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Helicobacter pylori antigen in stools of children: A cross-sectional study in two regional hospitals in the South West region of Cameroon

Antigène d'Helicobacter pylori dans les selles des enfants : Une étude transversale dans deux hôpitaux régionaux du Sud-Ouest du Cameroun

Djike Puepi F.Y[1], Nsoli Mbebi H.M[2], Aghoagni Gouajio G.G[1], Yanelle Wandji L[1], Noukeu Njinkui D[3], Andang P[2], Eloumou Bagnaka[4].

Article Original

1. Department of Internal Medicine and Paediatrics, Faculty of Health Sciences, University of Buea, Cameroon
2. Faculty of Health Sciences, University of Buea, Cameroon
3. Department of Pediatrics, Faculty of Medicine and Pharmaceutical Sciences, University of Dschang, Cameroon
4. Department of Internal Medicine, Faculty of Medicine and Pharmaceutical Sciences, University of Douala, Cameroon

Corresponding author:
Djike Puepi Fokam Yolande, Department of Internal Medicine and Paediatrics, Faculty of Health Sciences, University of Buea, Cameroon Email: yolandep2000@yahoo.fr, Tel : 677836217

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ABSTRACT

Background: Helicobacter pylori infection (HPI) is usually acquired during childhood and most often, it is asymptomatic in children. The aim of this study was to identify the presence of Helicobacter pylori antigen in stools of children.

Methods: We conducted a cross-sectional study from February to April 2023 at the Buea and Limbe regional hospitals. Children aged 6 months to 15 years were included. The diagnosis was made by performing the H. pylori stool antigen rapid test, using monoclonal antibodies. Data were entered into Excel spreadsheet and analysed using SPSS version 25.0. Chi square test was used to compare variables, and logistic regression by univariate and multivariate analyses were used to test for associated factors. Statistical significance was set at p-value<0.05.

Results: Three hundred and twenty-one participants were recruited. The mean age was 6.15 years ± 4.46. The overall prevalence of HPI was 28.7%. Male children represented 53.3% (n=171). Among the positive cases, 50% were symptomatic with abdominal pains emerging as the most common symptom. The associated factors to HPI were family history of HPI AOR=1.85 (95%CI [1.08-3.18], P=0.025) and increasing age, with age groups]5-10] years AOR=1.96 (95%CI [1.06-3.62], P=0.032) and]10-15] years with the AOR=2.67 (95% CI [1.46-4.91], P=0.002).

Conclusion: The overall prevalence of HPI was 28.7%. Age groups]5-10] years and]10-15] years, and family history of H. pylori infection were identified as risk factors associated to HPI.

RESUME

Introduction : L'infection à H. pylori (IHP) est contractée pendant l'enfance. Elle est asymptomatique chez les enfants. Le but de cette étude était d'identifier la présence de l'antigène d'Helicobacter pylori dans les selles des enfants.

Méthodes : Nous avons mené une étude transversale de Février à Avril 2023 dans les hôpitaux régionaux de Buea et de Limbé. Les enfants âgés de 6 mois à 15 ans étaient inclus. Le diagnostic était posé par le test rapide H. pylori stool antigen. Les données étaient analysées à l'aide de la version 25.0 de SPSS. Le test du chi carré était utilisé pour comparer les variables, et la régression logistique pour tester les facteurs associés. Une valeur p<0,05 était significative.

Résultats : Trois cent vingt et un participants étaient recrutés. L'âge moyen était de 6,15 ± 4,46 ans. La prévalence de l'IPH était de 28,7 %. Les garçons représentaient 53,3 % (n=171). Parmi les cas positifs, 50 % étaient symptomatiques. Les douleurs abdominales étant le symptôme le plus fréquent. Les facteurs associés à l'IPH étaient les antécédents familiaux d'IPH (AOR=1,85 (95%CI [1,08-3,18], P=0,025) et l'âge, avec les groupes d'âge]5-10] ans AOR=1,96 (95%CI [1,06-3,62], P=0,032) et]10-15] ans avec l'AOR=2,67 (95% CI [1,46-4,91], P=0,002).

Conclusion : La prévalence de l'IPH est élevée. Les groupes d'âge]5-10] ans et]10-15] ans, et les antécédents familiaux d'infection à H. pylori étaient les facteurs de risque associés à l'IPH.

Introduction

Helicobacter pylori is a Gram-negative flagellated bacterium of the family of Helicobacteraceae [1–3], described for the first time in 1982 by Marshall and Warren [4,5]. It was isolated from the mucosa at the antral portion of the human stomach, and it has been associated with gastroduodenal diseases worldwide [4,6]. The mode of transmission remains controversial, though it is believed to be interhuman [7]. Children become infected in the first few months of life; as many as 50% are infected by the age of 5 years, and up to 90% are infected by the time they reach adulthood [7].

H. pylori infection (HPI) is one of the commonest chronic human bacterial infection, with prevalence of infection higher in low and middle-income countries [4]. The prevalence of HPI in children has globally decreased from 39.0% before the year 2000 to 26.0% in 2010. It later fluctuated about 33% in 2016. In 2022, its overall prevalence had decreased to 32.4% [8]. Some studies have shown variability in prevalence, in which the highest prevalence was detected among the Bangladesh children (80%) followed by the Indians (57%) [9]. The burden of H. pylori infection in Africa remained high in 2022 (70.1%) though some variability exists [10,11]. Data on prevalence of this infection in Cameroon is limited. A community-based study in asymptomatic children in Buea and Limbe health districts in 2004 revealed a prevalence of 52.3% [1]. Multiple studies have shown positive relation between H. pylori infection and age, and prevalence increases with age [7]. Gender predominance is unclear, but it is considered that both sexes have equal risk of infection. The prevalence is higher in low and middle income countries [4]. The main risk factors associated with infection include low socioeconomic and poor sanitation and hygiene [12]. Gastrointestinal complications include diffuse chronic antral gastritis, Peptic Ulcer Diseases, MALT lymphoma, and gastric carcinoma [7,13–15].

Diagnostic methods can be invasive (endoscopy and histology, culture, PCR or rapid urease test) and non-invasive (stool antigen test, serology and urea breath test) [8,12,16]. The gold standard is histology [8,17]. However detecting bacterial antigens in stool offers an alternative non-invasive diagnostic test that can replace endoscopy and biopsy, and yields reliable results [12,13,18]. Helicobacter pylori stool antigen test (SAT) is a method used for direct detection of H. pylori antigen, which exists in the faeces [12], using monoclonal and polyclonal antibodies [19]. This study is relevant because the burden of HPI in children is still alarming, mostly in developing countries. Helicobacter pylori infection in children seems to be neglected in our settings. In addition, there is limited data on the burden of this infection in children in Africa in general and for Cameroon, data is outdated, with the most recent data dating back 20 years ago. Therefore, in this study, we aimed to determine the prevalence, risk factors of HPI and its clinical aspects in children aged 6 months to 15 years.

Materials and Methods

Study setting

The study was carried out at Buea and Limbe regional hospitals (henceforth BRH and LRH), located in the South-West region of Cameroon. Limbe and Buea regional hospitals are tertiary level hospitals (according to Cameroon health pyramid) found in Fako division. Fako is one of the six administrative divisions of the South-West region, and has 4 health districts including Buea, Limbe, Muyuka and Tiko. The BRH is the second referral level hospital in this region following LRH. Buea has an estimated population of 200,000 as per 2005 projections of the Central Bureau for census and population studies. The paediatric unit has three wards respectively dedicated to prematurity, neonatology and older children. It is headed by a pediatrician, 4 general practitioners, 10 senior nurses and assistant nurses. The LRH is located in the zone two health area of the Limbe Health District. Limbe is a coastal community situated in Fako division; it has an estimated population of 120,000 inhabitants. LRH is the principal referral level hospital in the region. The paediatric unit has three wards respectively prematurity, neonatology and older children. The paediatric unit is headed by one paediatrician who follows up the children, assisted by two general practitioners and 10 nurses.

Study design

This was a prospective hospital-based cross-sectional study with collection of data from caregivers of children who presented at BRH and LRH from February to April, 2023. All children aged 6 months to 15 years whose carers granted informed consent were included in the study. Children who had been treated with antibiotics (amoxicillin, metronidazole, or clarithromycin) within the last four weeks and/or with proton pump inhibitors (PPI) within the last two weeks respectively, were excluded.

Data collection and procedure

The caregivers of all children 6 months to 15 years who presented at the outpatient or paediatric unit were approached. An information leaflet was given to caregivers by the investigators, alongside presentation and verbal explanation of the research. After the written consent was granted, questionnaires were filled with information such as patients' and parents' demographic data, environmental factors around patients' area of residence, past medical history, and symptoms displayed. Stool samples were collected from each participant conveniently. The stool samples were analysed within six hours of collection. The presence of H. pylori in stool was

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determined by an immune-chromatographic assay using a commercially available monoclonal antibody kit (OEM/ODM, model number KST-41 from Zhejiang Plastic, China), following manufacturer's protocol. The test kits were stored at 4-30°C. After stool specimens were provided by participants in stool sample cups (about 2-5 spoons), the specimens were collected from the cups by dipping the collection stick (which is the buffer bottle) into 3 different spots of the stool sample to ensure homogeneity of the sample and the material obtained was placed in a sample bottle. We then chronologically shaked the specimen collection tube to mix the specimen and the extraction buffer, removed the test kit from the sealed pouch and used it as soon as possible (within 1 hour after preparation of the solution). We did not touch the membrane of the strip, held the specimen collection tube upright and carefully unscrewed the tip of collection tube, squeezed 2-3 drops of the sample solution to the test sample well of the device, then started the timer. We avoided trapping air bubbles in the specimen well, waited for the colored lines to appear and read the test result in 15-20 minutes. We did not read after 20 minutes. For the interpretation, negative test result (1 colored band on the control region); Positive test result (In addition to control band, a distinct colored band also appeared in the test region); Invalid (if no bands appeared, or a test band appeared without control band).

Case definition

Was considered infected a child with positive HpSA test; and non-infected a child with negative HpSA test. Children were categorized into three age groups

Data analysis

The data collected were entered using Epi info version 7.2.1 and exported as an excel spreadsheet, then saved in laptops, USB keys for storage and easy access. The data were analysed using SPSS version 25.0. Dependent variable was Helicobacter pylori infection. Independent variables were age and gender categories, age and level of education of mother, type of toilet used, type of drinking water, number of people living in the house, number of bedrooms, finger suckling. Continuous variables were summarized as means or median and standard deviation, Categorical variables were summarized as frequencies, percentages and proportions. Chi-square statistical and Fisher exact tests were used to compare variables and logistic regression (Univariate and multivariate analyses) were used to assess for association between H. pylori infection and different associated factors. Statistically significance was set at p-value <0.05.

Ethical considerations

The study commenced after obtaining ethical approval from the Institutional Review Board at the Faculty of Health Science (FHS) of the University of Buea (Ref: 2023/1896-01/UB/SG/IRB/FHS) and administrative authorisations from the FHS (Ref: 2023/265/VD/RC/FHS) and the Regional Delegate of Public Health for the South West region (Ref: R11/MINSANTE/SWR/RDPH/PS/338/370). Written informed consent was granted by carers. Ethical principles such as respect for autonomy, confidentiality, beneficence, non-maleficence, and justice were scrupulously observed.

Results

We initially planned to include 346 children (according to sample size calculation). Was considered positive a child with a positive HpSA test. We approached 386 children among whom, 24 (6.2%) had exposure to antibiotics in the past 4 weeks and/or to PPI in the past 2 weeks, and 41 (10.6%) refused to participate. Thus, we finally included 321(83.2%) children in this study; 193 (60.1%) from Buea, 104 (32.4%) from Limbe, and 24 (7.5%) from elsewhere.

Table 1: Distribution of participants age and gender, and mothers age and level of education

Variables	Frequency (n)	Percentage (%)
Participants (N= 321)		
Age (Years)		
≤5	176	54.8
]5-10]	74	23.1
]10-15]	71	22.1
Gender		
Female	150	46.7
Male	171	53.3
Residence		
Buea	193	60.1
Limbe	104	32.4
Elsewhere	24	7.5
Mothers (N= 321)		
Age (Years)		
≤30	116	36.1
[31-40]	159	49.5
[41-50]	38	11.8
>50	8	2.5
Level of education		
Primary	42	13.1
Secondary	191	59.5
Tertiary	87	27.1
No formal education	1	0.3

Of the 321 children, Male participants represented 53.3% (171 of 321), with a male to female ratio of 1.14:1. Children of age <=5 years represented 54.8% (176 out of 321), those of]5-10] years represented 23.1% (74 out of 321), and those of]10-15] years represented 22.1% (71 out of 321). The mean age of children was 6.15 years ±4.46. The mean age of the

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mothers was 33.5±7.25, and the age group [31-40] years represented 49.5% (see table 1).

Table 2: Determinants of HPI

Category	Frequency (n)	Percent (%)
Type of toilet used		
Water system	243	75.7
Traditional pit	76	23.7
No toilet	2	0.6
Number of persons in the house		
1-3	38	11.8
>3	283	88.2
Number of bedrooms in the house		
1	43	13.4
>1	278	86.6
Source of drinking water		
Mineral	86	26.8
Pipe borne	229	71.3
Stream	6	1.9
Breastfeeding time		
< 6 months	10	3.1
> 6 months	303	94.4
Finger suckling		
No	279	86.9
Yes	42	13.1
Family history of H-pylori infection		
No	234	72.9
Yes	87	27.1
Abdominal pain		
No	255	79.4
Yes	66	20.6
Vomiting		
No	265	82.6
Yes	56	17.4
Diarrhea		
No	284	88.5
Yes	37	11.5
Abdominal bloating		
Yes	316	98.4
No	5	1.6

Up to 59.5% (191 out of 321) of mothers had stopped at the secondary level of education (see table 3), against 27.1% and 13.1% who stopped at the tertiary and primary levels respectively.

We looked into some environmental and behavioural factors that could show the interhuman transmission of HPI. Of the 321 children recruited, household of 75.7% of them used water system toilet while 23.7% used traditional pits; 88.2% lived more than 3 in the house. Enrolled children had various sources of drinking water, and 71.3% drank pipe borne water, while 26.8% and 1.9% of participants drank mineral and stream water respectively. 94.4% of children were breastfed for more than 6 months; 27.1% (87) of the study population had a family history of HPI.

Table 3: Comparing HPI and mother level of education

Category	Negative	Positive	Total	P-value
Level of education				
Primary	28 66.7%	14 33.3%	42 100%	0.47
Secondary	130 68.0%	61 31.9%	191 100%	0.11
Tertiary	71 81.6%	16 18.4%	87 100%	0.01
No formal education	0 0.0%	1 100%	1 100%	0.11

Table 4: Comparing HPI and other variables

Variable	Negative	Positive	Total	P-value
Type of toilet used				
Water system	176 72.4%	67 27.6%	243 100%	0.44
Traditional pit	51 67.1%	25 32.9%	76 100%	0.35
No toilet	2 100%	0 0.0%	2 100%	0.36

Although HPI is usually asymptomatic in children, 50% of the positive cases reported symptoms such as abdominal pains and/or vomiting.

Table 5: Comparing HPI and symptoms

Variables	Negative	Positive	Total	P-value
Symptoms				
Absent	151 65.94%	46 50%	197	0.008
Present	78 34.06%	46 50%	124	
Abdominal pain				
No	191 83.41%	64 69.57%	255	0.001
Yes	38 16.59%	28 30.43%	66	
Vomiting				
No	187 81.66%	78 84.78%	265	0.51
Yes	42 18.34%	14 15.22%	56	
Diarrhea				
No	202 88.21%	82 89.13%	284	0.80
Yes	27 11.79%	10 10.87%	37	

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The prevalence of H. pylori infection was 28.7% (92 with positive test, out of 321).

The frequency of HPI varied with age groups; older children are apparently more infected than the younger ones: 33.78% (25 out of 74) and 43.66% (31/71) of positive cases were respectively children aged]5-10] and]10-15] years, against 20.45% (36/176) in those ≤5 years.

The prevalence also varies with the mothers' level of education: it is lower in children of mothers who have reached the tertiary level of education than those of mothers who stopped at the primary and secondary levels of education. From our study, 33.3% and 31.9% of mothers who stopped respectively at the primary and secondary levels of education against 18.4% of those who reached the tertiary level were positive for HPI (table 3).

Thus, we notice that the proportion of positive cases is higher in children of mothers with primary and secondary levels of education than that of tertiary level of education.

Although not statistically significant, it came to our notice that 34.5% (79/229) of children who drank pipe-borne and 33.3% (2/6) of those who drank stream water, compared to 12.8% (11/86) of those who drank mineral water were positive. Also, 29.68% of those who lives more than 3 in the same household were positive to HPI.

Regarding the factors associated with HPI in univariate analyses, two factors were fouund associated. Those factors were the family history of positive H. pylori with COR 1.95(95% CI 1.15-3.28, P=0.013), and age groups]5-10] years with COR 1.98 (95%CI [1.08-3.63], P=0.026) and]10 to 15] years with COR 3.01 (95% CI [1.66-5.47], p<0.001); with their p-values less than 0.05. The significant statistical values in the univariate analyses, were re-analysed with co-founders using multivariate analyses, which confirmed an association between H. pylori infection and family history of H. pylori infection; and between HPI and age groups]5-10] years and]10-15] years.

The risk of a child having H. pylori infection increased in those who had a family history of H. pylori infection with AOR 1.85 (95%CI [1.08-3.18], P=0.025); and in those in the age groups]5-10] years with AOR 1.96 (95%CI [1.06-3.62], P= 0.032) and]10-15] years with AOR 2.67 (95%CI [1.46-4.91], P= 0.002).

There was a significant association between the occurence of symptoms in children and H. pylori infection, with a p-value of 0.008. Abdominal pain was the commonest complaint with a p value of 0.001. We realised in this study that 30.43% (28 out of 92) of children who were found positive, and 16.59% (38 out of 229) who were negative reported abdominal pain.

Table 6: Factors associated with H. pylori infection in the univariate and multivariate analyses

Variable	Inf/Ex	Prev	cOR (95%CI)	P-value	aOR (95%CI)	P-value
Age						
≤5 years	36/176	20.45	–	–	–	–
]5-10] years	25/74	33.78	1.984 (1.083-3.634)	**0.026**	1.958 (1.060 – 3.618)	**0.032**
10-15] years	31/71	43.66	3.014 (1.662 - 5.465)	**<0.001**	2.674 (1.455 – 4.914)	**0.002**
Gender						
Female	48/150	32.00	1.358 (0.836 - 2.206)	0.216	–	–
Male	44/171	25.73	–	–	1.816 (0.491 - 1.355)	0.431
Number of people in the house						
1 – 3	8/38	21.05	0.632 (0.278 - 1.435)	0.273		
>3	84/283	29.68	–	–		
number of bedrooms						
1	7/43	16.28	0.442 (0.189 - 1.032)	0.059	1.877 (0.786 - 4.484)	0.156
>1	85/278	30.58	–	–	–	–
Source of drinking						
Mineral	11/86	12.79	0.293 (0.048 - 1.795)	0.185		
Pipe borne	79/229	34.50	1.053 (0.189 - 5.877)	0.953		
Stream	2/6	33.33	–	–		
Breastfeeding time						
<6 months			0.592 (0.123 - 2.841)	0.512		
>6 months			–	–		
Finger sucking						
No	80/279	28.67	1.005 (0.49 - 2.061)	0.989		
Yes	12/42	28.57	–	–		
History of recurrent NSAID						
No			0.935 (0.237 - 3.699)	0.924		
Yes			–	–		
Family history of H pylori infection						
Yes	58/234	24.79	1.947 (1.154 - 3.284)	0.013	1.853 (1.079 - 3.183)	0.025
No	34/87	39.08	–	–	–	–

COR: crude odd ratio; CI confidence interval; LCL: 95% lower confidence level; UCL: 95% upper confidence level; AOR: adjusted odd ratio; Inf/Ex: Infected/exposed; Prev: prevalence

Discussion

We conducted a cross-sectional study at the Buea and Limbe Regional hospital to determine the prevalence, clinical presentation, and factors associated with Helicobacter pylori infection in children. We targeted children who came to the hospital for consultation because it was easier to get consents/assents, since they visited the hospital because they needed medical

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attention; compared to meeting them in their various homes, where due to their carers cultural beliefs, wouldn't have given consents for sample collection. The gold standard to diagnose HPI is endoscopy and biopsy for histological study, and/or culture plus antibiogram[8,17]. Endoscopy is an invasive method that has specific indications before being carried out; it is also not always available, difficult to carry out, very uncomfortable for the patient, and needs qualified personnels. However detecting bacterial antigens in stool offers an alternative non-invasive diagnostic test that can replace endoscopy and biopsy, and yields reliable results [12,13,18]. Helicobacter pylori stool antigen test (SAT) is a method used for direct detection of H. pylori antigen, which exists in the faeces [12] using monoclonal antibodies; and HpSA test kits were bought and successfully shipped and made available for this study before recruitment started.

We enrolled 321 children among who 28.66% were positive. 50% of positive case were symptomatic, and most presented with abdominal pains. This study identified the following as factors associated with HPI in children: family history of HPI and age groups]5-10] years and]10-15] years.

The prevalence of HPI in children in our study was 28.66%. This finding is approximately similar to the worldwide prevalence of HPI (32.4%) in children in 2022 [8]. This high prevalence is common to developping countries. In the same line, a prevalence of 30.9% was found in Nigeria by Etukudo et al. in children aged 0.5 to 15 years in 2012 [4]. This can be explained by the fact that HPI in children was detected by the same tool in a similar type of setting (semi-urban areas in developing countries). Some developing countries have prevalences even higher than the prevalence found in our study and that of the worldwide. This is the case in Asia, of the study done in Bangladesh which revealed a prevalence of 80%, far higher than the prevalence in our study. Also, in Africa, studies conducted in Libreville Gabon by Ategbo et al. in 2012 [16] and Ethiopia in 2020 by Schalcher et al. [20] revealed respective prevalences of 62 % and 65.7%. These differences could be explained by the difference in years of study, difference in sample sizes, and the sampling method. A study conducted in Buea and Limbe health districts, Cameroon in 2004 by Ndip et al. reported a prevalence of 52.27% [1], which is higher than that of our study, although both carried out in the same semi-urban areas . The difference in prevalence of this study compared to our study may be due to differences in the sampling method, difference in HpSA testing kits used, the time gap between the two studies. Also the fact that the actual prevalence is lower could be explained by the fact that as times moves on, parents are more and more educated.

The frequency of HPI increases with age. In our study, children of age groups]10-15] years were more infected than those]5-10] years who in their turn were more touched than those ≤5 years. This finding is similar for many studies[1,4,16,20]

According to literatures, children with HPI are usually asymptomatic [16]. In our study, there was a strong correlation between the symptomatology and the positivity of HpSA with a P-value of 0.008. 50% of positive cases were symptomatic, and the commonest symptom was abdominal pain. This is in line with a study done by Martinez et al. in Mexican children where many children apparently healthy reported recurrent abdominal pains. We re-iterate on the fact that abdominal pain in the presence of H. pylori is non-specific.

Although not statistically significant, the frequency of infection was higher in females than in males. This is similar to the study carried out by Martinez et al. in 2020; but differs from the studies carried out by Ndip et al. in Cameroon, and Ategbo et al. in Gabon where the proportion of positive cases was higher in males than females. This difference can be explained by the difference in sample sizes and that in the study population.

HPI can be transmitted within the same household, from one person to another, given its fecal-oral and oral-oral routes of transmission. This was confirmed by our study that showed a significant association between H. pylori infection and a family history of H. pylori infection. This, although not exactly described the same, has been also found by other researchers such as Ategbo et al. who showed the role of family-related factors in HPI acquisition. HPI increases with increasing age. It is shown in our study that older children have more positive test than the younger ones.This is in line with most studies like those conducted in Gabon by Ategbo et al., in Cameroon by Ndip et al. and in Mexico by Martinez et al. where it was identified that HPI increases with age. The lower prevalence in the children ≤5 years could be explained by the elimination of the germ by these children by a natural physiological process as stated in the literatures.

Not like the study carried out by Schacher et al. and Ategbo et al., where using traditional pit toilet was a significant risk factor to HPI in children, our study did not show much differences in the frequences of infection between those using water system toilet and those using traditional pits. This could be explained by the difference in sample sizes and the difference in the total number of children using a specific type of toilet. In our study for example, up to 75.7% of the studied population used water system toilet; while in the study conducted in Ethiopia by 94.9% of the studied population used traditional pits and only 5.1% used flushing toilet.

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The link between HPI and the source of drinking water has been established. We found out in our study that drinking pipe borne water or stream water was associated with increased risk of positivity, compared to drinking mineral water. This is in line with the findings by Etukude et al. in Nigeria, where the population that drank borehole or well water presented a higher risk of HPI than those who drank pipe borne water.

Limitations

The short period of study; the difficulties in having access to certain health areas of the region preventing us from covering the overall southwest region of the country; and the refusal of some caregivers to participate due to cultural beliefs, were the limitations to this study.

Conclusion

H. pylori infection is a frequent infection in children in Buea and Limbe. Its diagnosis in our setting is possible by simplified tests which are still expensive. The prevalence of H. pylori infection in our study was higher in females than males. A significant association was found between H. pylori infection and increasing age, between H. pylori infection and family history of positive H. pylori test. For symptomatic cases, most presented with abdominal pain. This infection was commoner in those using traditional pit and in those drinking pipe borne and/or stream water. HPI remains a public health problem which should be considered in children. Carrying out this study in other regions of the country, including rural areas should be considered in the future.

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