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Seroprevalence of Leptospirosis and Risk Factor Analysis in Flood-prone Rural Areas in Lao PDR

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Abstract. A cross-sectional seroprevalence study on leptospirosis, using microscopic agglutination test (MAT), was conducted in rural villages in Khammouane Province, Lao People's Democratic Republic, in December 2006. The overall prevalence of leptospiral infection among 406 subjects was 23.9% (95% confidence interval [CI] 19.7–28.0%). Independent risk factors for the infection, identified by multivariate logistic regression, were male sex (odds ratio [OR], 1.92; 95% CI: 1.24–2.98), recent flooding on one's own property (OR, 2.12; 95% CI: 1.25–3.58), and collecting wood in the forest (OR, 1.90; 95% CI: 1.17–3.09). Age, occupation, and animal ownership were not associated with seropositivity. Flooding was associated with the risk of infection particularly for women, whose behaviors or activities involving contact with floodwater were presumed to play an important role. This study showed that leptospirosis is endemic in Khammouane Province and that local flooding plays an important role in the transmission of the disease.

INTRODUCTION

Leptospirosis is a zoonosis caused by pathogenic spirochetes of the genus *Leptospira*.[1–3] Many wild and domestic animals are potential reservoirs of the bacteria, and transmission usually results from direct or indirect exposure to the urine of infected animals. People working with livestock and wild animals are at great risk of infection because of the high opportunity for direct exposure.[4] Indirect exposure (*i.e.,* contact with contaminated water and soil) has caused numerous outbreaks[5,6] and also plays a crucial role in endemic settings.[7] Clinical presentations of human leptospirosis range from asymptomatic infection to potentially fatal manifestations; however, the majority of infections are subclinical or a mild self-limiting systemic illness presenting as fever, malaise, and muscle pain.[2]

Although leptospirosis is one of the most widespread zoonoses in the world, it is more common in the tropical regions, because of the longer survival of leptospires in the environment and frequent human exposure to contaminated environments. However, because it is most prevalent in areas where diagnostic capabilities are limited, few reliable data on its incidence and prevalence in developing countries are available.[1]

There was a marked increase in the number of febrile patients at Khammouane Provincial Hospital in Khammouane Province in Lao People's Democratic Republic (Lao PDR), after severe floods had hit many parts of the province in August 2005. Screening for acute leptospirosis was carried out using locally available rapid diagnostic tests, and 81 of 327 (24.8%) turned out to be positive, suggesting that leptospirosis is quite common in Khammouane Province (technical report of Khammouane Provincial Health Office, unpublished data). Local flooding was presumed to play an important role in the transmission of the bacteria, although no epidemiologic studies were carried out to investigate risk factors of the disease.

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The objectives of this study were to estimate the prevalence of leptospiral infection among people in rural villages of Khammouane Province in Lao PDR and to identify risk factors for the infection, including local flooding and other environmental and behavioral factors.

MATERIALS AND METHODS

A random cross-sectional survey was carried out in two districts in Khammouane Province, Lao PDR, in December 2006. Khammouane Province, with a population of 340,000, is located ~250 km east-southeast of the Lao PDR's capital city, Vientiane (Figure 1). Among the nine districts in Khammouane Province, Thakhek and Nongbok Districts were selected because many cases of leptospirosis were diagnosed in those districts during the possible outbreak in 2005. The total population of the two districts is ~150,000, and most inhabitants of the districts are members of the Tai Lao group, the largest ethnic community in the country.[8] Villages in the two districts are located near streams or rivers and surrounded by irrigated or rainfed rice paddies. Rice farming is the primary occupation in Khammouane's villages, although many villagers also are engaged in other work such as vegetable and fruit gardening, livestock farming, fishing, and weaving. Houses are usually built high-floored on high wooden or concrete poles, with floor and walls of wood or bamboo. Roofing is of thatch, leaves, and recently of corrugated tinplate. Cattle and water buffalo are reared both in sheds and free range around the village. Pigs, goats, and chickens are also kept by many households, and they are usually reared free range around the houses.

A total of 406 persons ≥ 15 years of age (200 males and 206 females) were selected using a two-stage random cluster sampling technique. In the first stage, 24 villages (clusters) were selected from 213 villages in Thakhek and Nongbok districts by random sampling with probability-proportional-to-size (PPS).[9] From each selected village, 16–20 individuals, ≥ 15 years of age, were randomly selected per cluster, using a list of village inhabitants kept by the village leader. If the sampled individual was not present, an available person from the same household or in the immediate neighborhood was selected, with the same sex and closest in age.

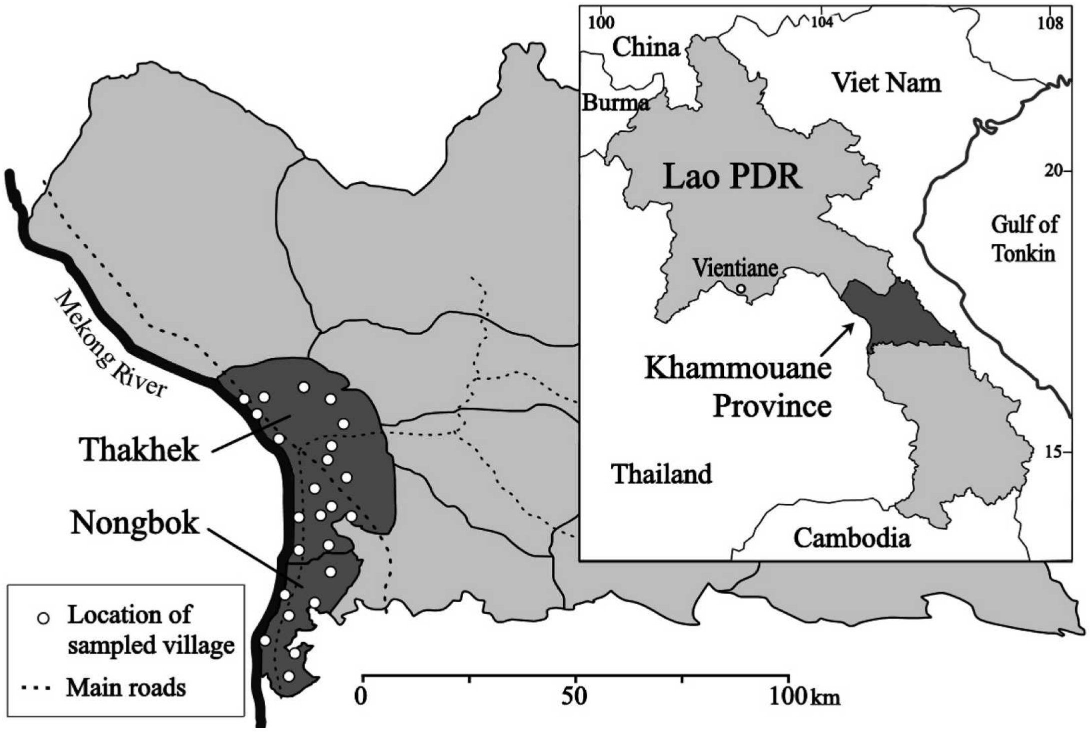

FIGURE 1. Khammouane Province, Lao People's Democratic Republic.

One individual face-to-face interview was carried out with each person selected for inclusion in the sample, using a structured questionnaire to collect information on that person's potential risk factors for leptospiral infection, such as occupation, ownership of different kinds of animals, activities associated with water and livestock, and the environmental conditions of the house and the village. After the interview, a venous blood sample was collected from each participant for determination of past leptospiral infections. A written informed consent was obtained from each participant before the interview and blood collection. Additional sessions of focus group discussion were held in two selected villages in July 2007 to obtain information regarding lifestyles and daily behaviors of the village populations. Ethical clearances for this study were obtained from the National Ethics Committee for Health Research, Ministry of Health, Lao PDR, and from the Ethics Review Committee of Nagoya University School of Medicine, Nagoya, Japan.

Frozen serum samples were sent to the laboratory of the National Institute of Infectious Diseases (NIID), Tokyo, Japan, for serologic analysis of leptospiral antibodies. The microscopic agglutination test (MAT) was performed for all serum samples, using a battery of 18 live *Leptospira* serovars from 15 serogroups, recommended by the World Health Organization.[10] A reactive antibody titer $\geq$ 1/100 was considered positive, based on previous serosurveys conducted in other developed and developing countries.[11] The serovar giving the highest titer was considered to indicate the presumptive serovar infecting the subject.

Collected data were entered in Epi Info version 6.4 (Centers for Disease Control and Prevention, Atlanta, GA). STATA release 9.2 (Stata Corp., College, TX) was used to derive descriptive statistics and in subsequent multivariate analyses. The considered risk factors were subjected to univariate analysis using Wald χ^2 and Fisher's exact tests for the whole study population and for selected strata. Multivariate analysis using a logistic regression model was performed with the laboratory results, with seropositive or seronegative as dependent variables and with age, sex, and other behavioral, socioeconomic, and environmental variables as independent variables. The model was adjusted for the cluster sampling by the svy: command in STATA program, setting village as the primary sampling unit. A hierarchical backward elimination approach was used to identify significant interaction terms and exposure variables that were strongly associated with seropositivity for leptospirosis. Variables and interaction terms with Wald $P \leq 0.05$ were considered significant.

RESULTS

Of the 406 serum samples tested, 97 (23.9%) were seropositive for antibodies against *Leptospira* (95% confidence interval [CI]: 19.7–28.1%) with agglutination titers ranging from 1/100 to 1/800 (Table 1). Of the 15 serogroups tested, 12 were detected among the samples. The most prevalent serogroups were Panama, Autumnalis, Hebdomadis, and Icterohaemorrhagiae, which together accounted for 84% (81/97) of the seropositive samples. The prevalence among males (28.5%) was significantly higher than among females (19.4%). Seropositivity rates were almost uniformly distributed among all age groups.

Table 2 indicates the univariate associations between exposures and leptospiral infection. The median age of the infected was 35 years (range, 15–78 years), and median age among the non-infected was 36 years (range, 15–81 years). The age group of 35–44 years, which had the lowest seropositive rate (18.6%), was used as the reference group for the

TABLE 1
Prevalence of *Leptospira* antibodies in Khammouane Province: by sex and age

Variable	Subjects with antibodies	Total (N)	Prevalence (%)	95% Confidence interval	P*
Total	97	406	23.9	19.7–28.0	
Sex					
Male	57	200	28.5	22.2–34.8	
Female	40	206	19.4	14.0–24.9	0.032
Age (years)					
15–24	21	88	23.9	14.8–32.9	
25–34	26	85	30.6	20.6–40.6	
35–44	18	97	18.6	10.7–26.4	
45–54	18	73	24.7	14.5–34.8	
≥ 55	14	63	22.2	11.7–32.8	0.44

* The Pearson χ^2 test was used to calculate P values.

statistical comparisons, but none of the other age groups were found to be significantly associated with infection ($P > 0.05$) compared with the reference group.

Being male was associated with high seropositivity (odds ratio [OR], 1.65; 95% CI: 1.04–2.63). Seventy-seven (19%) of the sample population answered that at least some part of their land had been flooded at some time in the previous 2 years. Having experienced flooding of their land was slightly more frequent among non-infected persons than among infected persons (20.1% versus 15.5%), although the association between flooding and seropositivity was not significant. Living in Thakhek District was very strongly associated with infection (OR, 2.80; 95% CI: 1.54–5.07; $P = 0.0004$). The seroprevalence in Thakhek district (28.9%) was 2.3 times higher than in Nongbok district.

Univariate analysis indicated that infected persons were significantly more likely to walk barefoot around the house (OR, 2.11; $P = 0.024$). Gathering wood in the forest was also associated with high OR (OR, 1.84; $P = 0.027$). Activities involving water and animal contact were not associated with infection. Keeping dogs, cattle, and pigs was not associated with infection. Having chickens and ducks around the house was more frequent among non-infected than infected persons (87.7% versus 77.3%; $P = 0.012$). Occupation, household water source, and condition and type of sanitary facility did not show associations with infection.

The result of multiple logistic regression analysis is shown in Table 3. Contrary to the result from the univariate analysis, recent history of flooding on the respondent's property was found to have a positive association with the infection (OR, 2.12; 95% CI: 1.25–3.58). Other risk factors for infection were being male, collecting wood in the forest, living in Thakhek district, and keeping no poultry at home. It is noteworthy that a strong interaction was observed between sex and a history of flooding on the respondent's property in the multivariate regression analysis. Both factors were independently associ-

TABLE 2
Univariate results of potential risk factors among persons MAT positive and MAT negative for leptospirosis

	MAT result						
	Positive (N = 97)		Negative (N = 309)				
Variable	N	Percentage	N	Percentage	OR	(95% CI)	P*
Age groups (years)							
35–44	18	(18.6)	79	(25.6)	1 (reference)		
15–24	21	(21.6)	67	(21.7)	1.38	(0.67–2.80)	0.38
25–34	26	(26.8)	59	(19.1)	1.93	(0.96–3.89)	0.059
45–54	18	(18.6)	55	(17.8)	1.44	(0.68–3.02)	0.34
≥ 55	14	(14.4)	49	(15.9)	1.25	(0.57–2.76)	0.57
Sex							
Female	40	(41.2)	166	(53.7)			
Male	57	(58.8)	143	(46.3)	1.65	(1.04–2.63)	0.032
District							
Nongbok	16	(16.5)	110	(35.6)			
Thakhek	81	(83.5)	199	(64.4)	2.80	(1.54–5.07)	0.0004
Recent flooding on one's own property	15	(15.5)	62	(20.1)	0.73	(0.39–1.35)	0.31
Individual activities							
Collect water from stream	26	(26.8)	105	(34.0)	0.71	(0.43–1.18)	0.19
Swim in stream	52	(53.6)	164	(53.1)	1.02	(0.65–1.62)	1.62
Walk barefoot	85	(87.6)	238	(77.0)	2.11	(1.09–4.11)	0.024
Collect wood in the forest	77	(79.4)	209	(67.6)	1.84	(1.06–3.19)	0.027
See rodents around house	94	(96.9)	288	(93.2)	2.28	(0.66–7.87)	0.18
Household animal ownership							
Dogs	55	(56.7)	180	(58.3)	0.94	(0.59–1.49)	0.79
Cattle	52	(53.6)	194	(62.8)	0.68	(0.43–1.09)	0.11
Pigs	22	(22.7)	64	(20.7)	1.12	(0.65–1.95)	0.68
Poultry	75	(77.3)	271	(87.7)	0.48	(0.27–0.86)	0.012
Occupation							
Rice field farmer	86	(88.7)	280	(90.6)	0.81	(0.39–1.69)	0.57
Vegetable/fruit farmer	24	(24.7)	102	(33.0)	0.67	(0.40–1.12)	0.13
Livestock farmer	9	(9.3)	30	(9.7)	0.95	(0.43–2.08)	0.90
Fisher	5	(5.2)	14	(4.5)	1.15	(0.40–3.27)	0.80
Household water source							
Well	91	(93.8)	272	(88.0)			
River, lake	6	(6.2)	37	(12.0)	0.48	(0.20–1.19)	0.11
Household sanitary facility							
Toilet/latrine	40	(41.2)	138	(44.7)	0.87	(0.55–1.38)	0.55

* The Wald χ^2 test was used to calculate P values.

TABLE 3
Risk factors for leptospiral infection by multivariate logistic regression

Variable	Adjusted OR (95% CI)		P
Sex (female = 0, male = 1)	1.92	(1.24–2.98)	0.005
Recent flooding on one's own property	2.12	(1.25–3.58)	0.007
Collect wood in the forest	1.90	(1.17–3.09)	0.012
Reside in Thakhek district	2.80	(1.90–4.12)	< 0.001
Keep no poultry at home	2.22	(1.10–4.48)	0.029
See rodents around house	2.63	(0.73–9.44)	0.13
Walk barefoot	1.58	(0.72–3.46)	0.24
Collect water from stream	0.83	(0.49–1.41)	0.48
Swim in stream	0.86	(0.50–1.48)	0.58
Sex × flood	0.26	(0.11–0.63)	0.005

ated with high risk of infection (OR, 1.92 and 2.12, respectively). However, if the person was male and had also experienced flooding of his land, the OR dropped to 1.06 (1.98 × 2.11 × 0.26). Seeing rodents around the house, walking barefoot, gathering water from the stream, and swimming in the stream did not show significant associations with seropositivity. However, they were found to confound other variables and therefore were retained in the model.

DISCUSSION

This is the first study that investigated both seroprevalence and risk factors for leptospiral infection in Lao PDR. Only a limited number of case reports and hospital-based studies for leptospiral infection in Lao PDR have been published.[12,13] We found that the overall prevalence of infection among rural populations in two districts of Khammouane Province was 23.9%. A serosurvey on leptospiral infection had been conducted previously among the general population in four different provinces in Lao PDR in 2000 and 2001, using the IgG ELISA to detect antibodies.[14] In that survey, the four provinces' prevalence rates ranged from 19% to 45%. Although different diagnostic methods were used, our findings are comparable to those of the previous survey, which suggests that leptospirosis is widely distributed in Lao PDR.

Southeast Asia is recognized as a leptospirosis-endemic region,[14–16] and several epidemiologic studies have been conducted in the area. A survey in the Mekong Delta, Vietnam, found a prevalence of 19%,[17] whereas a cross-sectional survey of 315 persons involved in high-leptospirosis-risk activities in Thailand reported a prevalence as high as 41%.[18] Leptospirosis is now increasingly recognized as an important cause of acute febrile illness,[14,19] among other febrile diseases such as malaria, dengue fever, scrub typhus, and other rickettsial diseases, which are commonly observed in Southeast Asia.[13,20–22] In a study in Thailand, 37% of patients with acute undifferentiated febrile illnesses were found to have leptospirosis.[21] Our findings also indicate that it is important to consider leptospirosis in the differential diagnosis of febrile illness in Lao PDR.

In this study, males had a significantly higher risk of infection (OR, 1.92), which suggests that males are likely to have contact with leptospires through their daily activities or occupational exposures,[23–25] because there were differences in certain daily activities between males and females. For example, the proportion of barefoot walkers was significantly higher in males than in females (86% versus 73%; $P = 0.002$ with χ^2 test). Swimming in streams and collecting wood were also common in males. Previous studies in Central America and South Asia showed that certain activities influence the transmission of leptospires and possibly contribute to the male predominance in seropositivity.[7,25–27] Among those activities, walking barefoot has been considered to be one of the important risk factors for the infection, especially in developing countries.[7,18,25,28] Behavioral changes for reducing environmental exposure to the bacteria are therefore likely to contribute to the prevention of leptospiral infection in Lao PDR. Regarding occupational exposures, on the other hand, we found that the proportion of persons engaged in rice farming was almost equal among males and among females (92% and 89%, respectively), as was also the case in other occupations, because most of the occupational activities in the region are uniformly performed by males and females.[8] Thus, occupation is not an important factor related to the male predominance in seropositivity in Lao PDR.

This study showed a strong association between a history of recent flooding of the participant's own land and leptospiral infection (OR, 2.12). Most of the sampled villages are located in the lowlands along the Mekong River and its tributaries. Because of poor drainage in those areas, many villages are hit by floods every year or two, caused by high seasonal rainfall. Flooding sometimes lasts up to 2 weeks, with water rising as high as 60–90 cm above ground level, which might cause extensive contamination of soil and of water systems by leptospires. Local flooding is known to play an important role in the transmission of leptospirosis in both epidemic and endemic settings.[5,29,30] Therefore, flood control and other environmental modifications are expected to reduce the risk of leptospiral infection.

We found that a history of recent flooding was associated with seropositivity, especially for women, with a strong interaction between sex and flooding. In the group discussion held in July 2007, villagers commented that men usually stay at home when the land is flooded, whereas women need to walk into the stagnant water to feed their livestock. Women's behaviors and daily activities during floods might increase their exposure to the bacteria and consequently increase their risk of infection.

The reason why the disease prevalence and risk are so much higher in Thakhek District than in Nongbok District is unknown, because cultural background, lifestyles, and economic status are quite similar among the villages of the two districts. Non-obvious differences in behavior, in environmental conditions, or in the distributions of reservoir animals could play important roles in this difference in seroprevalence, and further study of those factors is needed.

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