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Epidemiology of *Helicobacter pylori* infection in Kinh and Khmer Children in Mekong Delta, Vietnam

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Abstract

Background: *Helicobacter pylori (H. pylori)* infection varies among ethnic or racial groups. This study aimed to evaluate the seroprevalence and factors associated with *H. pylori* infection among Khmer and Kinh children living in the same natural and social conditions in Mekong delta, Vietnam.

Methods and Findings: This cross-sectional study was conducted between October and December 2012 in the Mekong delta. We collected data using a structured questionnaire on social-economic and health status, lifestyle and living conditions from 683 Khmer and Kinh children aged between 6 months and 18 years. Additionally, data were collected from 913 adults of 485 households settling together in the same rural communes. All the participants in the study had *H. pylori* serology (i.e. ELISA) tested on blood samples obtained by venous puncture. *H. pylori* serology data were analyzed using χ^2 test and logistic regression models. In our study, the overall *H. pylori* seroprevalence was 36.7% (32.1% in children from 6 months to 18 years *versus* 40.2% in adults ($p<0.05$), with a sharp increase in the prevalence around 3 years of age, without significant difference between Kinh and Khmer ethnics. Blood group B, absence of prejudicial habit of taking food by hand, good practice of hand washing after defecation were identified as protective factors for *H. pylori* infection [OR (95% CI): 0.50 (0.32-0.79), 0.48 (0.29-0.79), 0.79 (0.41-0.98); respectively]. In addition, *H. pylori* infection in mothers, in first sibling and in two first siblings in particular were found as high risk factors for *H. pylori* infection in children [OR (95% CI): 1.98 (1.12-3.18), 2.12 (1.25-4.12), 4.39 (2.81-6.94); respectively].

Conclusions: Results of our study showed low and indifferent rates of *H. pylori* seropositivity in Kinh and Khmer children. Data from this study strongly suggest that environmental andindividual hygiene and some lifestyle practices should be improved to lower *H. pylori* infection in this population.

Keywords: Children, Mekong delta, *H. pylori* infection, Prevalence, Kinh, Khmer, Risk factors

Introduction

Helicobacter pylori (*H. pylori*) infection is considered as a major cause of chronic gastritis, peptic ulcer, and gastric cancer [1]. *H. pylori* infection is less frequent in developed countries and the overall infection rate is declining worldwide [2,3]. However, *H. pylori* infection is prevalent around 75% of the population in developing countries [3]. Southeast Asia is the region where gastric cancer is the most frequent in the world, probably related to highly oncogenic characteristics of *H. pylori* isolates prevailing in this region [4]. Racial and ethnic differences in *H. pylori* infection in adults and in children had been reported and described worldwide [5-11]. To find explanatory reasons of this phenomenon, numerous putative factors had been hypothesized and studied in relation to either socioeconomic status [3-11] or characteristics of the host (human genetic and geographic distribution, migration) [12-14] or the bacterium (*H. pylori* genome evolution, genetic affinities, changes during chronic infection) [13]. In looking for preventive measures of *H. pylori* infection, it is reasonable for low-income nations to invest their modest resources in identifying risk factors for the infection in relation to the socioeconomic status of their own population.

Vietnam, a tropical country situated in South East of Asia, consisted of 54 ethnic groups with different cultures among 86 million inhabitants, of which 75-80% living in rural or remote areas [15,16]. So far in Vietnam, several institution-based and community-based studies have shown high seroprevalence of *H. pylori* infection, varying between 50 and 80% in adults and around 26 to 71.4% in children [17-21]. Different risk factors for has been identified for *H. pylori* infection in Kinh ethnic majority [17-21] and in some among 53 other ethnic minorities [22,23]. As the socioeconomic level and lifestyle vary considerably among ethnic groups of the country, the prevalence of *H. pylori* infection and particularly risk factors for this infection were different in previous studies [17-23]. The Khmer is the fourth largest among 53 ethnical minority groups in Vietnam. Although living together with Kinh in the fertile delta of the Mekong River for centuries, the Khmer have their own habits, customs and cultural practices that differ from those of Kinh co-habitants [15,16]. Moreover, their economic condition is different than the Kinh people [15,16]. That made it appealing to study the prevalence of *H. pylori* infection and risk factors for this infection to develop appropriate preventative measures for managing the *H. pylori* infection in this subpopulation in the future. The aim of the present study was then to evaluate the seroprevalence of *H. pylori* infection and factors associated with the *H. pylori* infection among Khmer and Kinh children and adults living in the Mekong delta.

Population and Methods

Study population

According to the 2009 census, there are 1,260,640 Khmer inhabitants in Vietnam [16]. One of the most important aggregation of Khmer was found in Travinh province (310,000 habitants), where they regroup in 2 communes (i.e. Hamtan and Hamgiang) of Tracu district where we implemented the study, enrolling 1,596 members of all generations living together in the same home from 485 households.

To avoid a selection effect, children aged less than 6 months were excluded due to the likelihood of residual maternal *H. pylori* antibody, as well as those with severe diseases or immuno-compromised status due to the possibility of an altered immune response.

Data were collected after obtaining written consents from local administration and health authorities. Informed consent was obtained from each household member. The study was approved by the ethics committee of Hanoi Medical University.

Data Collection

In this cross-sectional study, door-to-door sampling method was adopted. We used a structured questionnaire, as in previous studies [17-23] for data collection on socio-demographic, health status and potential exposure. The questionnaires were completed by the study investigators and information was collected from the head of the households. Blood samples were collected from all participants (i.e. *all members of each household*) by venous puncture and were immediately centrifuged. Sera were separated and preserved in vaccine thermos, then sent to the reference laboratory (Microbiology Division of Digestive Diseases, National Institute of Epidemiology and Hygiene) on the same day where sera were stored at -20°C and processed as previously described [24].

Variable definitions

H. pylori infection was determined by in-house ELISA for dosage of *H. pylori* IgG antibody against specific *H. pylori* antigen. The ELISA was carried out in the reference laboratory (Microbiology Division of Digestive Diseases, National Institute of Epidemiology and Hygiene) using sonicated Swedish and Vietnamese *H. pylori* strains as antigens prepared and validated in the Microbiology Department, Karolinska Institute, Stockholm for use in Vietnamese adults and children (sensitivity of 99.6%, and specificity of 97.8%) [24]. Children's sera with a value equal to or over 0.18 optical density (OD) unit were classified positive.

Socio-demographic variables consisted of age and sex of all children and adults in every household, monthly family income classified into three categories (i.e. <500.10^3 VND, actual national minimal monthly income, 500-1,000 or >1,000.10^3 VND), household space (in 3 categories, i.e. up to 10m^2/capita according to national mean standard, 10-20 or >20 m^2/capita), parents' occupation and parents' education level [16].

Potential exposure variables were divided into three major subgroups:

1. *Environmental, individual hygiene and life-style* including water sources classified in 2 main sources (family well and others such as streams, raining or collective well), latrine in 2 main types (existence or not), pet (dog or cat) in the house in 2 categories (presence or not), behavior of children on hand washing before meal and after toilet in 2 categories (*not regular* for those who practiced less frequently than one per twice, and *regular* for those who practiced every time or every of other time), mouth-to-mouth feeding from mothers or caregivers to child in 2 categories (*regular* or not), breast feeding duration in 2 categories (*shorter* or *longer* than 12 months of age).

2. Promiscuity including sibling size in 2 categories (≤ 2 or ≥ 3), sharing a bed in 2 categories (with 1 and with ≥ 2 persons), collective life initiation in 2 categories (<3 years and ≥ 3 years of age).

3. Health status variables consisted of *H. pylori* infection status in parents and sibling (*H. pylori* sero positivity or sero negativity), *g*astro-duodenal history of child and parents (defined as having had a gastro-duodenal disease if this disease was diagnosed and treated by health-givers from district health center or higher levels) in 2 categories (presence or absence), and history of child's antibiotic use during the previous 6 months determined from individual health booklets or in-depth interviews of the main caregivers, in 2 categories (absence or presence).

Statistical analysis

First, study population characteristics were compared according to their *H. pylori* infection status using the Chi square (χ^2) test. As one of the major aims of our study was to detect the factors potentially influencing *H. pylori* infection in this subpopulation, the appropriate strategy of analysis must be able to control for mutual confounding, point out the risk factors and avoid overlooking important associated variables. We then analyzed separately the associations between *H pylori* infection status with the demographic and socio-economic variables and with variables related to potential exposure. Analysis was performed firstly by univariate technique, by adjusting in each group on every variable, and finally by using backward stepwise conditional logistic regression to select variables importantly associated with *H. pylori* infection within each group, including all variables significantly associated with *H. pylori* seropositivity after adjustment and those with *p* values less than 0.20 by Chi square test. Associations were expressed as odds ratio (OR) and their confidence intervals (95% CI). Finally, backward stepwise conditional procedures were used again to include in the final model not only variables independently associated with *H. pylori* serological status in each group, but also those known to be important for transmission pathways. Statistical significance was set up at the 0.05 level. All *p* values were 2-tailed. Data were analyzed using SPSS software (SPSS® for Windows™ version 16.0 Copyright SPSS Inc.).

Results

Among 1,596 individuals enrolled in the study (Khmer accounting for

1,116 or 69.9%), 586 (36.7%) were seropositive for *H. pylori* infection. *H. pylori* seroprevalence was 40.2% (367/913) in adults and 32.1% (219/683) in children ≤ 18 years old (*p<0.05*). *H. pylori* seroprevalence in relation to sex and ethnicity are presented in **Table 1**.

There were no significant differences in *H. pylori* seropositivity based on sex (*p>0.05*), or ethnicity (*p>0.05*). *H. pylori* seropositivity in children in relation to socio-demographic variables are presented in **Table 2**.

We observed a sharp increase in the infection earlier moment, with a *H. pylori* seropositivity rate of 34.8% in children under 3 in contrast to 27.8% between 3 and 6 years, then being stable between 32.7% and 32.8% between 6 and 18 years of age.

No significant relationship was found between *H. pylori* seropositivity and sex, ethnicity, age groups of children or variables related to socio-economic status of their household. However, mother's education of lower secondary level was significantly related to higher rate of *H. pylori* infection in children (*p=0.049*). In addition, the rate of *H. pylori* seropositivity in children with blood group B was significantly lower than those in children with other blood groups (*p=0.003*). *H. pylori* seropositivity in children in relation to environmental, individual hygienic status and children life style are reported in **Table 3**.

Children in families without latrine and those with habit of taking food by hand were more likely to have higher *H. pylori* seropositivity than others (*p=0.003* and *p=0.001*, respectively). Moreover, children with good practices of hand washing (regular hand washing before meal and after defecation) were less likely to *H. pylori* seropositive (*p=0.048* and *p=0.012*, respectively). *H. pylori* seropositivity in children in relation to variables related to promiscuity, status of *H. pylori* infection and gastro-duodenal disease in parents as well as antibiotic use in children are presented in **Table 4**.

We did not observe any relationship between *H. pylori* seropositivity in children and variables related to promiscuity (i.e. *H. pylori* infection in fathers, gastroduodenal disease status in parents and history antibiotic use within 6 months in children). However, children whose mother or older siblings were infected by *H. pylori* were more likely to have *H. pylori* infection than others (*p=0.004* and *p=0.001*, respectively). Final results of logistic regression model are presented in **Table 5**.

In the final model of multivariate analysis, low risk for *H. pylori* infection in children was independently associated with blood group B, 'without taking food by hand', 'regular washing hand after making stool'. In contrast, *H. pylori*infection in mother and in older siblings in particular was independently associated with higher risk for *H. pylori* infection in children.

Discussion

To date, most of the population based *H. pylori* prevalence studies are exclusively done on adults or children and were used small sample size [17-22]. In those small studies it was difficult to interpret the result considering close host-bacterium-environment interaction. In this large cross-sectional community based-study with 1,596 participants we showed the seroprevalence of *H. pylori* infection among adults and children in the Mekong delta. To our knowledge this is one of the largest studies on *H. pylori* infection to date in Vietnam. Our results showed a *H. pylori* seroprevalence of 36.7% (32.1% in children from 6 months to 18 years *versus* 40.2% in adults; *p<0.05*), with a sharp increase in *H. pylori* seroprevalence around 3 years of age, without significant difference between Kinh majority and Khmer minority ethnics. Our study allowed identifying blood group B, absence of bad habit of taking food by hand and good practice of hand washing after defecation as protective factors for *H. pylori* infection [OR (95% CI): 0.50 (0.32-0.79), 0.48 (0.29-0.79) and 0.79 (0.41-0.98); respectively]. On the other hand, *H. pylori* infection in mothers, in the first sibling and in two first siblings in particular were identified as risk factors for infection in *H. pylori* children [OR (95% CI): 1.98 (1.12-3.18), 2.12 (1.25-4.12) and 4.39 (2.81-6.94); respectively].

The overall seroprevalence of *H. pylori* infection of 36.7% with 32.1% in children under 18 in this study was comparable to the low overall rate of 38.5% (301/781) with 26.7% (109/408) in children under 18 observed in our previous study in 2007 carried out in a population of Kinh and 3 other minority ethnics in a mountainous area of northern border (Laocai province) [22]. However, the rate of *H. pylori* seroprevalence in the present study was far lower than those reported in all other community-based studies in Vietnam [17-21,23]. In a community-based study in Kinh majority ethnic in Hanoi (2003), Hoang et al. reported a rate of *H. pylori* seroprevalence of 58.2% (310/533) with 47.3% (112/327) in children [17]. In another community-based study in Kinh of a rural area from Nghean (2007), we observed an overall rate of *H. pylori* seroprevalence of 61.5% (525/854) with 55.5% (213/384) in children [21]. Recently, in their community-based study carried out in a population of Kinh and 4 other minority ethnics in central highland (Taynguyen), Le et al. (2012) reported an overall rate of 45.2% (892/1,968) with 40.0% (476/1,186) in children under 15 [23].

An increase in rate of *H. pylori* infection along with increasing age in children was confirmed in Vietnam and worldwide [18-23,25-28]. In addition, all studies on the infection prevalence reported that subjects were infected with *H. pylori* early in childhood (before 5 years old) [18-23,25-28]. Although the difference in rates of *H. pylori* seoprevalence in children under 3 and in those aged 3 to 6 did not reach significant level (*p=0.051*), the earlier peak before 3 years old of *H. pylori* infection in this study was in agreement with what reportedby Okuda et al. (2007) in Japanese children [27] and it likes a mosaic mark depicted on a homogenous picture describing a gradualacquisition up to 5 years old of*H. pylori* infectionin children according to data reported in all previous studies in Vietnam [18-19,21-23] and in several oversea studies [25-28]. In above-mentioned hospital-based study in 2006 with a study population

Table 1 *H. pylori* seroprevalence in study population in relation to sex and ethnicity.

	Total		Adults >18 years		Children ≤18 years	
Variables	N (%)	HP(+) (%)	n (%)	HP(+) (%)	n (%)	HP(+) (%)
Sex						
Male	608 (38.1)	230 (37.8)	290 (31.8)	128(44.1)	318(42.8)	102 (32.1)
Female	988 (61.9)	356 (36.0)	623 (68.2)	239(38.4)	365(57.2)	117 (32.1)
Ethnic						
Kinh	480 (30.1)	173 (36.0)	278 (30.4)	109(39.2)	202(29.6)	64 (31.7)
Khmer	1,116 (69.9)	413 (37.0)	635 (69.6)	258(40.6)	481(70.4)	155 (32.2)
Total	1,596 (100)	586 (36.7)	913 (100)	367 (40.2)*	683 (100)	219 (32.1)*

HP (+): *H. pylori* seropositivity

*****$p<0.05$ between 2 subgroups

Table 2 *H. pylori* seropositivity in children in relation to socio-demographic variables and blood groups.

Variables		*H. pylori* seropositivity in children			
		n	HP (+) n(%)	*p*	OR (95% CI)
Sex	Male	318	102 (32.1)	NS	1.00
	Female	365	117 (32.0)		0.99 (0.71-1.42)
Age group (year)	<3	141	49 (34.8)[#]	NS	1
	3-6	151	42 (27.8)[#]		0,72 (0.4-1.2)
	10-18	220	71 (32.7)		0,91 (0.6-1.4)
Ethnic	Kinh	202	64 (31.7)	NS	1.00
	Khmer	481	155 (32.2)		1.02 (0.71-1.52)
Monthly income (103 VND/capita)	<500	416	127 (30.53)	NS	1.00
	500-1.000	210	75 (35.7)		1.27 (0.89-1.80)
	>1.000	57	17 (29.8)		0.97 (0.53-1.77)
House space (m^2/ person)	<10	229	71 (31.0)	NS	1.00
	10-20	295	104 (35.3)		1.21 (0.84-1.75)
	>20	159	44 (27.7)		0.85 (0.54-1.32)
Father occupation	Peasant	209	53 (25.4)	NS	1.00
	Others	31	12 (38.7)		1.82 (0.85-3.92)
Mother occupation	Peasant	422	130 (30.8)	NS	1.00
	Others	62	24 (38.7)		1.42 (0.81-2.47)
Father education level	≥Secondary	212	54 (25.5)	NS	1.00
	<Secondary	28	11 (39.3)		1.91 (0.85-4.26)
Mother education level	≥Secondary	426	129 (30.1)	0.049	1.00
	<Secondary	58	25 (43.1)		1.75 (1.00-3.05)
Blood group	A	173	70 (40.5)	0.003	1.00
	B	219	52 (23.7)		0.45 (0.31-0.72)
	O	208	73 (35.1)		0.79 (0.51-1.23)
	AB	83	24 (28.9)		0.59 (0.32-1.14)

NS: not significant

HP (+): *H. pylori* seropositivity

[#]*p=0.051* between 2 subgroups

of 824 children, we observed a sharp increase of *H. pylori* seroprevalence from 22.6% in children under 3 to 32.9% in 3-6 year group [OR (95 CI): 1.7 (1.1-2.7] [19]. In a community-based study in 2007 enrolling 854 subjects (384 children under 18 and 470 adults from 236 households in Nghean, we noted again this sharp increase in *H. pylori* seroprevalence from 36.5% in children under 3 to 52.1% in 3-6 year group [OR (95 CI): 7.75 (1.4-38.1)] [21]. In another community-based study carried outin 2007 enrolling 784 members of 245 households (408 children under 18 and 373 adults) of Kinh majority and 4 other minority ethnics living in a mountainous area in northern border (Laocai), we reconfirmed this sharp increase in *H. pylori* seroprevalence from 9.2% in children under 3 to 21.5% in 3-6 year group [OR (95 CI): 3.4 (1.1-10.6)] [22]. A recent community-based study carried out in 2012 in Kinh and three minority ethnics in mountainous area of central highland (Taynguyen), enrolling 1,968 members of 498 households (1.186 children and 782 adults), we reconfirmed again this sharp increase in *H. pylori* seroprevalence from 28% in children under 3 to 38% in 3-6 year group [OR (95 CI): 1.6 (1.1-2.2)] [23].

Differences in prevalence of *H. pylori* infection among racial and ethnic groups have been so much described in the literature [9-11]. However, it is not clear yet to what extent such differences can be ascribed to socioeconomic factors and other possible risk factors. This community based-study, enrolling 1,596 members of all generations living together in the same home of 485 households of Khmer and Kinh cohabiting for centuries on the same ground, did not find any difference in *H. pylori* seropositive rates between 31.7% in Kinh children (64/202) and 32.2% in Khmer ones (155/481). Kinh and Khmer are naturally different in genetics and they may be influenced by different flows of migration and then bacterial sources: immigration of ancestors from India introducing hpEurope bacteria into Cambodia for Khmer, and from Austro-Asiatic speaking people into Vietnam carrying hspEAsia bacteria for Kinh, as reported recently by Breurec et al. [14]. In addition, several traditional habits and numerous practices in lifestyle are inevitably different between Kinh and Khmer. However, the fact that the two ethnics are subjects of similar *H. pylori* seropositive rates in our study suggests major role of similar socioeconomic conditions as well as events and suffering that they shared during long centuries of natural circonstances in close mutuality and cohabitation. This fact may be partly influenced by not only cultural but also biological crosses appeared between the two ethnics in the long history of cohabitation. In reality, inside many households, administratively fathers or mothers are Kinh or Khmer for actual generation, but biologically they inherited a repeatedly mixed ethnicity in the past during long history of living together. Even at present, as far as we know during the field work, cross marriage between Kinh and Khmer is not rare. Nevertheless, the same and the different rates of *H. pylori* infection in different ethnics observed in our population-based studies are always puzzled and perplexed facts. In our previous community-based study in 2007, enrolling 784 members of 245 households of Kinh majority and 4 other minority ethnics (Day, Hmong, Tay, Dao) living in a mountainous area in northern border, no significant difference in *H. pylori* seropositive rates between 41.0% (16/39) in Kinh and 38.1% (52/135) in Giay cohabiting in an agglomeration.

Table 3 *H. pylori* seropositivity in children in relation to environmental, individual hygienic status and children life style .

Variables		*H. pylori* seropositivity in children			
		n	HP (+) n (%)	*p*	OR (95% CI)*
Water sources	Family well	233	80 (34.3)	NS	1.00
	Others	450	139 (30.9)		0.85 (0.61-1.19)
Latrine presence	Yes	624	190 (30.4)	0.003	1.00
	No	59	29 (49.2)		2.18 (1.41-3.92)
Regular taking food by hand	No	140	26 (18.6)	0.001	1.00
	Yes	543	193 (35.5)		2.41 (1.65-4.25)
Regular hand washing before meal	No	180	69 (38.3)	0.048	1.00
	Yes	489	148 (30.3)		0.69 (0.48-0.99)
Regular hand washing after defecation	No	193	75 (38.9)	0.012	1.00
	Yes	485	143 (29.5)		0.65 (0.46-0.93)
Regular receiving chewed food	No	254	76 (29.9)	NS	1.00
	Yes	429	143 (33.3)		1.18 (0.81-1.69)
Dog in the house	No	303	86 (28.4)	NS	1.00
	Yes	380	133 (35.0)		1.24 (0.79-1.92)
Cat in the house	No	478	158 (33.0)	NS	1.00
	Yes	205	61 (29.8)		0.85 (0.56-1.63)
Breast feeding duration (month)	<12	305	87 (28.5)	0.035	1.00
	≥12	359	128 (35.7)		1.64 (1.09-2.71)

*Adjusted for sex, age groups and ethnicity

Table 4 *H. pylori* seropositivity in children in relation to variables related to promiscuity and health status of study population.

Variables		*H. pylori* seropositivity in children			
		n	HP (+) n (%)	*p*	OR (95% CI)*
Size of household	≤4	374	115 (30.7)	NS	1.00
	≥5	309	104 (33.6)		1.21 (0.87-1.72)
Size of sibling	≤2	395	121 (30.6)	NS	
	≥3	288	98 (34.0)		1.23 (0.89-1.78)
Collective life initiation	<3 years	253	78 (30.8)	NS	1.00
	≥3 years	408	137 (33.6)		1.14 (0.81-1.62)
Regular sharing bed	with 1	141	47 (33.3)	NS	1.00
	with ≥2	542	162 (29.9)		0.83 (0.69-2.18)
H. pylori infection in father	(-)	258	70 (27.1)	NS	1.00
	(+)	109	30 (27.5)		1.02 (0.62-1.81)
H. pylori infection in mother	(-)	289	81 (28.0)	0.004	1.00
	(+)	190	72 (37.9)		2.34 (1.22-3.81)
H. pylori infection in both parents	2 (-)	54	18 (33.3)	NS	1.00
	1 (+)	237	81 (34.2)		1.19 (0.61-1.92)
	2 (+)	31	9 (29.0)		0.88 (0.32-1.63)
H. pylori infection in sibling	1st sibling (-)	256	65 (25.4)	0.001	1.00
	1st sibling (+)	257	91 (35.4)		2.71 (1.58-5.52)
	1st & 2ndsiblings	156	63 (40.4)		6.63 (2.98-11.64)
GD disease in father	No	240	56 (23.33)	NS	1.00
	Yes	120	34 (28.33)		1.45 (0.71-2.53)
GD disease in mother	No	387	123 (31.78)	NS	1.00
	Yes	97	31 (31.96)		1.01 (0.92-1.61)
Antibiotic use within 6 months in children	No	669	216 (32.29)	NS	1.00
	Yes	14	3 (21.43)		0.57 (0.22-2.13)

*Adjustment for sex, age groups and ethnicity

Interestingly, these rates were far higher than 20.3% (15/74) in Dao and 16.7% (6/36) in Tay as well as 16.1% (20/124) in Hmong, who lived in remote areas, completely separately from the group of Kinh and Day ($p<0.001$) [22]. The thing is totally adverse in another community-based

Table 5 Results of final model analyzing association between *H. pylori* seropositivity in children and variables that were statistically significant in univariate analysis.

Variables	OR (95% CI)
Blood group B	0.50 (0.32-0.79)
No taking food by hand	0.48 (0.29-0.79)
Hand washing after defecation	0.79 (0.41-0.98)
H. pylori infection in mother	1.98 (1.12-3.18)
H. pylori infection in elder sibling	
Seropositivity in 1st sibling	2.12 (1.25-4.12)
Seropositivity in 1st and 2nd siblings	4.39 (2.81-6.94)

The variables in the final model were included using a backward selection algorithm among variables that were statistically significant in univariate analysis (blood group, father education level, latrine use, taking food by hand, hand washing before meal, hand washing after defecation, breastfeeding duration, *H. pylori* infection in mother, *H. pylori* infection in sibling) and adjusted for sex, age and ethnicity.

study recently carried out in central highland, enrolling 1,968 members of 498 households, including 782 adults and 1,186 children under 15 years old (381 Kinh, 342 K'ho, 212 Ede and 246 Raglai children). This study did not show any significant difference in *H. pylori* seropositive rates between King majority children (35.3%) and K'ho minority ones (36.0%) living together on the same mountainous ground nearby Dalat town. Surprisingly, *H. pylori* seropositive rates in children of two minority ethnics (47.6% in Ede and 47.2% in Raglai) living far away in very remote mountainous areas were far higher than that in Kinh and K'ho ($p<0.01$) [23]. Because no major differences in *H. pylori* seropositive rateswere found to be correlated with studied variables related to socioeconomic status, habitsand lifestyle of study populations, and data on bacterial nature as well as on human genetics of the study populations were not explored yet, it is impossible to propose any explanative hypothesis for the fluctuation in *H. pylori* seropositive rates in our studies.

Potential exposure variables in relation to environmental and individual hygiene as well as exclusive habits and life-style have been always sought by investigators in epidemiological studies on *H. pylori* infection [5,11,29-33], in developing countries in particular [3,18-23]. Their target was to identify risk factors that are potentially intervenable or preventable so as to feasibly interrupt transmission pathways of this ubiquitous infection. However, researchers worldwide had hardly identified or ascertained any consistent risk factor among numerous attributive variables. As mouth-to-mouth or feces-to-mouth transmission has beenascribed to the most important or unique way for *H. pylori* to get to the host, so much attention had been done by researchers worldwide to seek out the pejorative role played by different putative variables in relation to food manipulation and feeding, vegetables, water and toilet practices. Running water, raw vegetables, chop stick use, chewing feeding had been some where reported prejudicial as risk factors for *H. pylori* infection [3,29,30,32], they were, however, not at all consistent in other studies of the kind. In the present study, 'owing latrine in the family', 'regular washing hand before meal', 'breastfeeding <12 months', though significantly associated to lower *H. pylori* seropositivity in univariate analysis, were not likely to be risk factors for the infection in multivariate analysis, the same as observed in our previous studies [18-23]. In contrast, 'regular washing hand after defecation and 'no taking food by hand' appeared as protective factors for *H. pylori* infection in this population in the final model using multivariate logistic regression analysis. This is the first time these variables manifested its role on *H. pylori* infection, although that had been desperately sought out in our previous studies in Vietnam [18-23]. In our opinion, more confirmative studies seem warranted and reasonable to confirm the role these variables veritably play in *H. pylori* infection for children, because it would be extremely important for this fact in health education to this population.

Intrafamilial clustering of *H. pylori* infection with predominant role of mothers and siblings as sources of *H. pylori* transmission to children has been largely reported worldwide [33-40]. The fact had been confirmed in Vietnam by our previous studies [30-35] and reconfirmed by this study. Our data showed that *H. pylori* infection in the first sibling rendered his younger siblings twice more likely to be infected [OR (95 CI): 2.12 (1.25-4.12)], even more important than likelihood of being infected when the mother was infected [OR (95 CI): 1.98 (1.12-3.18)]. Moreover, if the two first siblings were all infected, the likelihood of being infected in their younger siblings would be doubled [OR (95% CI): 4.39 (2.81-6.94)]. The fact that *H. pylori* infection in siblings influenced more on the infection of other children than the infection in the mother might be explained by longer and closer contact between siblings than with mother, because in Vietnam, like in most developing countries, after the first year of age, children spent more time to play and to sleep together with siblings than with mother and with father.

Breastfeeding in early childhood had been regularly reported as protective factor in most of studies in Vietnam [18-21] and worldwide [41-45]. However, data from epidemiological studies suggested that breastfeeding duration shorter than 6 months or longer than 12 months seemed to reduce or void its protective effect against *H. pylori* infection in children [19,21,22,43,46]. Results from our present study showed that children breastfed longer than 12 months were likely to be more infected by *H. pylori* than those breastfed for a shorter duration [OR (95% CI): 1.64 (1.09-2.71)]. This fact had also been observed in another community-based study carried out in a mountainous area in northern border with Kinh and five others minority ethnics [22]. However, the results from one hospital-based study [19] and another community-based one [21], both in Kinh people, showed that breastfeeding longer than 6 months was protective factor for *H. pylori* infection in children; and the longer children breastfed, the lower rates of the infection they got. Results from our studies in Vetnam suggested that long breastfeeding may be beneficial in Kinh children whose mothers benefited from better socioeconomic conditions, but it becomes prejudicial or harmful for children of minority ethnics whose mothers and themselves lived in disadvantageous socioeconomic conditions. It is easy to understand the lack of protective effect of breastfeeding shorter than six months of age on *H. pylori* infection in children. However, it seems not simple at all to know why breastfeeding longer than 12 months of age lost its protective effect in children. Lack of hygiene in mothers while preparing bottle and feeding their children as reported by Kitagawa et al. in Japan (*J Obst Gynaecol Res* 2001;27:225-30) or while breastfeeding their children as suggested by Ndip et al. in Cameroon (*Trop Med Int Health*2004; 9:1036-40) was not excluded. In our opinion, nevertheless, prolonged contact to mothers who were infected by *H. pylori* in vast majority of cases in developing countries might be more intrigued or implicated. Prolonged intimate contact with their children through breastfeeding inevitably facilitated transmission of the germ from infected mother to baby.

Before *H. pylori* era, several epidemiological studies had found that nonsecretors of ABO blood group antigens and individuals of blood group O were overrepresented among patients with peptic ulcer (Clark et al.; Borén et al.). This fact encouraged so many researchers to investigate the relationship between ABO blood groups and their secretor status with peptic ulcer. Many authors had reported an association between blood group O and *H. pylori* infection [47-49], while others failed to find such an association [50-52]. For the first time in Vietnam, we explored the relationship between ABO blood groups and *H. pylori* infection in the present study. Our results showed that individuals with blood group B were significantly less prone to *H. pylori* infection than others [OR (95% CI): 0.45 (0.31-0.72)]. This finding was compatible with results reported recently by Jaff et al. (2011) in Iraq in which authors found that *H. pylori* seropositive rate was significantly higher in individuals with blood group O ($p=0.01$) and significantly lower in those with blood group B

(*p=0.007*) when compared with that in general population, and also when compared with that in seronegative individuals with *p=0.0397* and *p=0.0495*, respectively [48]. Nevertheless, Kanbay et al. (2005) reported that individuals with blood groups A or O were more prone to *H. pylori* infection and those with blood group AB was less prone to this infection [47]. More recently in Japan, Inoue et al. (2014) found that among the group of people taking yogurt or beverage containing lactic acid bacteria more than 5 times a week, individuals with blood group B less prone to *H. pylori* infection than those taking that stuff less than 3 times a week; and no difference in *H. pylori* infection between those with blood group O and those with other than O group (i.e. A,B and AB groups). Among individuals without taking that stuff, the tendency of being less prone to *H. pylori* infection disappeared and individuals with blood group O were more prone to *H. pylori* infection than those with other than group O [OR (95% CI): 2.59 (1.12-6.22)] [53]. Results observed by Inoue et al. strongly suggested that differences in intake of dairy products affected the relationship between *H. pylori* infection and the ABO blood group system. In a general meaning, that implied the important role of different daily intake that could change the interaction between the bacteria and host reaction. This topic seems very attractive and worthy to invest more investigation.

In this cross-sectional community based-study, we recruited a large population (1,596 individuals) who were all members of all generations living together in the same household. This method of sampling facilitated the data analysis and interpretation, provided a more comprehensive understanding about the interaction or interrelation between studied variables and *H. pylori* infection. Therefore, it rendered more feasible and more reliable for risk factors to be identified among studied socio-demographic and potential exposure variables. One of the weaknesses in our study was absence during investigating period of an important number of fathers (118/485 households) who left home for towns and cities to earn extra-income serving their poor family. That partly affected results of data analyses on *H. pylori* infection in fathers. Other weaknesses in our study are issued from the limitation of structured questionnaire reluctantly adopted according to human and financial conditions of the study. The first limitation resides in recall biases inevitably committed by respondents during interview. One more limitation is ambiguity and difficulty to calculate exact income per capita per month given diversity in homemade and self-serving products, very popular in this population living in remote area of Mekong delta. Another point of limitation issued from limited knowledge of participants in recognizing history of gastro duodenal disorders and loss of health booklet or medical prescription in many cases, sources of inaccuracy in deciding whether someone had suffered from digestive disease in relation to *H. pylori* infection.

As evidences from scientific data become only useful when results from academic studies are served as pivotal points for transforming community health through health care strategy of local health authorities by constructing appropriate models of health care management as developed for the first time by Wagner et al. (1998), generally conceptualized and recommended by WHO (2002) [54] and sucessfully implemented by Ciccone et al. (2010) [55]. Vietnam has been well known since 1970s for the highly effective and sustainable community (village) health system [56], that enables us to transfer evidences from scientific studies to improve national population health in general and better care for inhabitants in study sites in particular. In fact, results from our study not only served as baseline for building national strategy of public health policy, but also had been handovered to local health authority who informed the health care-givers team (composed by general practioners, trained general nurses and village health workers) working in the field to transfer scientific evidences into daily practice to improve the quality of care and to back up the health education activities.

Conclusion

This first community-based study of epidemiology of *H. pylori* infection in a population of Kinh and Khmer settled together in Mekong delta showed low and indifferent rates of seropositivity of *H. pylori* infection. Moreover, results of our study strongly suggest that environmental, personal hygiene and some life style practices should be improved to lower the prevalence of *H. pylori* infection in this population.

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