

SOME RELATED FACTORS TO LEPTOSPIRA INFECTION AMONG RESIDENTS IN THAI BINH, HA TINH AND CAN THO PROVINCES, 2019

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SUMMARY

Objectives: To determine factors related to *Leptospira* seropositivity among residents in Vietnam. **Subjects and methods:** This is a cross-sectional study involving 600 residents in Thai Binh, Ha Tinh provinces and Can Tho city, conducted from September 2019 to January 2020. Information on demography, occupational exposures, environmental factors and behaviors was obtained by a set of questionnaire. Microscopic agglutination test (MAT) was used to define the seropositivity to *Leptospira*.

Results: The mean age of the participants was 42.0 (SD 20.4). Subjects were mainly female, accounting for 60.5% and almost all of them were the Kinh (98.7%). There were 3 factors related to *Leptospira* infection, including occupation (farmer OR = 5.6; 95%CI: 2.0 - 16.2, health staff OR = 6.2; 95%CI: 1.7 - 27.0); involving contact with wastes of domestic animals (OR = 1.9; 95%CI: 1.1 - 3.3); contact with sewers, garbage (OR = 2.1; 95%CI: 1.2 - 3.6). There was no relationship of age, gender, educational level, status of household, source of water. **Conclusion:** The *Leptospira* infection was related to occupation, contact with wastes of domestic animals and sewers, garbage.

* **Keywords:** *Leptospira*; Related factors; Microscopic agglutination test (MAT).

INTRODUCTION

Leptospirosis is an acute infectious disease of zoonosis and a major public health issue worldwide. It is considered to be an important re-emerging disease, especially in tropical and sub-tropical countries. It is estimated that there are

1.03 million cases of leptospirosis worldwide annually, with an estimated death toll of about 58,900 [2]. Vietnam is known to be an endemic country for human leptospirosis. The morbidity caused by Leptospirosis has tended to increase in recent years in the country.

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In Vietnam, Leptospirosis is classified into B group, the dangerous infectious diseases that can spread quickly and possibly cause death, according to the law on prevention and control of infectious diseases. According to Victoriano et al (2009), Vietnam is one of the countries with high annual rates of the disease [4]. However, due to the inconsistency of the monitoring systems in localities, the incomplete data and the inadequate information on related factors of leptospirosis. Based on the links in the chain of leptospirosis, these factors can be divided into three groups: human factors, environmental factors, and animal factors.

The first study on *Leptospira* infection in Vietnamese was carried out from 1993 to 1997 by Laras et al in several hospitals in Hanoi, Ho Chi Minh, and An Giang [5]. Since then, there have been more studies on *Leptospira* infection, but most of them have not been conducted to identify related factors of the disease. Understanding these factors will be the basis for making policies and measures to prevent disease in the community. We conducted this study aiming: *To determine factors related to Leptospira infection among residents in Thai Binh, Ha Tinh, and Can Tho provinces in Vietnam.*

SUBJECTS AND METHODS

1. Location, time and subjects of study

* *Subjects:* Residents in Thai Binh, Ha Tinh provinces, and Can Tho City.

- Selection criteria: (1) residents aged ≥ 5 years; (2) living in the study areas for at least 6 months; (3) agree to participate in this study.

- Exclusion criteria: (1) not agree to participate in the study; (2) have immunodeficiency diseases.

* *Location and time of study:*

This study was carried out from September 2019 to January 2020 in Thai Binh, Ha Tinh provinces and Can Tho City. These provinces are characterized by the geography and climate of Northern - Central - Southern regions of Vietnam where their socio-economic conditions, husbandry-farming practices are different.

2. Methods

* *Study design:*

A cross-sectional study.

* *Sampling method and sample size:*

- Sample size: Its formula based on the estimated percentage of people with the seropositivity to *Leptospira* in the community.

$$n = Z_{1-\alpha/2}^2 \frac{p \cdot (1 - p)}{d^2}$$

n: Minimum number of subjects recruited into the study.

$Z_{1-\alpha/2}$: reliability coefficient. In this study, we chose $\alpha = 0.05$, so $Z_{0.975} = 1.96$.

P: Estimated percentage of residents with *Leptospira* seropositivity. We used $p = 0.188$ according to Cao Thi Bao Van's study [1].

d: Absolute error, we chose $d = 0.035$.

Based on this formula, the minimum of sample size was 479 people. This study was carried out in 3 provinces/city. We recruited 200 people from each province and the total number recruited was 600 people.

- Sampling method: A multi-stage one was used. In each province, 02 districts were selected randomly for the study, of which one commune or ward was chosen randomly in each district. In each commune of Thai Binh and Ha Tinh provinces, take 2 villages at random and in each ward of Can Tho city, select randomly 01 village/hamlet. Make a list of people living in the selected villages. From that list, randomly selected 50 people each village (in Thai Binh and Ha Tinh provinces) and 100 people per village (Can Tho city) to enter the study.

3. Procedure of study and data collection

- Procedure of data collection:

+ Residents met the full criteria of the study.

+ Directly interviewed and recorded information in the questionnaire.

+ Taken blood and tested to confirm the seropositivity to *Leptospira*.

* *The techniques applied in study:*

- Information extraction: Based on the designed questionnaire, its content included:

+ Demography: Full name, age, gender, ethnicity, educational level, occupation.

+ Conditions of household: Number of households, houses close to pond or river, water source, waste collection, households in flood-prone area...

+ Risk factors of infection: Wading in rivers, ponds, lakes or fields; having contact with wastes of domestic animals; having contact with sewers, garbage.

+ Preventive measures: Use of personal protective equipment at work; having a shower after working in the fields, gardens...

- Test to confirm the presence of antibody to *Leptospira* in serum by microscopic agglutination test (MAT): MAT method is still considered the gold standard in the diagnosis of leptospirosis today. A positive MAT indicates that there is high concentration of leptospiral antibodies in human's serum, meaning that the patient infected or has been infected recently.

* *Principle:*

Based on a specific combination of antibodies to *Leptospira* in serum and live *Leptospira* antigens. Observe the mobility of spirochetes to assess whether agglutination occurs or not.

* *Procedure:*

Blood samples were collected and then serum was separated from them. Serum samples were stored at 2 - 8°C temperatures.

The set of 26 *Leptospira* reference strains was used in the study for MAT method. They were cultured in EMJH medium (Ellinghausen - McCullough - Jonson - Harris) and kept at 28°C in 5 - 7 days until the bacterial concentration reached 1 - 2 × 10⁸ bacteria/mL. Serum samples were diluted at concentrations of 1:25. Then they were put in 96 wells plate (Nunc - Germany) with live *Leptospira* bacteria, incubated at 37°C for 2 hours. Agglutination reaction was examined under a dark-field microscopy at 40X power magnification.

The results of tests were evaluated by observing the cell-free *leptospira* bacteria in the field. If no mobile free leptospira were observed on the field, it meant complete agglutination. However, in order

to facilitate the assessment process, the comparison with the negative control was as follows: The rate of free cell *Leptospira* bacteria was between 50 - 100%, the result was negative; if this percentage was less than 50%, the result was positive. Determine the positive values of MAT method in the following cases: i) stage of acute infection: when titre reaches ≥ 400 ; ii) or fourfold increase between paired sera in antibody titer (between acute and convalescent serum samples); iii) or infected with titer ≥ 100 .

In this study, serum samples with a titre ≥ 100 were considered positive.

4. Statistical analysis

The data were entered and analysed by using SPSS version 22. All continuous variables were described using $\bar{X} \pm SD$, whereas frequencies and percentages were used for categorical variables. Determine the association between factors and outcome variable using univariate and multivariate logistic regression models.

RESULTS

Table 1: Characteristics of the study participants.

Characteristics		n	%
Age group (years)	≤ 18	118	19.7
	19 - 60	369	61.5
	> 60	113	18.8
	$\bar{X} \pm SD$	42.0 \pm 20.4	
Gender	Male	237	39.5
	Female	363	60.5
Ethnicity	Kinh	592	98.7
	Others	8	1.3
Educational level	\leq Primary school	176	29.3
	Secondary/high school	364	60.7
	Colleges/university	34	5.7
	University/postgraduate	26	4.3
Occupation	Farmer	234	39.0
	Worker	44	7.3
	Business	37	6.2
	Healthcare staff	28	4.7
	Officers/students	128	21.3
	Others	129	21.5

Number of family members		4.0 (1.6)	
Family's economic status	Rich	58	9.7
	Middle	499	83.2
	Poor/near-poor	43	7.1
Appearance of fever in the past 2 weeks	Yes	58	9.7
	No	542	90.3

The mean age of residents was 42.0 (SD 20.4). They were mainly female (60.5%) and the vast majority were Kinh ethnicity (98.7%).

The majority of subjects had an educational level at secondary or high school (60.7%). Farmers were the most popular occupation (39.0%).

*Table 2: Factors related to *Leptospira* seropositivity.*

Factors		Seropositive	Seronegative	Crude OR (95%CI)	p-value
Age		45.7 (17.8) ^a	41.6 (20.7) ^a	-0.9 - 9.2 ^b	0.108
Age group	18 - 60	39 (68.4)	330 (60.8)	1.4 (0.8; 2.5)	0.261
	Others ^c	18 (31.6)	213 (39.2)		
Gender	Male	21 (36.8)	216 (39.8)	0.9 (0.5; 1.6)	0.666
	Female ^c	36 (63.2)	327 (60.2)		
Ethnicity	Kinh	57 (100.0)	535 (98.5)		> 0.999
	Others ^c	0	8 (1.5)		
Educational level	≤ Primary school	13 (22.8)	163 (30.0)	2.0 (0.3; 15.9)	0.515
	Secondary/high school	39 (68.4)	325 (59.9)	3.0 (0.4; 22.8)	0.288
	Colleges/university	4 (7.0)	30 (5.5)	3.3 (0.4; 31.8)	0.295
	University/postgraduate ^c	1 (1.8)	25 (4.6)		
Occupation	Farmer	36 (63.2)	198 (36.5)	5.6 (2.0; 16.2)	0.001
	Worker	2 (3.5)	42 (7.7)	1.5 (0.3; 8.4)	0.660
	Business	3 (5.3)	34 (6.3)	2.7 (0.6; 12.8)	0.202
	Healthcare staff	5 (8.8)	23 (4.2)	6.7 (1.7; 27.0)	0.007
	Officers/students ^c	4 (7.0)	124 (22.8)		
	Others	7 (12.3)	122 (22.5)	1.8 (0.5; 6.2)	0.368

Number of family members	> 3	38 (66.7)	355 (65.4)	1.1 (0.6; 1.9)	0.846
	≤ 3 ^c	19 (33.3)	188 (34.6)		
Households close to pond or river	Yes	34 (59.6)	387 (71.3)	0.6 (0.3; 1.0)	0.068
	No	23 (40.4)	156 (28.7)		
Water source using	Rain or tap water	45 (78.9)	453 (83.4)	1.3 (0.7; 2.6)	0.393
	Water in wells, ponds or lakes ^c	12 (21.1)	90 (16.6)		
Waste collection	Yes	46 (80.7)	449 (82.7)	1.1 (0.6; 2.3)	0.707
	No	11 (19.3)	94 (17.3)		
Wading in rivers, ponds, lakes, fields	Yes	27 (47.4)	208 (38.3)	1.5 (0.8; 2.5)	0.184
	No	30 (52.6)	335 (61.7)		
Having contact with wastes of domestic animals	Yes	34 (59.6)	240 (44.2)	1.9 (1.1; 3.3)	0.028
	No	23 (40.4)	303 (55.8)		
Having contact with sewers, garbage	Yes	31 (54.4)	198 (36.5)	2.1 (1.2; 3.6)	0.009
	No	26 (45.6)	345 (63.8)		
Barefoot	Yes	25 (43.9)	199 (36.6)	1.4 (0.8; 2.3)	0.286
	No	32 (56.1)	344 (63.4)		
Use of personal protective equipment at work	Yes	29 (50.9)	236 (43.5)	1.3 (0.8; 2.3)	0.285
	No	28 (49.1)	307 (56.5)		
Having a shower after working in the fields or gardens	Yes	40 (70.2)	308 (56.7)	0.6 (0.3; 1.0)	0.053
	No	17 (29.8)	235 (43.3)		
Households in the flood-prone area	Yes	8 (14.0)	84 (15.5)	0.9 (0.4; 2.0)	0.775
	No	49 (86.0)	459 (84.5)		

^a Mean (SD), ^b mean difference (95%CI), ^c reference group, OR: Odds ratio, CI: confident interval

Through a univariate analysis, the results showed that occupation was a related factor for *Leptospirosis* infection. Our results indicated that farmers and health staffs had a higher risk. Regarding behavior-related factors of infection, we also found that people who sometimes or regularly have contact with livestock waste or sewers and garbage also increases the risk of the infection. The results of univariate logistic regression analysis of that factors were statistically significant with $p < 0.05$.

Table 3: Multivariate analysis for factors associated with *Leptospira* seropositivity.

Related factors		Adjusted OR	95%CI	p-value
Age		0.99	0.97 - 1.01	0.189
Occupation	Farmer	8.78	2.15 - 35.9	0.003
	Worker	1.99	0.32 - 12.42	0.460
	Business	3.62	0.68 - 19.20	0.130
	Healthcare staff	6.05	1.23 - 29.71	0.027
	Officer/student ^c			
	Others	2.87	0.67 - 12.34	0.157
Households close to pond or river		0.67	0.37 - 1.20	0.177
Wading in rivers, ponds, lakes, fields		0.91	0.48 - 1.75	0.781
Having contact with wastes of domestic animals		1.02	0.48 - 2.16	0.957
Having contact with sewers, garbage		1.45	0.72 - 2.91	0.300
Having a shower after working in the fields, gardens.		0.94	0.44 - 1.99	0.862

^c reference group

The multivariate logistic regression models reveals that only occupation was found to be related to the seropositivity to *Leptospira*. Farmer and healthcare staff are subjects at higher risk.

DISCUSSION

This is the first study conducted to identify the related factors of leptospirosis in different regions in Vietnam. A total of 600 residents participated in the study. The results found that farmers and health staffs have a higher risk of leptospirosis. There are also two risk factors that are related to *Leptospira* infection, including having contact with wastes of domestic animals and sewers, garbage.

Leptospirosis is considered to be a primarily occupational disease. Depending on the features of the job, people are at risk of exposure to pathogenic leptospire.

High-risk occupations include farmers, miners, abattoir workers, veterinarians, sewer workers, military personnel, rangers... In this study, we evaluated the relationship between occupation and *Leptospira* seropositivity and found that compared to officers/students, those who did the farming (OR = 5.6; 95%CI: 2.0 - 16.2) and health staffs (OR = 6.7; 95%CI: 1.7 - 27.0) were more likely to have leptospirosis infection. Our results are similar to the study by Sohail et al and many other studies [6].

Residents who involve direct contact with livestock wastes or expose to sewers, garbage also have a higher risk

of leptospirosis according to our results, with OR 1.9 (95%CI: 1.1 - 3.3) and 2.1 (95%CI: 1.2 - 3.6), respectively. In leptospirosis, the main source of transmission is wildlife and livestock. *Leptospira* exist and develop in the renal tubules of these animals and eliminated through the urine, which exists in the environment (water, soil, mud...). Spirochetes enter the body through cuts and abrasions, mucous membranes or conjunctivae. In Vietnam, in both urban and rural areas, sewers and garbage are rich sources of rat waste - the main rodent and also the main source of this disease. Therefore, having contact with livestock waste; exposure to sewage, garbage will increase the likelihood of infection with disease. In the study by Sharman et al, despite not determining related factors to *Leptospira* infection, he found that people exposed to sewage had a relatively high rate of antibodies in serum (39.4%) [7]. A result of systematic review and meta-analysis also indicated that contact with livestock was a risk factor of *leptospirosis* with OR =1.95 (95%CI: 1.26 - 2.64) [3].

When analyzing all study subjects, we found no relationship between age and the likelihood of leptospirosis. Sohail et al's findings showed that age is a related factor of leptospirosis and this disease is mainly found in people of working age [6]. There is a greater risk of exposure to leptospira at this age group due to longer duration of employment and higher labor intensity. Moreover this might be due to fact that children and the elderly are given

better care when it comes to the working environment. However, in rural Vietnam, residents usually start working at younger age and stop working later compared to the working age. This may be the reason for the difference between our results and other studies.

This study could not find any association between environmental factors and anti-leptospiral antibody in human's serum. This finding was different from Fiji's [8], where significant association between living near streams and leptospirosis infection was found. Some other studies indicated that living close to pond or river, sources of water and flood-prone area are related factors to *Leptospira* infection [9,10].

The only significant determinant in the multivariate logistic regression model was the occupation of the participants. The model explained that farmers are 8.78 times (95%CI: 2.15 - 35.9) and health staffs are 6.05 times (95%CI: 1.23 - 29.71) at higher risk of contracting *leptospirosis* infection as compared with officers or students.

CONCLUSION

We determined three factors related to *Leptospira* infection, including occupation; contact with wastes of domestic animals; contact with sewers, garbage. These factors increased the likelihood of leptospirosis. This study provides data for public health staffs to evaluate current programmes of leptospirosis control in Vietnam in order to offer effective policies and health program in the future.

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