Original article

Seroprevalence and serovar distribution of Leptospirosis among healthy people in Vietnam: Results from a multi-center study

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ABSTRACTS

Objective: This is the first study was conducted to identify the Leptospirosis seroprevalence and serovar distribution among healthy people in different regions in Vietnam.

Methods: This cross-sectional study was conducted with 600 asymptomatic people in three provinces, representing for the North, the northern Central, and the South of Vietnam from November to December 2019. Each participant was collected a 3 ml venous blood sample by a certified phlebotomist. The microscopic agglutination test (MAT) was applied to determine the leptospiral seroprevalence and serogroups with a cutoff titre for seropositivity of $\geq 1:100$.

Results: The overall seroprevalence of leptospiral antibody among healthy Vietnamese people was 9.5%. In total eleven serovars of *Leptospira* were isolated. The most frequent serovars identified were strains of *Hebdomadis* (14.0%), *Pomona* (14.0%), *Saxkoebing* (12.3%), and *Panama* (8.8%). Among seropositive cases, farmer was majority, accounting for 63.2%, other jobs accounted from 3 to 8%.

Conclusions: Overall seropositivity Leptospirosis among healthy people was relatively high in Vietnam. Diverse serovars of *Leptospira* have been confirmed to circulate in different regions in the country. Further research need to be conducted to determine factors for high seroprevalence in the population of Vietnam. In addition, health education programs should be considered to prevent leptospirosis among Vietnamese people, especially for farmers, in the future.

1. Introduction

Human Leptospirosis is a zoonotic disease caused by a variety of leptospiral serotypes. It is a neglected disease, occurring worldwide especially in the tropical and subtropical areas with heavy rainfall, and affecting vulnerable populations in rural settings and semi-urban slum.¹ A paper published recently found that 318 Leptospirosis outbreaks were reported during 1970–2012. The number of outbreak increased over the study period in both tropical and non-tropical regions.² A systematic of the global morbidity and mortality of human leptospirosis estimated that annually 1.03 million cases (95% CI 434,000–1,750,000) and 58,900 deaths (95% CI 23,800–95,900) due to leptospirosis globally.³ Southeast Asian countries, including Vietnam, is one of the regions with highest estimates of disease morbidity and mortality.³ In terms of

Disability Adjusted Life Years (DALY), the research of global burden of leptospirosis suggested approximately 290 million DALYs are lost per year.⁴ The actual number of leptospirosis cases might be higher than reported. The underreporting is often because of the infection being clinically unapparent, misdiagnosed as dengue fever or other endemic diseases.¹ Moreover, lacking referenced laboratory to perform confirmatory tests of *Leptospira* may also contribute to the underestimation.⁵

Leptospirosis is highly prevalent in the Asia Pacific region, especially in developing countries.⁶ Research on seroprevalence of Leptospirosis among healthy people have been conducted in several countries, especially in the Asian Pacific Region. Studies in Malaysia reported high prevalence of leptospiral antibodies among healthy municipal service workers (34.8%),⁷ market workers and food handlers (46.3%),⁸ or in wet market workers in this country.⁹ Similarly, human leptospirosis is a

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public health problem,⁵ and remains the important cause for hospitalization in Indonesia,¹⁰ India.¹¹ Large outbreaks of Leptospirosis after severe floods were reported in other countries in the region, including Fiji,¹² and New Caledonia.¹³

In Vietnam, knowledge on human Leptospirosis is poorly understood.¹⁴ Due to the limitation of laboratory capacity for confirmation of acute leptospiral infection, data on the disease is still limited. It is, therefore, this disease has been considering as a neglected disease in Vietnam.¹⁵ A study conducted in 1998 the Mekong delta reported that 18.8% people aged 15–60 years were leptospiral seropositive using the microagglutination test (MAT), suggesting the important of the circulation of *Leptospira* among the population living in the South of Vietnam.¹⁴ The study conducted by Thai KTD. et al. (2006) also emphasized the important of Leptospirosis among children in Vietnam.¹⁶ In this study, 12.8%, and 5.4% children aged 7–14 years were positive with *Leptospira* IgG, and *Leptospira* IgM, respectively.¹⁶ Little know about the diversity of serogroups of *Leptospira* in Vietnam. Two studied conducted in Vietnam identified different serogroups, including *Australis*, *Bataviae*, *Canicola*, *Icterohaemorrhagiae*, *Javanica*, *Pykogenes*, *Tamssovi*, *Cynopteri*, *Louisiana*, *Panama*, *Sejroe*, *Hursbridgem*, and *Semarang*.^{14,15}

This is the first research was conducted in different locations, representing for the North, the northern Central, and the South in Vietnam, aiming to assess the seroprevalence and serovar distribution of human Leptospirosis among healthy people in the country.

2. Methods

2.1. Study locations and participants

This cross-sectional study was conducted from October 2019 to December 2019 involving 600 healthy people aged ≥ 5 years living in three provinces (200 adults each province), including Thai Binh, Ha Tinh, and Can Tho province, representing for the North, northern Center, and the South of Vietnam, respectively (Fig. 1). They were systematic randomly selected from the list of habitants living at least 6 months in the communities.

Non-Vietnamese citizen and people who were not available during the study period were excluded from the study. Replacements were made by randomly selected from the remaining list.

2.2. Blood sample and data collection

A 3 ml venous blood sample was collected from each participant by certified phlebotomists by standard procedure.¹⁷ The sample then was coagulated by centrifuging at 4500 rpm for 5 min. The resulting serum was stored in 2 ml Eppendorf tubes, then transported in a cool box to the laboratory and stored at -30°C until be tested.

General information, including age, gender, ethnicity, educational level, and occupation of the participants were also be gathered by trained interviewers.

2.3. Laboratory procedures

A microscopic agglutination test (MAT) was carried out at the National Institute of Hygiene and Epidemiology, Hanoi followed standard methods.¹⁸ The antigen panel included the following 26 serovars reference strains (Table 1). The strains are generally diluted 1:2 using PBS (Phosphate Buffer Saline) to obtain a density of 1.2×10^8 *Leptospira*/ml. The cell density of each strain is individually verified. Various dilutions (1:100, 1:200 and 1:400) of the sample of serum which serve as positive controls were mixed with live *Leptospira* to detect agglutinating antibodies. The cutoff point was dilutions $\geq 1:100$ and the predominant serovar was that with the highest dilution. This cut off point had also been used in recent seroprevalence studies.^{7,9}

Fig. 1. Study locations of the study.

3. Data analysis

Descriptive analysis was applied in this study. Numerical variables were presented as means and standard deviations (SD) whereas categorical data were presented as frequencies and percentages. The seroprevalence was calculated by dividing number of positive MAT over total samples and presented as number and percentage for each provinces, and total 3 provinces. Data were analyzed using the IBM SPSS package version 22.0.

4. Ethical issue

All the participants involved voluntarily signed the informed consent form after they were given a detailed explanation about the procedure and adequate time to make a decision. The research protocol was approval by the Ethic Board in Human Research at the National Institute of Hygiene and Epidemiology, Hanoi, Vietnam.

5. Results

Table 2 showed the sociodemographic characteristics of the studied participants. The mean age was 42 years, with the majority of the participants was female (60.5%). The educational level of the participants was mainly completed secondary or high school (60.7%). More than one-third of the study participants was farmer (39.0%).

Fig. 2 demonstrated the seroprevalence of *Leptospira* among the studied participants. Overall the positive seroprevalence of *Leptospira* was 9.5%. Ha Tinh province had the highest positive seroprevalence (14.0%) compared to Thai Binh province (9.0%) and Can Tho City, only 5.5%.

Table 3 demonstrated the serovar distribution of leptospirosis among positive cases. Eleven serovars were found. The most prevalent being

Table 1List of *Leptospira* reference strains.

No.	Species	Serogroup	Serovar	Strain
1	<i>L. interrogans</i>	AUSTRALIS	australis	Ballico
2	<i>L. interrogans</i>	AUSTRALIS	Bratislava	Jez-Bratislava
3	<i>L. interrogans</i>	CYNOPTERI	cynopteri	3522 C
4	<i>L. interrogans</i>	CANICOLA	canicola	Hond Utrecht IV
5	<i>L. interrogans</i>	HEBDOMADIS	hebdomadis	Hebdomadis
6	<i>L. interrogans</i>	AUTUMNALIS	autumnalis	Akiyami A
7	<i>L. interrogans</i>	BATAVIAE	bataviae	Van Tienen
8	<i>L. interrogans</i>	ICTEROHAEMORRHAGIAE	copenhageni	Wijnberg
9	<i>L. borgpetersenii</i>	BALLUM	castellonis	Castellon 3
10	<i>L. interrogans</i>	PYROGENES	pyrogenes	Salinem
11	<i>L. weilii</i>	TARASSOVI	vughia	LT 89-68
12	<i>L. biflexa</i>	SEMARANGA	Patoc	Patoc I
13	<i>L. borgpetersenii</i>	TARASSOVI	tarassovi	Mitis Johnson
14	<i>L. noguchii</i>	PANAMA	panama	CZ 214K
15	<i>L. noguchii</i>	LOUISIANA	louisiana	LSU 1945
16	<i>L. borgpetersenii</i>	JAVANICA	javanica	Veldrat Bataviae 46
17	<i>L. interrogans</i>	POMONA	Pomona	Pomona
18	<i>L. interrogans</i>	ICTEROHAEMORRHAGIAE	Icterohaemorrhagiae	Verdun
19	<i>L. kirschneri</i>	GRYPOTYPHOSA	gryppotyphosa	Moskva V
20	<i>L. borgpetersenii</i>	sejroë	hardjobovis	Sponselee
21	<i>L. interrogans</i>	sejroë	Wolffi	3705
22	<i>L. interrogans</i>	sejroë	Saxkoebing	Mus 24
23	<i>L. weilii</i>	CELLEDONI	Celledoni	Celledoni
24	<i>L. interrogans</i>	DJASIMAN	Djasiman	Djasiman
25	<i>L. borgpetersenii</i>	MINI	Mini	Sari
26	<i>L. Santarosai</i>	SHERMANI	Shermani	1342K

Table 2

Characteristics of the participants (n = 600).

Variables	Thai Binh	Ha Tinh	Can Tho	Overall
Age	40.01 ± 20.47 (5–81)	42.83 ± 19.67 (7–86)	43.05 ± 21.11 (6–89)	41.95 ± 20.44 (5–89)
Gender				
Male	95 (47.5%)	71 (35.5%)	71 (35.5%)	237 (39.5%)
Female	105 (52.5%)	129 (64.5%)	129 (64.5%)	363 (60.5%)
Ethnicity				
Kinh	200 (100%)	199 (99.5%)	193 (96.5%)	592 (98.7%)
Others	0	1 (0.5%)	7 (3.5%)	8 (1.3%)
Educational level				
≤Primary school	43 (21.5%)	28 (14.0%)	105 (52.5%)	176 (29.3%)
Secondary/High school	140 (70.0%)	147 (73.5%)	77 (38.5%)	364 (60.7%)
Colleges/University	11 (5.5%)	15 (7.5%)	8 (4.0%)	34 (5.7%)
University/Postgraduate	6 (3.0%)	10 (5.0%)	10 (5.0%)	26 (4.3%)
Occupation				
Farmer	83 (41.5%)	110 (55.0%)	41 (20.5%)	234 (39.0%)
Worker	24 (12.0%)	9 (4.5%)	11 (5.5%)	44 (7.3%)
Business	7 (3.5%)	9 (4.5%)	21 (10.5%)	37 (6.2%)
Healthcare staff	5 (2.5%)	13 (6.5%)	10 (5.0%)	28 (4.7%)
Officer/Students	48 (24.0%)	37 (18.5%)	43 (21.5%)	128 (21.3%)
Others	33 (16.5%)	22 (11.0%)	74 (37.0%)	129 (21.5%)

Hebdomadis (14%), and *Pomona* (14.0%), followed by *Saxkoebing* (12.3%), and *Panama* (8.8%). These four serovars accounted for 50% of the seropositive cases. In addition, 28.1% positive cases were not determined their serovars.

Table 4 reported the sociodemographic characteristic of *Leptospira* seropositivity among positive cases. Majority of seropositive cases was females, accounting for 63.2%. In terms of occupation, 63.2% the seropositive cases was farmer, followed by healthcare staff (8.8%).

6. Discussion

To the best of our knowledge, this is the first study was conducted to assess the seroprevalence of Leptospirosis in different regions in Vietnam. The results found that almost ten percent of the people in the

community were seropositive with *Leptospira*, ranging from 5.5% in Can Tho province to 14.0% in Ha Tinh province.

Results of the current study support the previous studies that Leptospirosis is common in Vietnam. Study conducted by Van et al. (1998) reported that 18.8% people aged 15–60 years in the Mekong delta were seropositive using MAT, with agglutination titres $\geq 1/100$.¹⁴ Similarly, high rates of *Leptospira* seropositivity in the general children was also reported previously in Vietnam increasing from 11% in 7 years–25% in 12 year children.¹⁹ However, the present study showed a lower seroprevalence of *Leptospira* among the Vietnamese people compared to studies in other Southeast Asian countries. Several studies conducted in Malaysia showed high seroprevalence of leptospiral antibodies among high-risk planters (28.6%),²⁰ or 33.6% among wet market workers.⁹ The lower positivity in the seroprevalence of leptospiral antibodies in the present study may be related to the studied group. This study randomly selected healthy people aged more than 4 years, and not high risk groups. It is, therefore, the seroprevalence could be lower than previous studies with high risk group.

Regarding frequency of serovars, 57 out of 600 participants had antibody against one *Leptospira* serovar with antibody titre was $1 \geq 100$. A high degree of diversity of serogroups was found in our study, including 11 serovars. The most common serovar detected was *Hebdomadis* (8 people), *Pomona* (8 people), and *Saxkoebing* (7 people). Findings of this study confirm previous studies regarding the circulation of *Leptospira* serovars in Vietnam in which 8 serovars, including *Canicola*, *Bataviae*, *Pyrogenes*, *Tarassovi*, *Panama*, *Louisiana*, *Pomona*, *Gryppotyphosa* were identified.^{14,15} Those serovars were identified in previous studies in the Asian countries, including Thailand,²¹ and Idian.²² Moreover, this study isolated 3 additional serovars, including *Bratislava*, *Hebdomadis*, and *Saxkoebing*. Two of them (*Hebdomadis*, and *Saxkoebing*) were the most common serovars identified among healthy people, accounting for 26.3% of the seropositivities. *Leptospira* diagnosis tests require complicated technique, and costly,²³ only some central laboratories in Vietnam can perform the confirmed test.²⁴ Therefore, most of studies on human *Leptospira* in Vietnam focused only on *Leptospira* seroprevalence using Enzyme-linked Immunosorbent assay (ELISA) test, just few studies identified serovars circulated among human. The current study provided important findings for human Leptospirosis in the country.

Fig. 2. Prevalence of leptospiral antibodies among healthy adults in Thai Binh, Ha Tinh and Can Tho provinces.

Table 3

Serovar distribution among positive MAT results on all serovars (n = 57).

Serovars	Thai Binh n (%)	Ha Tinh n (%)	Can Tho n (%)	Overall n (%)
Bratislava	0	0	1 (9.09%)	1 (1.7%)
Canicola	3 (16.67%)	0	0	3 (5.3%)
Hebdomadis	3 (16.67%)	3 (10.71%)	2 (18.18%)	8 (14.0%)
Bataviae	0	0	1 (9.09%)	1 (1.7%)
Pyrogenes	1 (5.55%)	1 (3.57%)	0	2 (3.5%)
Tarassovi	2 (11.11)	1 (3.57%)	0	3 (5.3%)
Panama	2 (11.11)	2 (7.15%)	1 (9.09%)	5 (8.8%)
Louisiana	0	2 (7.15%)	0	2 (3.5%)
Pomona	2 (11.11)	1 (3.57%)	5 (45.46%)	8 (14.0%)
Gryppotyphosa	0	1 (3.57%)	0	1 (1.7%)
Saxkoebing	0	7 (25.0%)	0	7 (12.3%)
Co-agglutinins (undetermined)	5 (27.78%)	10 (35.71%)	1 (9.09%)	16 (28.1%)

Table 4

Seropositive cases of Leptospirosis according to gender, and occupations (n = 57).

Variables	n (%)
Gender	
Male	21 (36.8%)
Female	36 (63.2%)
Occupations	
Farmer	36 (63.2%)
Worker	2 (3.5%)
Business	3 (5.3%)
Healthcare staff	5 (8.8%)
Officer/Students	4 (7.0%)
Others	7 (12.3%)

The majority of seropositive people was farmer indicating that this group is the occupational risk group for Leptospirosis infection. This result supports the previous studies performed in Central India,¹¹ and other studies in the Asian Pacific regions.⁵ Farmer working on the field could expose possibly Leptospirosis-contaminated environment that increase the risk of the infection.⁵ Leptospirosis has been documented to associate with occupations and activities that increase interactions between humans, animals and contaminated environments.²⁵ Working conditions of the farmer in Vietnam, mainly in the rice field or fruit farm with wet environment is suitable for survival of *Leptospira* where they can survive for several weeks in stagnant water and wet soil.²⁶

Strength of the current study is applying a standard method for *Leptospira* diagnosis to identify seroprevalence and serovar distribution of *Leptospira* among the general population. The limitation is the study was conducted only in 3 provinces representing for three regions in Vietnam, it not covers all regions which may have different in Leptospirosis epidemiology. Further study need to be performed in all regions representing for the whole country to provide more information on epidemiological characteristics of Leptospirosis in Vietnam.

7. Conclusion

The findings in the current study reported a relatively high rate of seropositivity of *Leptospira* among general population in Vietnam. Diversity of *Leptospira* serovars (11 serovars) has been confirmed in this study. There were four predominant serovars circulated among the general population in the country, including *Hebdomadis*, *Pomona*, *Saxkoebing*, and *Panama*. Majority of the seropositive people was farmer. Health promotion program and trainings in using personal protective equipment should be initiated among farmers to reduce risk of the infection.

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Declaration of competing interest

No conflict of interest is declared.

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