesus macaques that gastric pathology induced by *H. pylori* was related to proliferation and activation of CD4 cells through a TH1 pathway (20). *Ascaris* infection in humans can lead to a polarized TH2 cytokine response (5), and an extract of another nematode, *Nippostrongylus brasiliensis*, has been shown to act as an immunomodulator of murine B-cell responsiveness (8).

Second, in our population, BCG immunization protected against the development of gastroduodenal mucosal lesions in *H. pylori*-infected adults. BCG exposure may simply act as a marker of an otherwise unidentified aspect of environmental exposure, or BCG immunization may induce changes in the balance of TH1- and TH2-dominant responses which even now are not well understood. Furthermore, BCG immunization in HIV-infected adults has been noted in one report to protect against *Ascaris* infection (9), possibly through modulation of the TH1-TH2 cytokine balance in CD4 cells. Why it should confer reduced risk of gastroduodenal mucosal lesion in humans is unclear, as *Helicobacter*-induced gastric inflammation is TH1 mediated. In view of the finding that *H. pylori*-related gastritis is TH1 dependent, as claimed by Mattapallil et al. (20), we postulate that this immune-mediated mucosal damage allows transexudation of serum components which enhance colonization efficiency of the bacteria, and therefore processes which attenuate TH1-mediated responses will reduce both colonization intensity and pathology. In any case, these interactions were clearly significant and merit further evaluation, as there is much to be learned from them about the host-pathogen relationship.

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