



Seroprevalence of *Helicobacter pylori* IgG Antibodies and Associated Risk Factors among Asymptomatic Vegetable Sellers in Uyo, South Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors GMN and ANU designed the study. Authors GMN, IAA, NFJ, AOA, RSO and EOB did the data acquisition. Authors GMN, ENE, OJA and GEU did the analysis and interpretation of data. Authors GMN, ANU, ENE, OJA and EOB drafted the manuscript. Authors ANU, ENE, OJA and GEU did the critical revision. All authors read and approved the final manuscript.

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ABSTRACT

Background: Vegetables are essential part of people's diet and are sometimes consumed raw and often without heat treatment or thorough washing, hence some studies suggested it serves as vehicle for the transmission of pathogenic microorganisms like *Helicobacter pylori*. The source of contamination has also been traced to sellers of vegetables who are involved in unhygienic practices which contribute to the dissemination of pathogenic microorganisms. Therefore the aim of this study was to provide information on the seroprevalence of *H. pylori* IgG antibodies and associated risk factors among asymptomatic vegetable sellers in Uyo, Akwa Ibom State, Nigeria. **Materials and Methods:** Blood and stool samples from 142 participants were included in the study. Blood samples were analyzed using the rapid diagnostic method while the stool samples were analyzed using Enzyme linked immunosorbent Assays (ELISA). **Results:** A total of 85 (59.9%) were positive by rapid diagnostic test (RDT) while 53 (37.3%) were positive by stool ELISA test. Risk factors like age, marital status, residency family size, years in vegetable business, source of vegetable, wash before stalling etc were all statistically associated with ($p < 0.05$) with *H. pylori* prevalence. **Conclusion:** This study revealed that unhygienic behaviors by vegetable sellers are associated with high risk of *H. pylori* infection to vegetable consumers.

Keywords: Vegetables; Enzyme Linked Immunosorbent Assays (ELISA); *helicobacter pylori*; seroprevalence; antibodies.

1. INTRODUCTION

1.1 Background of the Study

Vegetables are the edible parts of plants which could be leaves, stems, roots, flowers, seeds, fruits, bulbs, tubers and fungi that are consumed wholly or in parts, raw or cooked as part of main dish [1]. In recent years, vegetables gain consumer's attraction due to their reputation of being healthy in combination with low energy density. However, since fresh produce is often eaten raw, it may also be a source for food borne illness [2]. Vegetables that usually make up this recipe include cucumber, carrots, green chilli, cabbage and lettuce. They are sold in almost every market and can be seen hawked around by traders [3].

The tropical and sub-tropical countries of the world are blessed with varieties of vegetables and Nigeria is known worldwide for its great biodiversity of plants used in several ways such as culinary, medicinal, therapeutic and nutritional

purposes [4,5]. In many parts of Nigeria, green leafy vegetables have gained widespread acceptance as dietary constituents, generally forming a substantial portion of the diet in the preparation of soups and stews [6].

Vegetables come in a close contact with soil manure, human stool and water through irrigation [7]. They can be source of *Helicobacter pylori* (*H. pylori*) infection when consumed raw without heat treatment or thorough washing [3]. *Helicobacter pylori* are microaerophilic gram negative bacteria known to be the causative agent of mucosa associated lymphoid tissue lymphoma [8]. *Helicobacter pylori* infection is one of the most prevalent chronic gastric infections affecting more than 60.3% of the world population [9]. Epidemiological studies have demonstrated that *H. pylori* infection is most prevalent in developing countries and among populations with low socioeconomic background [9]. According to reports, the main routes of infection remain unproven. However, its transmission occurs by

person to person either by faecal-oral or oral-oral routes [10] with education level, living standards such as sanitation and hygiene, crowding index and source of drinking water being part of *H. pylori* risk factor [11]. A study in Iran by Yahaghi et al. [12] reported *Helicobacter* seropositivity to be associated with consumption of uncooked vegetables, perhaps this was related to contaminated water used on the vegetables. Therefore, this research aimed at providing information on the seroprevalence and risk factor of *Helicobacter pylori* IgG antibodies among asymptomatic vegetable sellers in Uyo, Akwa Ibom State.

2. MATERIALS AND METHODS

2.1 Study Area and Design

This study was conducted in Uyo which is the capital of Akwa Ibom State, South-south part of Nigeria. Uyo lies between latitude 5.5°N and 6.0°N and longitude 6.0°E and 6.5°E of the Greenwich meridian. A cross sectional study design with a random sampling technique was used.

2.2 Study Population and Sample Size

This study involved 142 individuals from Uyo metropolis consisting of male and female sellers of vegetables and salads. This sample size was obtained using Godden's [13] formula from a prevalence rate of 89.7% in a study at Agbor in Delta state, Nigeria by Ophori et al. [14].

2.3 Inclusion and Exclusion Criteria

Eligible participants for this study were sellers of vegetables within Uyo metropolis, Akwa Ibom State.

Those excluded were people with known peptic ulcer condition or those with history of peptic ulcer and those on proton pump inhibitors, antibiotics, steroids or non-steroid anti-inflammatory drugs for the past one month.

2.4 Specimen Collection and Laboratory Analysis

2.4.1 Blood analysis

Five (5) millilitres of venous blood samples were collected into plain bottles and allowed to clot to get the sera following blood clotting, the serum would be separated by aspiration using Pasteur

pipette. The blood samples were quantitatively examined serologically for *Helicobacter pylori* IgG antibodies using PROMED Rapid Diagnostic test, Xinghu Avenue, Nantong226010, China. The analysis was carried out according to manufacturer's description. Two distinct red lines (one on the control C and the other on the test T) indicate positive result. One red line only on the control C indicates negative result. One red line only on the test T or no line at all on both control C and test T indicate invalid result and the test repeated with new test kit.

2.4.2 Stool specimen analysis

Stool specimen was collected from each participant using a sterile leak proof single universal bottle with a screw-capped lid. The stool specimen was qualitatively screened for *Helicobacter pylori* IgG antibodies using Accu-Diag TM ELISA kit Cat 1506 -11, USA. The analysis was carried out according to manufacturer's description. The minimum detection concentration was 0.5ng/ml, and results > 20ng/ml were considered positive.

2.4.3 Statistics

Data obtained was analyzed using chi square test through the Statistical package for social science (SPSS) version 25.0. A p-value of ≤ 0.05 was considered statistically significant.

3. RESULTS

3.1 Detection of *H. pylori* Antibody Based on Socio-Demographic Variables

Table 1 shows the positivity of *H. pylori* antibody across Socio-demographic variables and also the association between *H. pylori* and Socio-demographic variables. Variables that were statistically significant ($p < 0.05$) with positivity of *H. pylori* antibody were age, marital status, place of residence and family size. Variables that were not statistically significant ($p > 0.05$) with positivity of *H. pylori* antibody were gender and education.

3.2 Detection and Association of *H. pylori* positivity Across Vegetable Related Risk Factors

The distribution of *H. pylori* antibody positivity across vegetable-related risk factors are shown in Table 2. Variables that were statistically significant ($p < 0.05$) with positivity of *H. pylori* antibody were years in vegetable business,

source of vegetable, wash before stalling, eating vegetables while cleaning, use of cleaned instrument and frequency of vegetable consumption.

3.3 Distribution and Association of *H. pylori* Positivity across Behavioral Risk Factors

The distribution of *H. pylori* antibody across behavioral risk factors is listed in Table 3. Variables that were statistically significant ($p < 0.05$) with positivity of *H. pylori* antibody were convenience type, hand washing after convenience, spicy food intake and fried food intake. Hand washing before eating was not statistically significant ($p > 0.05$) with *H. pylori* antibody positivity.

3.4 Distribution and Association of *H. pylori* Positivity across Related Risk Factors

Table 4 shows the distribution of *H. pylori* positivity across *H. pylori* related risk factors. Variables that were statistically significant ($p < 0.05$) with positivity of *H. pylori* antibody were Number per room, Waste disposal. Variables that were not statistically significant ($p > 0.05$) with *H. pylori* antibody positivity were pet ownership and water source.

4. DISCUSSION

Helicobacter pylori are a common bacterium found in various sources including soil, animal manure, and human stool and as a result, cross-contamination of vegetables with *H. pylori* is unavoidable [15]. Consumption of contaminated materials, including fruits and vegetables, is a significant means of transmission for this infection, with a reported prevalence of 90% among regular consumers of these foods [16].

This study found the prevalence of *H. pylori* IgG antibodies by rapid diagnostic test (RDT) to be higher (59.9%) than stool ELISA test (37.3%). This may be due to the fact that serology tests detect IgG regardless of current infection, previous exposure, or treatment with proton pump inhibitors. However, the low rate by stool test may be due to the influence of relatively low bacterial load, use of antibiotics, and proton-pump inhibitors. The study's findings are supported by previous studies by Jemilohun and Otegbayo [17] and Sabbagh et al. [18]. A study

conducted in Owerri, Nigeria by Emerenini et al. [19] reported a lower RDT rate of 20%.

H. pylori cases were more prevalent in females than male, which could be attributed to the fact that the study focused on vegetable sellers who are mostly women. This finding is consistent with other studies by Goto et al. [20] and Zawaya et al. [21] where the majority of *H. pylori* cases were found in females.

The prevalence of *H. pylori* was 56.6% for those between the ages of 36-45 years and this was found to be highest among the participants. This may be because people in this age range are more involved in agriculture or vegetable industries. However, this rate is consistent with previous studies by Okezie et al. [22] and Zawaya et al. [21], where the highest rates of infection were found among individuals aged 31-40 years (27.9%) and 35-44 years (27.8%), respectively.

The prevalence of *H. pylori* did not show any significant correlation with an individual's level of education or knowledge about the infection. This suggests that regardless of education level, disregarding proper hygiene can increase the risk of infection. However, other studies by Mbang et al. [23] and Okoroiwu et al. [24] reported a high prevalence of *H. pylori* among individuals with a tertiary level of education or those belonging to the literate class, possibly because they assume immunity and overlook simple hygiene procedures for handling vegetables.

There was a statistical correlation between marital status and *H. pylori* infection, with higher rates observed among singles compared to those who are married. This could be due to behavioral differences, as single individuals may lack a sense of responsibility in ensuring healthy meals are consumed regularly. This finding is consistent with previous studies by Enitan et al. [25] and Okezie et al. [22], which also reported a higher prevalence of *H. pylori* among singles.

The length of time an individual has been involved in the vegetable business was found to have a statistically significant association ($p < 0.05$) with *H. pylori* infection. The study found that the rate of infection decreased as the number of years in business increased. It is possible that individuals who have been in the vegetable business for a longer period of time have been previously exposed to *H. pylori*, which may have created immunologic memory. This

Table 1. Detection of *H. pylori* antibody across Socio-demographic variables

Variables	Group	No. Screened (n=142)	Blood RDT Positive	p-value	Stool ELISA Positive (%)	p-value
Gender	Male	9	4 (44.4)	0.26	3 (33.3)	0.07
	Female	133	81 (60.9)		50 (37.6)	
Age-group	18-25	10	6 (60.0)	0.000	4 (40)	0.001
	26-35	52	25 (50.0)		10 (19.2)	
	36-45	49	43 (87.8)		30 (66.7)	
	>45	31	11 (35.5)		9 (29.0)	
Education	None	5	2 (40.0)	0.77	1 (20.0)	0.41
	Primary	24	14 (58.3)		6 (25.0)	
	Secondary	102	63 (61.8)		41 (40.2)	
	Tertiary	11	6 (54.5)		5 (45.5)	
Marital Status	Married	95	46 (48.4)	0.000	28 (29.5)	0.006
	Not Married	47	39 (83.0)		25 (53.2)	
Residency	Rural	29	11 (37.9)	0.007	4 (13.8)	0.003
	Urban	113	74 (65.5)		49 (43.4)	
Family Size	<5	96	41 (42.7)	0.000	20 (20.8)	0.001
	>5	46	44 (95.7)		33 (71.7)	
	Total	142	85 (59.9)		53 (37.3)	

immunologic memory could help the individual respond more effectively and rapidly in subsequent exposures.

The source of vegetables, washing before stalling, eating vegetables while cleaning, use of cleaned instruments, hand washing before eating, hand washing after convenience, waste disposal, and consumption of vegetables were all found to have a statistically significant association ($p < 0.05$) with *H. pylori* infection. The reason for this association may be due to the behavioral and hygienic conditions which promote infections as reported by Edem et al. [26] that unhygienic behaviors promotes high risk of infection. Krueger et al. [27] reported soil contact to be statistically associated with *H. pylori* prevalence, which agrees with our observations. Poor fecal disposal or management by vegetable sellers can also pose a risk to vegetable consumers, in line with the fecal-oral route of transmission. Etukudo et al. [28] found waste disposal and hand washing after convenience, respectively, to be associated with *H. pylori* prevalence. However, the habit of hand washing before eating was not found to be statistically associated ($p > 0.05$) with *H. pylori* infection in this study.

Participants who used pit toilets had a 50.9% prevalence of *H. pylori* infection, and there was a statistically significant association ($p < 0.05$) between the type of toilet and *H. pylori* infection. This finding supports the theory that *H. pylori* can be transmitted through the fecal-oral route and

that environmental sources can play a role in the spread of this infection.

The prevalence of *H. pylori* infection was not statistically associated with pet ownership. While Papiez et al. [29] proposed that pets may serve as a source of *H. pylori* transmission, this study found that individuals who had never owned a pet had the highest prevalence of *H. pylori* infection. However, our results are consistent with the findings of Mnena et al. [30], which also found no association between owning a pet and *H. pylori* infection.

There was no significant association between receiving the *H. pylori* vaccine, having a history of peptic ulcer, or having an underlying condition, and the prevalence of *H. pylori* infection. For participants who received the *H. pylori* vaccine, this lack of association may be due to the fact that the majority of those who received the vaccine still had a prevalence rate of 3.7%, which could be because the vaccine's weaning period may have elapsed. This finding is supported by Schacher et al. [31], who also reported a non-significant association between *H. pylori* vaccination and prevalence. The lack of association with history of peptic ulcer can be explained by the fact that those with no history of peptic ulcer had the highest *H. pylori* prevalence rate in this study. This reemphasizes the function of the immunologic memory on preexistence of antigen-specific lymphocytes that assists in tackling pathogens previously encountered in a more rapid and robust way [32].

Table 2. Prevalence and association of *H. pylori* across vegetable related risk factors

Variables	Group	No. Screened (n=142)	Blood RDT Positive (n=85) (%)	p-value	Stool ELISA Positive (n=53) (%)	p-value
Year in Veg. Business	≤1	56	48 (85.7)	0.000	33 (58.9)	0.001
	2-5	53	26 (49.1)		13 (24.5)	
	>5	33	11 (33.3)		7 (18.9)	
Source of Vegetable	Bush Market	56	48 (85.7)	0.000	32 (57.1)	0.001
	Open Market	86	37 (43.0)		21 (24.4)	
Wash before Stalling	Yes	118	65 (55.1)	0.010	45 (38.1)	0.001
	No	24	20 (83.3)		8 (33.3)	
Eating Veg. while cleaning	Yes	68	52 (76.5)	0.000	34 (50.0)	0.003
	No	74	33 (44.6)		19 (25.7)	
Use Cleaned Instrument	Yes	61	54 (88.5)	0.000	31 (50.8)	0.004
	No	81	31 (38.3)		22 (27.2)	
How often do you consume Vegetables	Never	10	6 (60.0)	0.006	2 (20.0)	0.001
	sometimes	75	36 (48.0)		20 (26.7)	
	Often	29	19 (65.5)		8 (27.6)	
	Always	28	24 (85.7)		23 (82.1)	

Table 3. Distribution and association of *H. pylori* positivity across behavioral risk factors

Variables	Group	No. Screened (n=142)	Blood RDT positive (n=85) (%)	p-value	Stool ELISA positive (n=53) (%)	p-value
Hand washing before eating	Never	5	2 (2.4)	0.76	2 (4.0)	0.24
	Sometimes	6	3 (3.5)		2 (4.0)	
	Often	35	21 (24.7)		9 (17.0)	
	Always	96	59 (69.4)		40 (75.0)	
Convenience Type	Pit Toilet	51	42 (49.4)	0.000	27 (50.9)	0.007
	Bucket	8	4 (4.7)		1 (1.9)	
	Water Closet	76	38 (44.7)		25 (47.2)	
	Others	7	1 (1.2)		0 (0)	
Hand washing after convenience	Sometimes	5	3 (3.5)	0.01	3 (5.7)	0.001
	Often	24	8 (9.4)		2 (3.8)	
	Always	113	74 (87.1)		48 (90.6)	
Spicy food intake	Never	32	14 (16.5)	0.02	9 (17.0)	0.001
	Sometimes	41	22 (25.9)		10 (18.9)	
	Often	41	26 (30.6)		12 (22.6)	
	Always	28	23 (27.1)		22 (41.5)	
Fried food intake	Never	12	7 (8.2)	0.01	4 (7.5)	0.001
	Sometimes	70	38 (44.7)		23 (43.4)	
	Often	25	11 (12.9)		4 (7.5)	
	Always	35	29 (34.1)		22 (41.5)	

Table 4. Distribution and association of *H. pylori* positivity across related risk factors

Variables	Group	No. Screened (n=142)	Blood RDT Positive (n=85) (%)	p-value	Stool ELISA Positive (n=53) (%)	p-value
Number per room	1	70	22 (25.9)	0.000	9 (17.0)	0.001
	2-3	33	26 (30.6)		14 (26.4)	
	>3	39	37 (43.5)		30 (56.6)	
Pet	Yes	7	4 (4.7)	0.88	2 (3.8)	0.62
	No	135	81 (95.3)		51 (96.2)	
Water Sources	Tap water	132	77(90.6)	0.18	50 (94.3)	0.80
	non-tap water	10	8 (9.4)		3 (5.7)	
Waste disposal	Sewage	52	49 (57.6)	0.000	30 (56.6)	0.002
	Non sewage	90	36 (42.4)		23 (43.4)	

There was a statistically significant association ($p < 0.05$) between a history of using proton pump inhibitors (PPIs) and *H. pylori* infection. This association may be due to the fact that long-term use of PPIs can induce a provocative reaction in the stomach mucosa, which can aid in *H. pylori* proliferation, as reported by Poly et al. [33]. However, it is possible that respondents who were unaware of PPIs may have been taking them without knowing, which could contribute to the high prevalence observed in this study. Interestingly, studies by Cheung et al. [34] and Poly et al. [33] supports our observation that there is a statistically significant association ($p < 0.05$) between a history of using PPIs and *H. pylori* infection.

5. CONCLUSION

In conclusion, this study has shown that *H. pylori* infection can be spread from vegetables through vegetable sellers to consumers. This was ascertained by the statistical associations of *H. pylori* infection and factors such as age, marital status, residency, family size, years in vegetable business, source of vegetables, hand washing habits, eating habits, and food intake. The data from this study have some limitations which include small sample size or measurement error, which could have affected the results.

CONSENT AND ETHICAL APPROVAL

Informed consent forms were issued to the participant before sample collections. Ethical approval was sought and obtained from the Akwa Ibom State Health Research Ethics Committee through their letter referenced AKHREC/15/7/21/011.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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