

Comparative Evaluation of Stool Antigen Immunoassay and Blood Antibody Test Methods for the Screening of *Helicobacter Pylori* Infection in Asymptomatic Adult Population in Delta State, Nigeria

Omosor Kingsley Ifeanyichukwu¹, Omosor Omasan Herrienta¹, Adejumo Babatunde Ishola Gabriel^{2*}, Ibeh Isaiah Nnana² and Dimkpa Uchechukwu³

¹Shalom Diagnostic Medical Laboratory, Warri, Delta State, Nigeria

²Medical Laboratory, Science Department, University of Benin, Benin City, Nigeria

³Physiology Department, Nnewi Campus, Nnamdi Azikiwe University, Awka, Nigeria

*Corresponding author: Adejumo Babatunde Ishola Gabriel, Medical Laboratory, Science Department, University of Benin, Benin City, Nigeria, Tel: +234800000000; E-mail: bigadejumo@yahoo.com

Received date: 27 February 2018; Accepted date: 23 March 2018; Published date: 30 March 2018

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Citation: Omosor K.I., Omosor O.H., Adejumo B.I.G., Ibeh I.N., Dimkpa U. (2018) Comparative Evaluation of Stool Antigen Immunoassay and Blood Antibody Test Methods for the Screening of *Helicobacter Pylori* Infection in Asymptomatic Adult Population in Delta State, Nigeria. J Mol Microbiol. Vol. 2 No. 1: 3.

Abstract

Aim: To compare the *H.pylori* positivity between stool antigen and blood antibody test methods and to determine the accuracy, sensitivity, specificity of the stool antigen and IgG serology tests as well as sex, age and geographical area variations with the rate of *H. pylori* positivity.

Methods: 408 asymptomatic volunteers (aged 32.55 ± 11.98 years) participated in this study. Stool and serum samples were obtained and assayed quantitatively using the stool antigen Accu-Diag *H. pylori* Antigen and Accu-Bind antibody ELISA Kits respectively. The stool samples were also assayed qualitatively using the monoclonal fecal *H. pylori* antigen rapid test kit (Aria *H. Pylori* Ag Combo Rapid Test).

Results: The overall positivity rate of *H. pylori* stool antigen test (28.2%) was significantly lower (P=0.02) compared to that of the serology test (48.3%). No statistical differences were observed between genders and among age groups and geographical areas in both the stool and serology tests. The positivity in age group of ≥ 60 yrs in the IgG antibody test was significantly (p<0.05) greater compared with age groups 18-39 years and 40-59 years respectively. The stool antigen test had a higher sensitivity (100% vs. 59.8%), specificity (98.7% vs. 57.9%), accuracy (99% vs. 60%) and area under the Receiver Operator Characteristic curve (99.3% vs. 61.4%) compared with the IgG serology test.

Conclusion: The present study suggests that *H. pylori* stool antigen test would be a more appropriate and reliable first-line routine test for diagnosis of *H. pylori*

infection in Delta region of Nigeria. The rate of positivity was not affected by sex and geographical areas, but high prevalence rate of *H. pylori* infection was observed within the oldest population.

Keywords: *Helicobacter pylori*; Stool sample; Enzyme linked immunosorbent assay; Blood antibody test

Introduction

Helicobacter pylori a Gram-negative bacterium, is recognized as a major cause of chronic gastritis, gastric carcinoma, peptic ulcer disease and lymphoid tissue lymphoma of the gastric mucosa [1,2]. It is estimated that half of the world's population are carriers of this bacterium [3,4]. The prevalence of *H. pylori* infection has been reported to be higher in developing countries relative to developed countries [5]. Different modes of transmission have been suggested by scientific literatures and these include, oro-oral, faeco-oral, gastro-oral, gastro-gastric and person-to-person transmissions [3,6]. Some of the risk factors for the transmission of *H. pylori* include, race, socioeconomic status, residing in rural area, age, poor sanitary conditions, overcrowding, poor diet, poor water supply and lower educational level of mothers [3,4,7-9].

The methods used for the diagnosis of *H. pylori* infection are classified into two major groups - the invasive and non-invasive tests [10]. The invasive methods are however considered more accurate than the non-invasive and involve culture, endoscopy and biopsy for histology and rapid urease test analysis. The non-invasive methods include serology, urea breath test and stool antigen analysis. Of the three non-invasive methods, urea breath test is reported to be the most accurate method used in determining *H. pylori* infection [11]. However, despite its accuracy, urea breath test has been found to be unreliable

in very young children, cumbersome, expensive and consequently unavailable in certain countries [12,13]. The serologic tests are usually performed to detect specific circulating antibodies against *H. pylori*. They are commercially available, easy to perform and inexpensive, but reported to be unreliable for the diagnosis of *H. pylori* since they cannot differentiate between active and asymptomatic colonization and past and current *H. pylori* infection [14]. The stool antigen test is used to detect traces of *H. pylori* antigens in the feces. It is a reliable and accurate test for diagnosis of the *H. pylori* infection and confirmation of its cure after treatment, since it avoids detection of previous *H. pylori* infection. It is convenient to the patients and can be easily performed even in small laboratories [15,16]. However its accuracy in different clinical situations and outside of controlled studies is a matter of concern [17,18].

There is paucity of studies aimed at determining *H. pylori* diagnostic tests accuracy among asymptomatic adult population in Nigeria. In the light of the rising incidents of *H. pylori* infection in Nigeria, there is the need for basic studies on accuracy of cost effective and available non-invasive diagnostic methods. In this study, we compared the *H. pylori* positivity between the stool antigen test and a blood antibody test method (IgG) in order to determine which method is a more efficient and dependable non-invasive test for detection of *H. pylori* infection in asymptomatic adult patients population in a Niger-Delta area of Nigeria. In addition, the present study determined the accuracy, sensitivity, specificity, positive and negative likelihood ratios of the stool antigen and IgG serology tests as well as sex, age and geographical area variations with the rate of *H. pylori* positivity.

Materials and Methods

Participants

Four hundred and eight apparently healthy, asymptomatic volunteers (aged 32.55 ± 11.98 years) were recruited and screened for *H. pylori* infection. The participants were randomly selected from the three senatorial districts of Delta State - Delta South, Delta Central and Delta North districts after accepting our request asking them to participate in the study. The health history and demographic information of the participants were obtained from a well-structured questionnaire administered to them prior to the study. The exclusion criteria for selection of participants included, history of gastritis, gastric or duodenal ulcers, previous diagnoses or treatment for *H. pylori* infection, those on medication with proton-pump inhibitors, antibiotic, steroids or non-steroid anti-inflammatory drugs for the past one month. We sought and obtained informed consents of the participants after the purpose of the research was explained to them. The study was approved by the Ethical Committee of the Ministry of Health, Delta State, Nigeria.

Sample collection

Five milliliters of blood was collected and dispensed into a plain container. The non anticoagulated blood was spun at 1500rpm for 10 minutes and the supernatant serum was separated into a separate sterile tubes. The serum was stored at -200°C for up to 2 weeks prior to analysis for *H. pylori* antibodies (IgG). The quantitative assays of the serum and plasma samples were done using the Accu-Bind ELISA Micro-wells (system code, 1425-300; Monobind Inc, Lake Forest, CA 92630, USA) for the detection of IgG antibodies to *H. pylori* in human serum.

Stool samples were obtained from the participants and a small piece of the samples (~ 5 mm in diameter; ~ 150 mg) transferred into 1 ml of Sample Treatment Solution and mixed thoroughly in a test tube. This was then quantitatively assayed using the monoclonal *H. pylori* stool antigen kit (Accu-DiagTM ELISA, Cat #1506-11, USA), which is based on enzyme-linked immunoabsorbent assay (EIA). The stool samples were also assayed qualitatively using the monoclonal fecal *H. pylori* antigen rapid test kit (Aria *H. Pylori* Ag Combo Rapid Test, Medtek, USA) based on the immunochromatography (ICA) technique. The performance of the quantitative enzyme immunoassay stool test and the IgG antibody serology test were determined with reference to the combined positive results of the EIA and ICA tests. The assay procedures were done according to the manufacturer's instructions. The analyses of all the samples were carried out at Shalom Medical Services Ltd, Warri – Delta State, Nigeria.

Data analysis

Categorical data were expressed as percentages. Comparison of *H. pylori* positivity between the stool antigen and blood antibody tests was done using chi-square test (goodness of fit). The sensitivity, specificity, positive and negative likelihood ratios of the quantitative stool antigen and IgG serology tests were determined with reference to the results of the combined positive results of the EIA and ICA tests. The area under a Receiver Operating Characteristic (ROC) curve for each test was determined (with 95% confidence interval) and was compared with each other. The test of statistical significance was set at $p < 0.05$. SPSS/IBM Statistical Software (version 20.0) was used to carry out all statistical analyses.

Results

Table 1 shows the socio-demographic characteristics of the study population. Data indicated that majority (81.6%) of the participants were aged between 18 – 39 years of age and followed by the age group, 40 – 59 years (13%). Those aged ≥ 60 years had the least percentage of the population (5.4%). The mean age of the study population was 32.55 ± 11.98 years (range 18-87 years). Majority (70.6%) of the study population were females, while the males constituted a smaller percentage (29.4%) of the population. The participants were

selected from Delta South (34.6%), Delta North (34.1%) and Delta Central (31.4%) districts.

Table 1 Socio-demographic characteristics of the study population.

Characteristics	Frequency	Percentage
Age Group		
18 – 39 yrs	333	81.6
40 – 59 yrs	53	13
≥ 60 yrs	22	5.4
Sex		
Males	120	29.4
Females	288	70.6
Location		
Delta Central	128	31.4
Delta North	139	34.1
Delta South	141	34.6

The overall prevalence of *Helicobacter pylori* in blood and stool of 408 volunteers is shown in **Figure 1**. The positivity rate of *H. pylori* EIA stool antigen test [28.2% (n=115)] were significantly lower (P=0.02) compared to that of the serology (IgG antibody) test [48.3% (n=197)]. Among the 408 subjects,

293 (71.8%) tested negative for EIA stool antigen test, while 211 (51.7%) tested negative for serology (antibody) test.

The prevalence of *H. pylori* according to sex of participants is summarized in **Table 2**. Data indicated no significant differences (P=0.09) in *H. pylori* positivity between the EIA stool antigen tests (32.5%) vs. blood (IgG antibody) test (47.5%) in males. In females, the *H. pylori* positivity in stool test (26.4%) was significantly (p=0.008) lower compared with IgG test (48.6%). Furthermore, higher prevalence rate of the *H. pylori* was observed in males in the stool antigen tests (32.5%) while in females it constituted 26.4%. In contrast, the *H. pylori* IgG test indicated higher prevalence rate in the females (48.6%) than the males (47.5%). However, no statistical differences were observed between the genders in both the stool and serology tests.

In **Table 3** the serology test indicated significantly greater *H. pylori* positivity compared with the stool antigen test in 18-39 yrs age group (47.7% vs. 27.6%; p=0.02); in 40-59 yrs age group (43.4% vs. 26.4%; p=0.04) and in ≥ 60 yrs groups (68.2% vs. 40.9%); p=0.01). Data further indicated that the highest positive result was found in the age group of ≥ 60 yrs, which indicated 40.9% positivity by stool antigen test, and 68.2% by the serology test. The positivity in age group of ≥ 60 yrs in the IgG antibody test was significantly ($\chi^2=6.60$; p<0.05) greater compared with age groups 18-39 years and 40-59 years respectively. In contrast, there was lack of significant differences among age groups in the stool antigen test ($\chi^2=4.18$; p=0.12).

Table 2 The prevalence rate of *H. pylori* according to sex of subjects.

Sex	Number of Subjects	EIA Stool Test	IgG Serology Test	χ^2	P-Value
Males	120	39 (32.5)	57 (47.5)	2.77	0.098
Females	288	76 (26.4)	140(48.6)*	7.05	0.008
Total	408	115 (28.2)	197 (48.3)		

* Statistical difference between *H. pylori* positivity between the stool antigen test and blood antibody test; (Abbreviations: EIA = Enzyme-linked immunoabsorbent assay; IgG = Immunoglobulin; χ^2 = Chi-square coefficient)

Table 3 The prevalence rate of *H. pylori* in different age groups.

Age Groups (years)	Number of Subjects	EIA Stool Test	IgG Serology Test	χ^2	P-Value
18 – 39	333	92 (27.6)	159 (47.7)*	5.26	0.022
40 – 59	53	14 (26.4)	23 (43.4)*	4.18	0.041
≥ 60	22	9 (40.9)	15 (68.2)*	6.68	0.01
Total	408	115 (28.2)	197 (48.3)		

* Statistical difference between *H. pylori* positivity between the stool antigen test and blood antibody test; (Abbreviations: EIA = Enzyme-linked immunoabsorbent assay; IgG = Immunoglobulin; χ^2 = Chi-square coefficient)

The prevalence rate of *H. pylori* according to the residence of participants is summarized in **Table 4**. *H. pylori* positivity in blood antibody test was significantly greater compared with the stool antigen test in Delta Central (59.4% vs. 32%; p=0.005), Delta North (44.6% vs. 25.2%; p=0.017), and Delta

South (41.8% vs. 27.7%; p=0.043) respectively. Data indicated that the highest prevalence rate for *H. pylori* was observed in Delta Central as detected by stool test (32%) and blood test (59.4%). However, no statistical differences were observed

among the geographical areas in both the stool and serology tests.

Table 4 The prevalence rate of *H. pylori* according to socio-economic stratified living region.

Residence of Participants	Number of Subjects	EIA Stool Test	IgG Serology Test	χ^2	P-Value
Delta Central	128	41 (32.0)	76 (59.4)*	8.01	0.005
Delta North	139	35 (25.2)	62 (44.6)*	5.71	0.017
Delta South	141	39 (27.7)	59 (41.8)*	4.08	0.043
Total	408	115 (28.2)	197 (48.3)		

* Statistical difference between *H. pylori* positivity between the stool antigen test and blood antibody test; (Abbreviations: EIA = Enzyme-linked immunoabsorbent assay; IgG = Immunoglobulin; χ^2 = Chi-square coefficient)

Table 5 Diagnostic characteristics of quantitative stool antigen test and quantitative IgG serology antibody tests for *H. pylori* infection.

Results	<i>H. Pylori</i> Tests	
	Stool Antigen Test	IgG Antibody Test
Sensitivity	1	0.598
Specificity	0.987	0.579

Positive Predictive Value	0.965	0.365
Negative Predictive Value	1	0.815
Accuracy	0.99	0.6
Area under the ROC curve	0.993 (0.986 – 1.00)*	0.614 (0.553 – 0.675)*
*95% confidence interval		

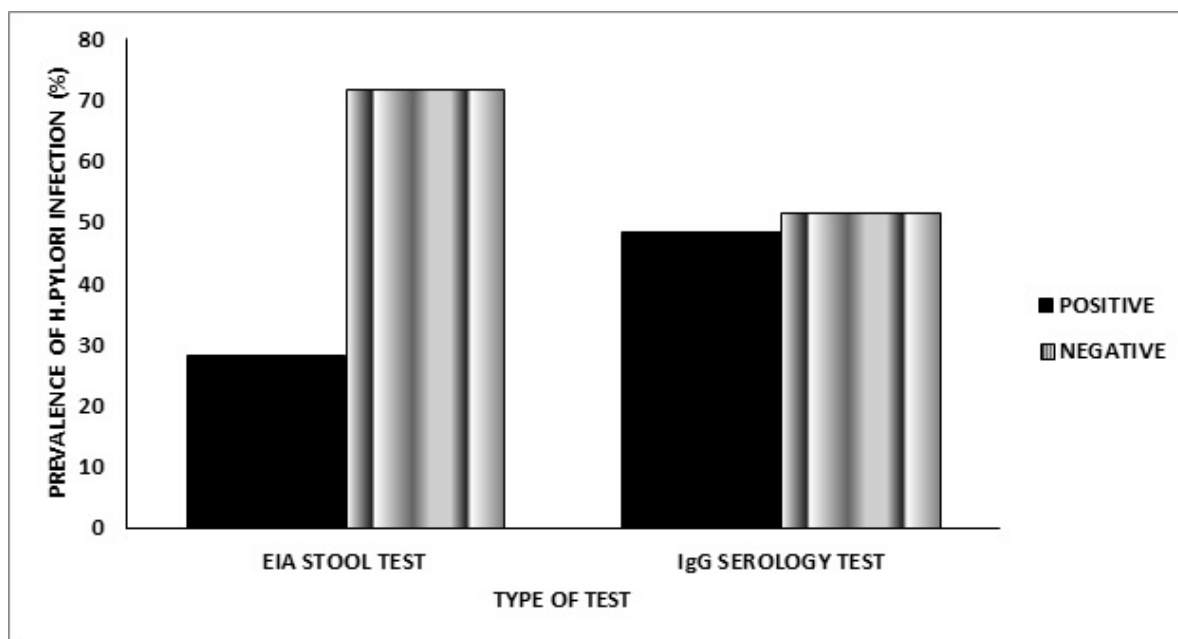


Figure 1 The prevalence of *H. pylori* in both blood and stool of the study population (EIA: Enzyme-linked immunosorbent assay).

Figure 2 shows the results from ROC curve analyses generated for each test by plotting the true-positive rate (sensitivity) against the false-positive rate (1-specificity). The

sensitivity, specificity, positive predictive value, negative predictive value, accuracy and area under ROC curve for each test, based on the predefined criteria, are shown in **Table 5**.

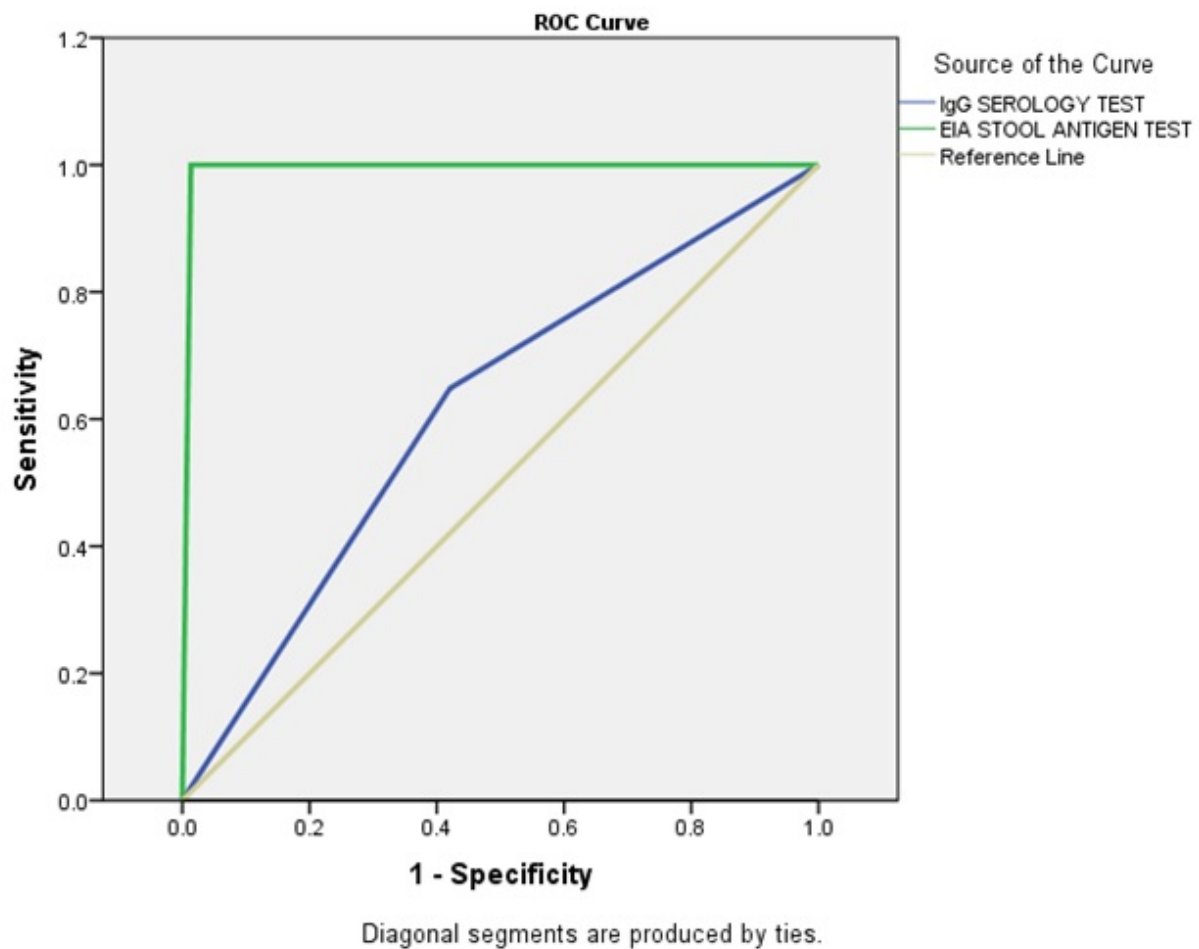


Figure 2 ROC curves of quantitative EIA stool antigen test and quantitative IgG serology antibody tests for *H. pylori* infection.

Discussion

Helicobacter pylori is a very common infection in developing countries, therefore a low-cost, rapid diagnostic technique may be useful for the management of *H. pylori* infection in both children and adults from these developing regions. Nigeria is one of the developing countries with rising incidents of gastritis and duodenal ulcers, as well as increasing prevalence of *H. pylori* infection hence the need to re-appraise the performances of different cost-effective, easy to use methods in diagnosis of *H. pylori* infection in the general population.

As expected, the EIA monoclonal stool antigen test was more accurate compared with the IgG serology test. The study showed high sensitivity (100%), high specificity (98.7%) and high accuracy (99%) of the quantitative enzyme-linked stool antigen test (Accu-Diag™ ELISA). However, this result applies only to this particular kit. In addition, the area under the curve of the ROC also indicates that the EIA monoclonal stool test is a more reliable diagnostic test for *H. pylori* diagnosis compared with the serology test. Previous studies [18-21] using monoclonal stool tests have shown sensitivities of between of 88.5%-98% and specificities of between 93.8%-99% in adults.

The monoclonal stool antigen test has also been shown to have an initial diagnostic accuracy comparable with that of urea breath test and higher than both serologic and urine antibody tests [22], and may provide a viable alternative to the Urea Breath Test, especially in developing countries. The high accuracy, sensitivity and specificity observed in the present study makes the enzyme-linked stool antigen test suitable for use in clinical practice for primary screening of asymptomatic adult populations in Niger Delta region of Nigeria. The low accuracy, sensitivity and specificity of the serology test also suggest that this method may not be suitable for primary diagnosis of *helicobacter pylori* infection in this region.

In this study, the overall *H. pylori* positivity rate was higher in the serology test compared to the stool antigen test. The same trend was also observed in tests involving males, all age groups and all selected geographical areas. Previous studies have also reported lower positivity in stool antigen tests compared to the serology tests [23,24]. This may be attributed to the fact that *H. pylori* stool antigen test is a more accurate indicator of active disease compared to serology test, which may not measure active disease. *H. pylori* stool antigen test has been shown to be rapid, easy to use and differentiates between active and latent infection; whereas, serology only

detects exposure and are not suitable to diagnose active infection or follow-up of eradication because of its low accuracy [25,26].

There were no significant differences between the two sexes with respect to *H. pylori* positivity in both stool antigen and serology tests. Previous studies [24,27] have also reported lack of gender differences in the rate of *H. pylori* positivity in both stool antigen and serology tests. In contrast, a study by Alim et al. [28] showed a significantly higher prevalence in female gender in both stool and serology tests. It is noteworthy that a greater number of females [n=288 (70.6%)] participated in this study. This may be a reflection of a greater consciousness of females on issues relating to their health.

The rate of *H. pylori* positivity showed a trend towards increase in rate of infection with the advancing age. The highest percentage positivity was observed in the age group ≥ 60 years compared with 18-39 years and 40-59 years respectively. The present study is in agreement with previous studies [29,30] which found that the age of *H. pylori* infection is more likely in patients over 50 years of age than in younger patients. However, another study [27] involving a mass survey of healthy adults, reported lack of age difference in *H. pylori* positivity.

H. pylori prevalence rates are reported to vary widely between different geographical regions and ethnic groups [31]. The risk of *H. pylori* prevalence by geographical areas may reflect differences in social and/or hygiene factors [32] and also related to differences in cultural background, social, dietary and environmental factors [33]. In the present study, we categorized geographical areas or residences of participants into three senatorial zones (North, South and Central) of Delta state, Nigeria. Our result indicated that the highest prevalence rate for *H. pylori* was observed in Delta Central as detected by stool test and blood test. However, when compared, no statistical differences were observed among these geographical areas in both the stool and serology tests. This may suggest that differences in social and cultural backgrounds or dietary and environmental factors among the Delta state people may not play a significant role in the rate of prevalence of *H. pylori* infection.

Conclusion

Although serologic testing is the most common noninvasive diagnostic method for *H. pylori* and is relatively inexpensive and convenient, the present findings indicate that the quantitative enzyme-linked stool antigen test (Accu-Diag™ ELISA), would be a more appropriate and reliable first-line routine test for diagnosis of *H. pylori* infection in large population surveys in Niger Delta region. It also provides a viable alternative to the more expensive and inconvenient 13C-UBT and endoscopy-based tests especially in developing countries. Our data also supports high prevalence rate of *H. pylori* infection within the oldest population.

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