

ORIGINAL RESEARCH ARTICLE

## ***Helicobacter pylori* among patients with symptoms of gastroduodenal ulcer disease in rural Uganda**

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**Introduction:** To meet key millennium development goals, the rural population needs to be reached for health assessment and service delivery. Gastroduodenal ulcer disease is a common ailment affecting the health of people in Uganda. A cross-sectional study was conducted at Bwera Hospital in Kasese district of western Uganda, to establish the prevalence and predisposing factors of *Helicobacter pylori* among gastroduodenal ulcer disease patients.

**Methods:** A sample of 174 patients with symptoms of gastroduodenal ulcer disease was purposively obtained. Using two laboratory test methods, the prevalence of *H. pylori* among these patients was determined. A structured questionnaire was administered to participants to establish their demographic background and selected aspects of their lifestyle. Finally, the results obtained by enzyme-linked immunosorbent assay (ELISA) and immunochromatographic rapid test (IRT) were compared.

**Results:** We established the prevalence of *H. pylori* as 29.9% (52/174) by ELISA and 37.4% (65/174) by IRT. Cigarette smoking, poor sanitation, and lack of formal education were the significant predisposing factors with *p*-values <0.05. The two tests gave identical results in 87.9% of the patients.

**Discussion:** The prevalence of *H. pylori* by IRT and ELISA test methods was similar to what has been reported elsewhere in developed countries; but was lower than previously reported in developing countries including Uganda. The previous studies in Uganda were carried out in the urban population and on young children; and some used antibody-detection methods only, therefore leading to different prevalence as a result of difference in study population and methods.

**Keywords:** *Helicobacter pylori*; peptic ulcers; symptoms; gastroduodenal ulcer; chronic gastritis; smoking

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**H***elicobacter pylori* is believed to be responsible for the majority of peptic ulcers in patients with chronic gastritis and active ulcer disease (1). *H. pylori* is associated with many diseases of the gastrointestinal tract and high prevalence has been reported in many developing countries (2). *H. pylori* is believed to infect about half of the world's population (3), with prevalence of the infection, however, varying within geographical settings. Some developing countries have figures as high as 90%, whereas developed countries have values ranging from 25 to 40% (3). In Uganda and Africa at large, data on the determinants of infection with *H. pylori* are scanty. A previous study in Kampala, Uganda, revealed a prevalence of the organism among patients with stomach

cancers as 87% (4). This was broadly comparable to what had been reported elsewhere in the African continent: 79% in Algeria, 71% in Ivory Coast (Cote D'Ivoire), 79% in DR Congo, 85% in Nigeria, and 86–93% in South African Blacks (5).

*H. pylori* bacterium is a known causal agent for chronic gastritis, peptic ulcer disease, and gastric cancer. Its transmission has been reported to be promoted by low socio-economic status, poor quality of drinking water, overcrowding, poor personal and environmental hygiene, animal faecal matter, and food contamination. The bacteria is said to be mostly acquired in childhood, but it has also been reported to be sexually transmitted (6). The ulcer disease and the tests for diagnosis have received

insignificant concern; yet, its poor management is said to contribute to cancers of various organs such as the stomach, duodenum, and perhaps the pancreas. Other long eventualities are associated with cardiovascular diseases (5, 6).

Despite the ubiquity of *H. pylori* bacterium, there is lack of specific information on its prevalence and the predisposing factors among patients with gastroduodenal ulcer disease; especially in western Uganda. A previous study conducted among three ethnic groups of Uganda revealed that the severity of gastritis correlated with the presence of *H. pylori* in Baganda and Banyarwanda, whereas intestinal metaplasia and gastric atrophy (GA) were observed among Banyarwanda and Banyankole. The bacteria were detected more frequently in the severe form of GA and with a higher prevalence in areas of volcanic mountains (4). The same study reported that in Uganda, 87% of people with stomach cancer were seropositive for antibodies against *H. pylori* antigens. There are no such studies targeting the rural setting of Uganda. This study was conducted to investigate the prevalence of *H. pylori* infection and associated factors among adult patients presenting with symptoms of gastroduodenal ulcer disease, using the immunochromatographic rapid index test (IRT) and enzyme-linked immunosorbent assay (ELISA).

## Materials and methods

### Study population and sample selection

A cross-sectional study was conducted and patients with gastrointestinal complaints were purposively selected. The study was conducted at Bwera Hospital in Kasese district, western Uganda. To undertake the study, we obtained permission from the management of Bwera Hospital and from the research and ethics committee of the College of Veterinary Medicine, Animal Resources & Biosecurity of Makerere University, Kampala, Uganda. Participants were informed about the purpose of the study and what would be involved before consenting. In total, 174 adult patients, aged 18 and above, were enrolled in the study. Individuals were observed only once based on their clinical presentation of the disease. The study took place during a 5-month period.

### Questionnaire and laboratory examination

A structured questionnaire was administered to obtain information about the habits and family history of the patients. Information sought included past and recurring clinical symptoms such as epigastric pain, nausea, flatulence, vomiting, heartburn, and abdominal distension or any symptoms of gastroduodenal ulcer disease. Stool and venous blood samples were collected from all 174 patients for laboratory examination. We also sought respondents' demographic information and specific personal habits, as presented in Tables 1 and 2. Bwera Hospital offered a

physician to help in diagnosis based on tests and patient symptoms.

### Detection of *H. pylori* antibodies by IRT

The aliquoted sera were analysed serologically by IRT using a commercial hexagon SD BIOLINE *H. pylori* Ag kit (Standard Diagnostics Inc., Yongin, Korea) of sensitivity 98.4% and specificity 100% as per manufacturer's specification. Using a Pasteur pipette, five drops of serum were transferred to the sample well of the test strip followed by an addition of three drops of assay diluents, and the results were read macroscopically after 10 min. A positive result was that in which two pink/red bands (control line and test line) appeared in the result window of the test cassette, whereas the negative one was that in which one pink/red band was seen in the control window. An invalid result was that in which no pink/red band appeared in the control window of the strip, in which case the analysis was repeated.

### Testing for the presence of *H. pylori* antigens in stool by ELISA

*H. pylori* antigens in stool were detected by the commercial antigen capture ELISA technique using SD *H. pylori* Ag ELISA (Standard Diagnostics Inc.) of sensitivity 99.2% and specificity 96.0%, as per manufacturer's specification. This method was chosen for its ability to detect the antigen of *H. pylori* in stool samples, whereas the IRT method detects antibodies in blood serum. The patients' stool samples were diluted in phosphate-buffered solution (PBS) in ratio of 1:100, and 100 µl of the diluted patient's stool samples was added and incubated in the plate wells at room temperature for 20 min. The plate was then manually washed six times using a multichannel pipette with 350 µl of wash buffer (PBS) to remove unused substances, followed by complete drying by inverting the microwells. This was done by tapping on an absorbent pad and firmly compressing the holder to prevent wells from falling out. Then, 100 µl of the horseradish-peroxidase-conjugated monoclonal antibody was added and the plates were re-incubated for 10 min at room temperature. The unbound conjugate was manually washed away with the wash buffer and 100 µl of the premier substrate tetramethylbenzidine was added. Colour changes were observed and read within 10 min after adding 100 µl of 0.1 N sulphuric acid premier stop solution.

The results were read by visual observation for colour change and the actual OD values were read after 1 hour using the VMax Kinetic ELISA Microplate Reader (Molecular Devices, Sunnyvale, CA, USA) at 450 nm wavelength. Negative results had colourless wells while positive ones showed definite yellow colour, indicating the absence and presence of *H. pylori* antigens, respectively. The data were entered into EPINFO statistical programme and analysed using SPSS for windows version 11.5

(SPSS Inc., Chicago, IL) and chi-square test at a confidence level of 95%.

## Results

The description of the study population is shown in Table 1. The study represented both male and female patients and the majority who tested positive for *H. pylori* were younger than age 21. There was a female preponderance (F:M = 5:2) among positive patients.

### Prevalence and predisposing factors to *H. pylori* infection

Fifty-two patients (29.9%) tested positive with stool ELISA and 65 (37.4%) were reactive by the IRT. Four patients (2.3%,  $n = 174$ ) tested positive with stool ELISA after negative results by IRT and 17 (9.8%,  $n = 174$ ) were reactive by the IRT and negative by ELISA. The two tests gave identical results in 87.9% of the patients and differed in 12.1% of the cases. Among the predisposing factors to *H. pylori* infection, cigarette smoking, poor sanitation, and lack of formal education were found to be significant factors with a  $p$ -value  $< 0.05$  as shown in Table 2.

### Reported disease manifestation and symptoms among tested patients

As presented in Table 3, most patients who tested positive reported gastritis, peptic ulcers, and presented with bloody stool, burning abdominal pain, and epigastric pains. Notable clinical symptoms were fever, nausea, and heart burn and only one patient presented with bloody vomit. Only one case with cancer as disease manifestation was encountered, and this patient was positive by both test methods. Many patients did not respond to most of the questions, as shown in Table 3. Hence, those patients were not considered in the analysis; however, the test results were of value for disease diagnosis.

## Discussion

The prevalence of *H. pylori* was 29.9% by stool ELISA and 37.4% by IRT. This prevalence was low compared to other studies done elsewhere in the developing world (5, 7). The difference in results is probably due to the variations in the study population, such as the urban dwellers, and the age and health conditions of the patients. In addition, differences in the geographic regions, the type of test

Table 1. Biodata for positive *H. pylori* patients by rapid test kit and stool ELISA

Demographic aspect	Positive <i>H. pylori</i> $n = (\%)$ by:			$p$
	Rapid test (Index)	ELISA test (Reference)	Chi-square (95% CI)	
<b>Sex</b>				
Male ( $n = 58$ )	19 (32.8)	14 (24.1)	0.0047	0.945
Female ( $n = 116$ )	46 (39.7)	38 (32.8)		
<b>Age (years)</b>				
Below 21 ( $n = 29$ )	22 (75.9)	17 (58.6)	6.9828	0.3224
22–32 ( $n = 42$ )	15 (35.7)	9 (21.4)		
33–43 ( $n = 54$ )	10 (18.5)	10 (18.5)		
44–54 ( $n = 30$ )	8 (26.7)	7 (23.3)		
Above 55 ( $n = 19$ )	10 (52.6)	9 (36.8)		
<b>Marital status</b>				
Single ( $n = 49$ )	19 (38.8)	16 (32.7)	0.2654	0.9664
Married ( $n = 89$ )	27 (30.3)	23 (25.8)		
Divorced ( $n = 19$ )	10 (52.6)	7 (36.8)		
Widow ( $n = 17$ )	9 (52.9)	6 (35.3)		
<b>Type of marriage</b>				
Monogamous ( $n = 64$ )	17 (26.6)	13 (20.3)	0.0302	0.862
Polygamous ( $n = 25$ )	10 (40.0)	10 (40.0)		
<b>Place of residence</b>				
Village (rural) ( $n = 106$ )	40 (37.7)	34 (32.1)	0.0556	0.8136
Town (urban) ( $n = 68$ )	25 (36.8)	18 (26.5)		
<b>Formal education</b>				
None ( $n = 64$ )	17 (26.6)	5 (7.8)	5.9097	0.1161
Primary ( $n = 50$ )	22 (44.0)	20 (40.0)		
Secondary ( $n = 41$ )	19 (46.3)	17 (41.5)		
Tertiary ( $n = 19$ )	7 (36.8)	10 (52.6)		

Values computed at significance level of 5%.

Table 2. Predisposing factors to *H. pylori* infection

Predisposing factors	<i>H. pylori</i> test by ELISA n (%)		Chi-square (95% CI)	p
	Negative 122 (70.1)	Positive 52 (29.9)		
Do you smoke any kind of cigarette?				
Yes (n = 106)	65 (61.3)	41 (38.7)	8.966	0.003*
No (n = 68)	57 (83.8)	11 (16.2)		
Do you take alcoholic drinks?				
Yes (n = 114)	74 (64.9)	40 (35.1)	3.581	0.058
No (n = 60)	48 (80.0)	12 (20.0)		
Are you employed?				
Yes (n = 76)	57 (75.0)	19 (25.0)	1.151	0.283
No (n = 98)	65 (66.3)	33 (33.7)		
Do you consider your work stressful?				
Yes (n = 77)	49 (63.6)	28 (36.4)	2.240	0.1345
No (n = 97)	73 (75.3)	24 (24.7)		
How do you rate your sanitation?				
Poor (n = 92)	56 (60.9)	36 (39.1)	7.0548	0.007*
Good (n = 82)	66 (80.5)	16 (19.5)		
What is your source of drinking water?				
Risky source (river, lake, stream) (n = 157)	110 (70.1)	47 (29.9)	0.055	0.815
Non-risky source (tap) (n = 17)	12 (70.6)	5 (29.4)		
How do you treat your drinking water?				
Reliable method (boiling) (n = 41)	26 (63.4)	15 (36.6)	0.769	0.381
Non-reliable method (filtering, chemicals, none) (n = 133)	96 (72.2)	37 (27.8)		
How many meals do you take a day?				
One (n = 19)	13 (68.4)	6 (31.6)	1.586	0.453
Two (n = 79)	52 (65.8)	27 (34.2)		
Three or more (n = 76)	57 (75.0)	19 (25.0)		
Formal education				
None (n = 64)	46 (71.9)	5 (7.8)	14.619	0.002*
Primary (n = 50)	33 (66.0)	20 (40.0)		
Secondary (n = 41)	31 (75.6)	17 (41.5)		
Tertiary (n = 19)	12 (63.2)	10 (52.6)		
How often do you take non-steroidal anti-inflammatory drugs?				
Every 2 weeks (n = 66)	46 (26.4)	20 (11.5)	0.376	0.945
Once a month (n = 72)	51 (29.3)	21 (12.1)		
Every 2 months (n = 19)	14 (8.0)	5 (2.9)		
Every 4 months (n = 17)	11 (6.3)	6 (3.4)		

Values computed at significance level of 5%; \*values lower than 0.05.

specimens (stool and blood), the analytical methods used, and the target molecules, that is, antigen versus antibodies, are likely to have influenced the differences in the study findings. The current study population was mainly above 18 years of age, hence a lower prevalence than that reported in other developing countries. This is supported by the findings of studies by Newton et al. (4), Jackman et al. (8), Brandi et al. (9), and Hestvik et al. (10). The 9.8% cases positive by IRT and negative by ELISA are likely to be due to the test methods detecting antibodies and antigens, respectively. It is most likely due to the extended occurrence of antibodies after the infection or

antigens have been eliminated. The 2.3% that had positive ELISA and negative IRT results were probably recent infections before development of detectable immune response. Alternatively, cross reactions between *H. pylori* and other stomach and intestinal normal microflora may have resulted in false-positive results. This was also experienced in a study done in Europe; which found out that peptic ulcer patients harbour other gastrointestinal bacteria that give false-positive serological test results (11, 12). A study by Triantafyllopoulou et al. (13) revealed that most adults infected with *H. pylori* organism are asymptomatic and may therefore test negative by the antibody-detection tests and

Table 3. Reported disease manifestation, clinical symptoms in relation to test positivity and respondents' response

Disease manifestations reported	Positive <i>H. pylori</i> reaction <i>n</i> (%) by	
	ELISA 52 (29.9)	Rapid 65 (37.4)
Peptic ulcers		
Yes ( <i>n</i> = 20)	15 (75)	20 (100)
No ( <i>n</i> = 35)	33 (94.3)	35 (100)
No response ( <i>n</i> = 119)	4 (3.4)	10 (8.4)
Gastritis		
Yes ( <i>n</i> = 10)	10 (100)	10 (100)
No ( <i>n</i> = 23)	23 (100)	11 (47.8)
No response ( <i>n</i> = 141)	19 (13.5)	44 (31.2)
Cancer		
Yes ( <i>n</i> = 1)	1 (100)	1 (100)
No ( <i>n</i> = 7)	5 (71.4)	7 (100)
No response ( <i>n</i> = 166)	46 (27.7)	57 (34.3)
Other (intestinal perforation, etc.)		
Yes ( <i>n</i> = 4)	4 (100)	0 (0.0)
No ( <i>n</i> = 10)	9 (90.0)	10 (100)
No response ( <i>n</i> = 160)	39 (24.4)	55 (34.4)
Signs and symptoms		
Burning abdominal pain		
Yes ( <i>n</i> = 24)	19 (79.2)	24 (100)
No ( <i>n</i> = 41)	33 (92.7)	41 (100)
No response ( <i>n</i> = 109)	0 (0.0)	0 (0.0)
Bloody vomit		
Yes ( <i>n</i> = 1)	1 (100.0)	1 (100.0)
No ( <i>n</i> = 4)	4 (100.0)	0 (0.0)
No response ( <i>n</i> = 169)	47 (27.8)	64 (37.9)
Bloody stool		
Yes ( <i>n</i> = 16)	10 (61.3)	16 (38.7)
No ( <i>n</i> = 25)	25 (83.8)	22 (16.2)
No response ( <i>n</i> = 133)	17 (12.8)	27 (20.3)
Indigestion		
Yes (0)	0 (0.0)	0 (0.0)
No ( <i>n</i> = 1)	1 (100.0)	1 (100.0)
No response ( <i>n</i> = 173)	51 (29.5)	64 (36.9)
Other (epigastric pains, etc.)		
Yes ( <i>n</i> = 10)	0 (0.0)	10 (100.0)
No ( <i>n</i> = 3)	1 (33.3)	3 (100.0)
No response ( <i>n</i> = 161)	51 (31.7)	52 (32.3)

positive by the antigen-detection tests. Hence, the stool ELISA tests can aid in detection of actively infected or carrier individuals.

The majority of patients who tested positive using both tests were below 21 years of age, 75.9% by IRT. These findings are contrary to those reported by Kang et al. (14), where seroprevalence of *H. pylori* increased with age among the Chinese, Malay, and Indians. This current finding in the less than-21-year population can be due

to the early colonisation by *H. pylori* as reported by Hestvik et al. (11).

More female patients tested positive (39.7% by IRT and 32.8% by ELISA); however, this was not significantly different ( $p = 0.945$ ) to that of the male participants (32.8% IRT and 24.1% ELISA). In addition, none of the patients' biodata contributed to positivity of *H. pylori* using either test, as their  $p$ -values were above the level of significance of 0.05. However, Moayyedi et al. (15) reported that males, living with a partner, and poor adult socio-economic conditions are associated with increased risk of *H. pylori* infection.

Our study also examined possible predisposing factors to infection. Determinations of the significant factors were based on ELISA method although the study shows no significant difference between the two methods. It was found that cigarette smoking, poor sanitation, and lack of formal education were the predisposing factors to *H. pylori* infection with a  $p$ -value < 5% level of significance. This contrasts with findings by Ogihara et al. (16), where *H. pylori* seropositivity decreased linearly with cigarette consumption *per* day. Because Khalifa et al. (17) proved that there is no significant association between cigarette smoking with *H. pylori* infection, the most likely risk factor in the current study was poor living conditions, which is in agreement with findings from similar studies in other countries (18–21). Elsewhere in the world, Triantafyllopoulou et al. (13) reported that the acquisition of *H. pylori* could result from genetic, environmental, and occupational exposure factors, and poor community water supply.

Some patients who tested positive for *H. pylori* reported gastritis, peptic ulcers, and intestinal perforation among the disease manifestation and presented with bloody stool, burning abdominal pain, and epigastric pain. Basing on data collected, there was no significant association of the infection with a particular symptom.

The current study aimed at establishing the prevalence of *H. pylori* and the predisposing risk factors in rural western Uganda. The prevalence was less than what has previously been reported in other developing countries as well as in urban areas in Uganda. The main risk factor was poor sanitation.

## Conclusion and future perspectives

The prevalence of *H. pylori* among patients with gastrointestinal ulcer disease was established at 37.4%. Although both the IRT and ELISA tests targeted detection of different biomolecules, the results did not differ significantly. Cigarette smoking, poor sanitation, and lack of formal education were some predisposing factors. The study also demonstrates the need for a larger sample size as there are many cases of no response to some questions. Another suggestion is to redesign questionnaires in a suitable way to have a larger response rate. The high observed

prevalence in the lower age group 19–32, compared to the population, needs further investigation for explanation. A possible explanation is a difference in eating patterns, especially in school-going young people, with less affordability for meals in rural areas.

### Conflict of interest and funding

There is no conflict of interest whatsoever, regarding the study. Further, the authors have not received any funding or benefits from industry or elsewhere to conduct this study.

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