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Seroprevalence and Risk Factors of the *Helicobacter pylori* Infection in Bukavu City in the Democratic Republic of Congo

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Abstract

The prevalence of *Helicobacter pylori* (*H. pylori*) infection is high in Africa. However, local surveys are still needed to be conducted for filling up the gap existing in understanding the epidemiological situation of the infection within the continent. This study aimed to assess the seroprevalence of *H. pylori* and its determinant factors in Bukavu, the capital city of Southern Kivu Province in Eastern part of the Democratic Republic of Congo (DRC). A cross-sectional community-based study was conducted across the 3 communes of Bukavu. A two-stage cluster sampling process was performed at Health Area and Households levels by using a standard questionnaire addressing sociodemographic characteristics and gastrointestinal symptoms of participants. Sera samples were collected from each participant for the detection of anti-*H. pylori* IgG antibodies by an ELISA system. A logistic regression model was fitted to assess potential determinants of the anti-*H. pylori* positive serological status. Statistical analyses were conducted by SPSS software version 21 (IBM®, USA, 2012). The survey included 331 participants aged from 10 to 86 years old. The anti-*H. pylori*: seroprevalence was estimated at 89% [95% CI: 84.9% - 92.2%]. Alcohol consumption increased the

risk of anti-*H. pylori* seropositivity by 6 (OR = 5.73 [95% CI: 1.89 - 17.41]; p = 0.002), while illiteracy (OR = 0.41 [95% CI: 0.18 - 0.97]; p = 0.043) and smoking (OR = 0.16 [95% CI: 0.04 - 0.65], p = 0.010) had paradoxical protective effect against anti-*H. pylori* seropositivity. The seroprevalence of *H. pylori* is very high in Bukavu. Identification of risk groups has the potential for supporting publish health issues needed for fighting against this infection. We recommend implementing appropriate sanitation and hygiene activities, health education programs, and further investigations for addressing this endemic infection locally.

Subject Areas

Biochemistry

Keywords

Helicobacter pylori, Seroprevalence, Risk Factors, Bukavu

1. Introduction

Helicobacter pylori (*H. pylori*), a spiral-shaped gram-negative flagellated bacterium, colonizes the stomach of more than 50% of people worldwide. Its frequency varies widely with the geographical environment, ethnicity, age and socio-economic factors—it is high in developing countries and lower in developed countries [1] [2]. *H. pylori* infection is acquired in the majority of cases in childhood and, when not treated, leads to a lifelong infection [3]. Since its discovery in the early 1980s by Warren & Marshall [4], *H. pylori* have been associated with several gastrointestinal diseases including chronic gastritis, peptic ulcers, gastric adenocarcinoma, and gastric MALT lymphomas [4] [5]. *H. pylori* infection is ubiquitous in sub-Saharan Africa where the prevalence is the highest observed [6]. However, available African data most often arise from hospital-based studies with substantial methodological limitations [7] [8] [9] [10]. Actually, these studies generally involve a small sample size of symptomatic patients likely missing to capture the majority of *H. pylori* carriers who remain asymptomatic [11] [12]. In DR Congo there are very little epidemiological data published on the *H. pylori* infection. In fact, a former study carried out in 1991 in eastern DRC in Katana in rural South Kivu, reported a prevalence of *H. pylori* of 90% in gastric biopsy samples [13]. In the city of Bukavu, the capital of the province, no data are available. Thus, this study aimed to estimate the seroprevalence of H. pylori and identify its associated risk factors.

2. Methods

This study is reported following the Strengthening the reporting of observational studies in epidemiology (STROBE) Statement for Observational studies (Supplementary Appendix, STROBE Statement—Checklist) [14].

2.1. Setting Study

This study was carried out in Bukavu, the capital city of Southern Kivu province, located in the Eastern of the Democratic Republic of Congo. This city has 870,954 inhabitants and stretches over 60 km^2 at an altitude of 1498 m, on the Southwest coast of Kivu Lake. It is subdivided into 3 communes: Ibanda, Kadutu and Bagira corresponding to 3 Health Zones each divided into 12 health areas by the National Health Administration System.

2.2. Study Design

A cross-sectional mass survey was conducted from April 15 to May 23, 2015. This survey targeted residents selected in households, regardless of gender or age, who had been living in the study area for at least 12 months. Study participants had to sign an informed consent form to be included in the study. A legal respondent adult had to sign the informed consent on the behalf of participants who were under 18 years old.

Since a prevalence of 70.8% has been reported in a neighboring population from Burundi [7], we estimated that a minimum sample size of 163 participants was deemed to be sufficient for estimating the prevalence in Bukavu city with its 95% confidence interval while reaching a precision of 5%. A systematic random sampling scheme based on a two-stage cluster sampling process (the health area and the household levels) was applied for recruiting study participants. Therefore, the sample size of 163 was multiplied by 2 for reducing the clustering effect, reaching 326. And by assuming a 10% loss of participants, the study targeted a sample size of 354 participants. Finally, 327 participants could be recruited.

2.3. Data and Sample Collection

The data collection was conducted by 12 investigators organized into three teams targeting each of the three Communes of the Bukavu city. Each team included a physician, a nurse, a laboratory technician, and a local community member who had been trained for the conduct of the survey. Activities performed by investigators consisted of identifying households to target, applying selection criteria for including participants in the study, conducting interviews based on a standard questionnaire and collecting biological samples in lines with good clinical practice requirements. Prior to the survey, the study questionnaire had been translated into Kiswahili, the local language, and successfully pre-tested on the field. For collecting biological samples, 3 ml of whole blood were sampled from a vein of the right arm on each study participant and immediately put in an EDTA containing tube. Then, all the tubes with blood samples were transitorily kept into a cooler box before being transported to the Blood Bank Laboratory at the Provincial General Reference Hospital of Bukavu (PGRHB). At the PGRHB, the serum was extracted from each blood sample by centrifugation process and was stored at −40˚C until shipment under cold chain toward the Brugmann Hospital Laboratory in Brussels (Belgium) for biological analyses.

2.4. Biological Analyses

A serological test for detecting *H. pylori* infection was carried out on each serum sample by using an ELISA kit (Euroimmun©, Lubek, Germany) processed under an Etimax 3000 analyzer (Diasorin©, Saluggia, Italy) and following the manufacturers' instructions. This technique allowed in vitro quantitative measurement of specific anti-*H. pylori* IgG antibodies, with a sensitivity and specificity of 100% according to the manufacturer. The result interpretation was made in agreement with criteria that had been set by the manufacturer:

- <16 UR (Relative units)/ml: negative result;
- 16 - 21 UR/ml: doubtful result;
- ≥22 UR/ml: positive result.

2.5. Study Variables

The main variable of this study is the anti-*H. pylori* serological status which is categorical. Other variables were either categorical or numerical and included socio-demographic information (age, sex, address, number of people living in the household) and clinical features (alcohol consumption, tobacco consumption, level of promiscuity in daily life, drinking water source, and gastrointestinal symptoms) related to each study participant. Additional variables were operationally defined in the study as follows:

- A high level of promiscuity was defined as the presence of at least 3 people sharing one bedroom within a given household.
- The source of drinking water was considered as clean when water was from a tap (municipal water) or a harnessed water source, while non-potable drinking water was that coming from other types of sources (e.g. a lake, a river, a non-harnessed source or well).
- Based on the presence or the absence of gastrointestinal symptoms, two groups of participants were defined namely, symptomatic and asymptomatic participants.

2.6. Risk of Bias

Efforts to address potential sources of bias were made by the random sampling process likely avoiding selection bias in the study. Furthermore, eventual confounding factors were assessed statistically during the logistic regression modeling.

2.7. Statistical Analysis

All the data were recorded into an Excel database (Microsoft®, USA, 2010) before being analyzed using the Statistical Package for Social Sciences (SPSS) software version 21 (IBM®, USA, 2012). The distributions of quantitative variables were summarized using medians with their respective interquartile ranges while qualitative variables were reported as absolute and relative frequencies for different categories. The comparison between proportions was performed using the Pearson Chi-square test or the corrected Yates' Chi-square test. The research for

the factors associated with the anti-*Helicobacter pylori* positive serology was carried out by the logistic regression test in univariate analysis. When differences were observed between anti-*Helicobacter pylori* positive serology and the independent variables, the effect of potential confounders was investigated by logistic regression adjustment in multivariate analysis. Finally, the odds ratios (ORs) and their 95% confidence intervals (95% CIs) were calculated to determine the degree of association between anti-*Helicobacter pylori* positive serology and the independent variables. A p value < 0.05 was considered to be the threshold of statistical significance.

2.8. Ethical Considerations

This study was conducted in accordance with the standard ethical principles set out in the Declaration of Helsinki. The study protocol was approved by the ethical committee of the Catholic University of Bukavu. Free and informed consent was obtained from each study participant. The confidentiality of information collected from participants was observed. The collection of biological samples was carried out by trained and qualified investigators in fulfillment of standard good clinical practices (e.g. using disposable and sterile materials).

3. Results

3.1. Study Participants

A total of 331 participants was finally included, reaching a response rate of 93.5% (n = 331/354) compared to the targeted sample size.

3.2. General Characteristics of the Study Participants

The general characteristics of the study participants are presented in Table 1. We thus observed that participants were mainly young and female (sex ratio 2 women for 1 man). They had a low education level and low socioeconomic status. Almost all of them had access to drinking water and were non-smoker and tobacco users.

3.3. *H. pylori* Seroprevalence in Bukavu

The *H. pylori* seroprevalence rate in the general population of Bukavu was estimated at 89% [95% CI: 84.9 - 92.2].

3.4. Factors Predicting the risk of *H. pylori* Seropositivity in Bukavu

The univariate analysis associating different variables of interest to the anti-*H. pylori* seropositivity is shown in Table 2. It thus appears that the sex, the residence, the promiscuity, the number of people per household, the type of drinking water, the presence of digestive symptoms and the history of gastroscopy did not show any significant association with the anti-*H. pylori* seropositivity (p > 0.05). However, there was a significant association between the anti-*H. pylori* seropositivity and smoking, alcohol consumption as well as the university education level.

The risk of anti-*H. pylori* seropositivity increased by more than 2 and more than 3 respectively with smoking and alcohol consumption, and with the university education level. After adjusting for confounding factors (smoking and residence), the logistic regression modelling shown in Table 3 identified significant

Table 1. Baseline characteristics of the study population.

Characteristics	n	%	Med (min-Max)*
Sexe			
Female	215	65.0	
Male	116	35.0	
Age (in year)			30 (10 - 86)
<20	81	24.5	
21 - 30	88	26.6	
31 - 40	34	10.3	
40 - 50	22	6.6	
>50	106	32.0	
Profession			
University or high school student	118	39.1	
State civil worker	19	6.3	
Worker in private sector	109	36.1	
Unemployed	56	18.5	
Address			
Bagira	72	21.8	
Ibanda	145	43.8	
Kadutu	114	34.4	
Education level			
No education	44	13.8	
Primary school level	42	13.2	
High school level	143	44.8	
Professional/technical level	27	8.5	
College/University level	63	19.7	
People in the household			7 (1 - 21)
<5	101	30.9	
6 - 10	165	50.5	
>10	61	18.6	
Tobacco	18	5.4	
Alcohol	117	35.3	
Non-potable water drinking	18	5.4	
History of endoscopy	28	8.6	

*Med (min-max) = median (minimum and maximum).

Table 2. Distribution of Helicobacter pylori seropositivity among different categories of participants.

Characteristics	N	*H. pylori* seropositivity			
		n	%	OR (IC-95%)	p
Sex					
Female	215	187	83.2	1	
Male	116	107	86.2	1.8 (0.8 - 3.9)	0.15
Age					
≤20	81	71	87.7	1.7 (0.5 - 2.8)	0.71
21 - 30	88	80	90.9	1.6 (0.7 - 4.1)	0.49
31 - 40	34	31	91.2	1.7 (0.5 - 6.2)	0.56*
41 - 50	22	21	95.5	3.5 (0.4 - 27.7)	0.30*
>50	106	91	85.8	1	
Tobacco					
Yes	18	17	94.4	2.4 (1.2 - 3.3)	0.04*
No	313	277	88.5	1	
Alcohol					
Yes	117	112	95.7	3.9 (1.4 - 11.8)	0.003
No	214	182	85	1	
Education level					
No education	44	35	79.5	1	
Primary	42	37	88.1	1.9 (0.6 - 6.2)	0.28
Secondary	143	127	88.8	2.0 (0.9 - 4.5)	0.11
Professional	27	25	92.6	3.2 (0.6 - 16.2)	0.19*
University	63	59	93.7	3.7 (1.1 - 13.2)	0.04*
Residency					
Bagira	72	66	91.7	1.8 (0.6 - 5.2)	0.24
Ibanda	145	125	86.2	1	
Kadutu	114	103	90.4	1.5 (0.7 - 3.3)	0.31
Promiscuity					
Yes	108	94	87	0.8 (0.4 - 1.7)	0.47
No	223	200	89.7	1	
People in the household					
≤5	101	89	88.1	1	
6 - 10	165	146	88.5	1.0 (0.5 - 2.2)	0.92
>10	61	55	90.2	1.2 (0.4 - 3.5)	0.67
Source of drinking water					
Drinking water	313	279	89.1	1	
No drinking water	18	15	83.3	0.6 (0.2 - 2.2)	0.44*

Continued

Recurrent digestive symptoms					
Yes	248	218	87.9	0.7 (0.3 - 1.6)	0.36
No	83	76	91.6	1	
Previous gastroscopy					0.26
Yes	28	23	82.1	0.6 (0.2 - 1.6)	
No	296	264	89.2	1	

*Fisher exact.

Table 3. Final model of factors explaining *the H. pylori* seropositivity by a multivariate logistic regression*.

Factor	Adjusted OR (CI 95%)	p-Value
Alcohol consumption		
Yes	5.73 (1.89 - 17.41)	0.002
No	1	
Education level		
Primary-university	2.5 (1.1 - 5)	0.043
No education level	1	
(*) Adequacy of the model		0.0006

and independent predictors of anti-*H. pylori* seropositivity in the study population. Thus, the alcohol consumption increased by 6, while the primary-university education level multiplied by 3 the risk of anti-*H. pylori* seropositivity in the final model (validity of the model: $p < 0.001$).

4. Discussion

This is the first study providing the data related to the epidemiology of *H. pylori* in the community living in Bukavu, the capital city of the Southern Kivu province in the Eastern part of the DRC. The *H. pylori* seroprevalence in Bukavu was thus estimated at 89% [95% CI: 84.9 - 92.2]. A similar prevalence is often reported in serological surveys conducted in communities from emerging countries such as Turkey and China [14] [15] [16] or from developing countries such as Madagascar and Ethiopia [10] [17]. However, the observed prevalence is 2 to 9 times higher than that ranging from 10% to 30% often reported in populations from Western countries and [3] [18] [19]. The prevalence estimate observed in the current study is also higher to *H. pylori* infection rates reported during endoscopic surveys from Burundi (70.8%) [7] and Rwanda (75.3%) [8], two DRC's neighboring countries located close to Bukavu city. This very high *H. pylori* seroprevalence in Bukavu can be linked to several factors likely able to create conditions for a persisting high transmission level of *H. pylori* in the population. First, recurrent armed conflicts that have happened for more than 20 years in the Great Lakes Region where Bukavu city, Rwanda and Burundi have located re-

gion, have forced huge population displacements since the 1990s in promiscuity and poor hygienic conditions favoring for *H. pylori* spread [20] [21] [22]. Moreover, many existing habits in local cultures may be additional risk factors for *H. pylori* contamination in the community. For instance, small children are often fed with feeds pre-chewed by their mothers—a practice capable of maintaining intra-familial *H. pylori* transmission [23]. Other local dietary habits are worthy to be mentioned, such as the fact that it is very common for a child to use the same cutlery with their parents when they are eating together, or for parents or elders to taste the cooled porridge of infants with the same spoonful [23]. On the other hand, an Iranian team, after isolating *H. pylori* living strains in milk and dairy products samples, has shown that the consumption of raw milk and its derivative products by humans could transmit *H. pylori* [24]. Bukavu is mainly occupied by the *shi* ethnic group, a pastoral ethnic, known for consuming unpasteurized cow's milk in their daily diet. One could then wonder whether this factor could play a certain role in the *H. pylori* epidemiology in the Bukavu area [25]. Furthermore, Bukavu is located in a region known to be endemic for cholera [26], a diarrheal infection established Megraud F. *et al.* [27] as increasing the risk of oral-fecal transmission of *H. pylori*.

We assessed different factors eventually associated with the *H. pylori* seropositivity in Bukavu. We thus observed that the *H. pylori* seroprevalence was higher among people aged 20 - 50, who were more active, inclined to excess alcohol and smoking consumption [28], with an infection rate of around 95.5% at 40 years old. The lowest prevalence rates were observed among people aged < 20 and > 50 years old (86%). An increase of the *H. pylori* infection with age is often reported in the literature. The infection peak occurs in the forties in some studies [29] [30] but can vary according to studies, and generally concerns younger ages [9] [16] [31]. Globally, there is a tendency for the infection predominance to be in younger people in developing countries compared to developed countries [10]. The most common hypothesis explaining the increase in the prevalence of *H. pylori* as a function of age is the cohort effect [31] [32] [33] with the highest rate of infection among children born around 1965s in this study.

In the current study, men and women were equally infected with *H. pylori* (men: 86.2 versus women: 83.2%) resulting in a sex ratio of 1.04. There are controversies about the impact of sex on the seroprevalence of *H. pylori*. Actually, some studies support a neutral impact of sex [16] [32] [33] [34], while others show a predominance of *H. pylori* seropositivity in female [35] or male [9] [36] [37] [38]. Similar to the sex, there are controversies in the literature regarding a possible association between alcohol consumption or smoking, and *H. pylori* infection [9] [16] [32] [34] [35] [36] [37] [38]. Illustratively, while data from Japan [39], Sudan [39] and China [16] did not show any relationship between the *H. pylori* infection and tobacco, a recent meta-analysis of observational studies suggested that a reduction in *H. pylori* infection could be associated with moderate alcohol consumption [40]. Oppositely, the current study and other reports [23] [28] [39] show a rather significant association between excessive alcohol

consumption and *H. pylori* seroprevalence. Furthermore, our observations showed that excess alcohol consumption increased by six the risk of having a positive anti *H. pylori* serology in Bukavu. Traditional alcoholic beverages are frequently consumed in a group of people who happily share their glasses as a form of solidarity in local understanding in the Bukavu area. This practice could be seen as an additional factor of oro-oral *H. pylori* transmission, which can be evoked to explain the association between seropositivity and alcohol consumption.

In the literature, the low level of education with ignorance is predictive of *H. pylori* infection [16] [41] [42] [43]. However, the current study shows that the low level of education, without financial resources along with excess alcohol consumption would be protective. In our country, the westernization of society concerns more the most educated barn of the population that copies many practices from Western societies. Thus a practice such as deep kissing currently enhanced by educated people might be having a role in the spread of *H. pylori* and may explain our observations. Indeed, it is established that the oral cavity hosts and can be a source of infection and transmission of *H. pylori* [43]. However, further studies are still needed to provide factual explanations for the epidemiological role eventually played by deep kissing in *H. pylori* transmission. Other possible factors such as gastroesophageal reflux should be considered.

Despite the absence of a significant association between the residence and the seroprevalence of *H. pylori*, the present study found a slightly lower seroprevalence in Ibanda commune (86.2%), the highest rate observed in the city compared to the rate in the poor communes. Several authors in Africa have also not found a link between the seroprevalence of *H. pylori* and the rich or poor residing area [9] [10]. Even worse, Sathar M.A. *et al.* found no significant difference between urban and rural areas in South Africa [44].

Like Nurgalieva in Kazakhstan [45], the present study found no relationship between promiscuity and seroprevalence of *H. pylori*. However, several authors have found that promiscuity is closely related to *H. pylori* infection. This is the case of Füresz J. *et al.* among recruits of the Hungarian army [20], Kalaajieh WK *et al.* in Lebanon [21], or Malaty in Korea [22]. Unlike this study, different sources of water supply have been identified as potential reservoirs for acquiring the bacteria [46]. Thus, Nurgalieva ZZ found that the use of stream water was associated with a high prevalence of H. pylori [45]. As most of the literature data [46] gastric symptomatology was not associated with the seroprevalence of *H. pylori* in Bukavu. In addition, the history of upper gastrointestinal endoscopy has been described as a risk factor for *H. pylori* infection [32]. In the present study, however, the history of gastroscopy was not associated with *H. pylori* seropositivity.

5. Conclusion

In this study, the prevalence of *H. pylori* is high; it constitutes a public health problem in this city. This prevalence is associated with smoking, alcohol con-

sumption and education level. To address this problem, appropriate epidemiological strategies should be drawn up and put in place with active involvement of the community, clinicians and policymakers.

Authors' Contributions

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Conflicts of Interest

The authors declare no conflicts of interest.

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