ORIGINAL ARTICLE

Seroepidemiological study of leptospirosis among the communities living in periurban areas of Sarawak, Malaysia

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ABSTRACT

Introduction: Leptospirosis is endemic to tropical regions of the world and is re-emerging as a new danger to public health in Malaysia. The purpose of this particular study was to determine the common leptospiral serovars present in human communities living around wildlife reserves/disturbed forest habitats. The objective of this study was to estimate the seroprevalence of leptospirosis and finding infecting serovars in villages surrounded habitats where wildlife lives in Sarawak, Malaysia.

Methods: A cross-sectional serological survey of 198 humans was conducted in four villages around Kuching, Sarawak between January 2011 and March 2012.

Results: A seroprevalence of 35.9% (95%CI 29.2-43.0) to the MAT was detected in the tested humans. Antibodies to serovar Lepto 175 Sarawak were most commonly detected (31.3%; 95%CI 24.9-38.3) and were detected in individuals at all four locations. The presence of skin wounds (OR 3.1), farm animals (OR 2.5) and rats (OR 11.2) were all significantly associated with seropositivity in a multivariable logistic regression model.

Conclusions: The results of the current study are important as wildlife may act as reservoirs of leptospires for humans. Health authorities should expand disease control measures to minimise the spill-over from wildlife to humans visiting, living or working in the sampled locations. The pathogenic status of serovar Lepto 175 Sarawak also requires further investigation.

INTRODUCTION

Leptospirosis is disease affecting humans and animals and is found in most countries of the world. It is caused by members of the *Leptospira* genus and the disease is recognised as a direct anthropozoonosis[1, 2] and an occupationally acquired disease.[3] Infection results through contact with infected animals or urine-contaminated surfaces, such as soil or water.[4]

In Malaysia, leptospirosis is a notifiable disease in humans.[5] In Sarawak alone, the number of cases rose almost four-fold from 49 in 2010 to 186 in 2011.[6] It is widely believed that bats and rodents may be responsible for the spread of leptospirosis in Sarawak.[6] With increased urbanisation and rapid deforestation happening across the state, the possibility of people coming into contact with wildlife increases, resulting in greater risk of contracting leptospirosis. Domestic animals can also be exposed to a large number of leptospiral serovars and may act as maintenance hosts potentially resulting in infection in humans.[7]

Globally researchers have focussed on the pathogenic serovars and strains of *Leptospira* spp. and their zoonotic significance, epidemiology, control and prevention. There is a lack of studies on the intermediate pathogenic strains although these strains also plan an important role in disease outbreaks in communities. There are many technical challenges in confirming the virulence of intermediate strains, although genotyping and proteomic methods have been developed to investigate these factors.[8,9] However the pathogenicity of a serovar for humans can only be established after it has been isolated from a patient presenting with the clinical symptoms of leptospirosis.

The study described in this manuscript was conducted to assess the extent of exposure to leptospires in humans living on the fringes of forested areas and disturbed jungle environments in Sarawak. The major objective of this study was to determine which strains/serovars of *Leptospira* were infecting humans.

MATERIALS AND METHODS

Study areas

A cross-sectional serological survey was conducted in four different locations (Kampung (Kg) Sebayor, Kg Etingan, Mount Singai and Wind and Fairy Caves) in Sarawak between January 2011 and March 2012. The villages were selected based on their location adjacent to areas (with in 1000 m distance) where wildlife inhabited. Universiti Malaysia Sarawak (UNIMAS) is located in Kota Samarahan,

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approximately 30 kilometres east of the state capital Kuching. The campus grounds have a disturbed habitat or deforested area. Kampung (Village) Sebayor is located inside the campus and Kampung (Kg) Etingan is located close to UNIMAS campus. Mount Singai is located midway between the town of Bau and the Matang wildlife reserve. The Wind and Fairy caves are part of the Bau Formation, a narrow belt of limestone covering approximately 150 sq. km in south-west Sarawak (Figure 1).

Sample Collection
A total of 198 individuals over the age of 18 were sampled from four villages. A single venous blood sample (5 mL) was collected from each participant. Samples were maintained at 20°C for 6 hours to allow coagulation and then were centrifuged at 3,000 RPM for 1 minute to separate the sera. The serum was then removed and transferred to vials and stored at -70°C until testing was performed.

Questionnaire
A structured questionnaire was developed based on validated questionnaires performed by others.[10-12] The questions were categorised under six broad categories: general (age, sex, education and occupation); knowledge and symptoms of leptospirosis; exposure to animals; involvement in outdoor activities; potential sources of infection; and socio-economic status. All the questions, except for the source of drinking water and socio-economic status, were categorised as either a yes/no answer. Households were randomly selected for this study using the information (number of house hold and number of villagers living in that village) provided by the village chief of the respective village.

Serology
Anti-leptospiral antibodies were detected by using an IgG and IgM ELISA kit (CTK Biotech cat: E0331) indicating current or past leptospiral infection. The MAT was also performed on all samples to identify the serovar responsible for any infection. A titre ≥1:100 on the MAT was considered as evidence of recent or past infection with *Leptospira*.

Serum samples were tested by the MAT in the Leptospirosis Laboratory at the Institute for Medical Research (IMR), Kuala Lumpur, Malaysia. The MAT was performed using antigen from a collection of 17 live serovars of *Leptospira* that are common in Malaysia.[13] The ELISA was also performed at the IMR according to the standard procedure provided by the CTK Biotech Data sheet (Cat No: E0331). ELISA test was performed as preliminary evaluation (CTK Biotec Evaluation kit) and finally MAT was chosen as the depended variable to have accurate final results.

Statistical analysis
Data were entered into Microsoft Excel and statistical analyses were performed using SPSS version 22. The seroprevalence on the MAT test and their 95% confidence intervals (95%CI) were calculated. Categorical variables were summarised using percentages and compared using the Chi-square test. A multivariable logistic regression was performed to identify factors associated with seropositivity to leptospires. Odd ratios (ORs) and their 95% confidence intervals (CIs) were calculated for factors in the reduced subset model. Model building was performed by considering variables that were statistically significant (p values <0.20) in the univariable analyses, and including them in the preliminary multivariable logistic regression model. Once the preliminary main effects model was obtained (fully saturated model) a manual backward stepwise approach was used to remove non-significant variables and only variables with p values<0.05 were retained in the final model. Overall fit of the final logistic model was assessed with the use of the Hosmer-Lemeshow goodness-of-fit statistic. To determine possible interactions between the final set of factors, interaction factors between each remaining variable were added one at a time to the final model to determine the impact of the interaction factor on the model. Findings were presented with Odds ratio (OR), 95%CI and p-value.

Ethics Approval
This research was approved by the Murdoch University Human Ethics Committee (Permit number: 2010/240) and the Malaysian Research Ethics Committee (NMRR-10-879-7153).

RESULTS

Samples were collected from people living in Kg. Sebayor (n=27), Kg. Etingan (n=40), Mt. Singai (n=70) and Wind and Fairy Caves (n=61) for this study. Of the 198 participants, 126 (63.64%) were women and 72 (36.36%) men (Table I).

Seroprevalence
An overall seroprevalence of 35.9% (95% Confidence Interval (95%CI) 29.2-43.0) was obtained on the MAT. There was no significant difference (χ^2=3.04; d*f*=3; P=0.38) in the seroprevalence between the four sampled locations (Table II), with the highest seroprevalence (41%) (95%CI 28.6-54.3) obtained in people from the Wind and Fairy Caves.

Based on the results from the ELISA, 33.3% (95%CI 26.8-40.4) of samples were seropositive to leptospires (Table II). The highest percentage (41%; 95%CI 28.6-54.3) was again in residents from the Wind and Fairy Caves area. Only 5 of 40 respondents from Kampung Etingan (12.5%; 95%CI 4.2-26.8) were seropositive. The seroprevalence in Kg Etingan was significantly lower than that in Mt Singai and Wind and Fairy Caves ($p < 0.05$) (Table II).

Reactivity to serovar Lepto175 (Sarawak) was found in humans residing at all four locations. Overall the seroprevalence to serovar Lepto175 (Sarawak) was significantly different between locations ((χ^2=11.43; d*f*=3; P=0.010), being significantly lower in Kg. Etingan than in the other locations (Table II).

Antibodies to 17 different serovars were detected in the serum samples collected from humans from the four locations sampled. Antibodies to serovar Lepto 175 Sarawak were most commonly detected being found in 62 (31.31%; 95%CI 24.9-38.3) of all samples. Thirty-three samples (16.7%; 95%CI 11.8-22.6) showed reactivity to Djasiman, 15.7% (95%CI 10.9-21.5) were positive to Autumnalis and a similar percentage were positive to Canicola and 14.6% (95%CI 10.0-20.4) were positive to Australis (Table V). The antibody titres

Table I: Gender distribution of humans sampled at the four localities

Locality	Gender		Total
	Female	Male	
Kg. Sebayor	17 (63.0%)	10 (36.0%)	27
Kg. Etingan	21 (52.5%)	19 (47.5%)	40
Mt. Singai	47 (67.1%)	23 (32.9%)	70
Wind & Fairy Caves	41 (67.2%)	20 (32.8%)	61
Total	**126 (63.6%)**	**72 (36.4%)**	**198**

Table II: Seroprevalence (MAT; ELISA and Lepto 175 Sarawak-MAT) to leptospirosis in humans from different localities

Locality	MAT	ELISA	Lepto 175 (MAT)
Kg. Sebayor	33.3 (16.5-54.0)	33.3 (16.5-54.0)	33.3 (16.5-54.0)
Kg. Etingan	25.0 (12.7-41.2)	12.5 (4.2-26.8)	10.0 (2.8-23.7)
Mount Singai	38.6 (27.2-51.0)	38.6 (27.2-51.0)	34.3 (23.3-46.6)
Wind & Fairy Caves	41.0 (28.6-54.3)	41.0 (28.6-54.3)	41.0 (28.6-54.3)
Total	**35.9 (29.2-43.0)**	**33.3(26.8-40.4)**	**31.3 (24.9-38.3)**

Table III: Distribution of seropositive (MAT) reactions to Leptospira spp. in humans according to serovar and their respective titres

Serovars	1:100	1:200	1:400	Total	% (95%CI)
Lepto 175	42	17	3	62	31.3 (24.9-38.3)
Djasiman	28	4	1	33	16.7 (11.8-22.6)
Autumnalis	24	4	3	31	15.7 (10.9-21.5)
Canicola	23	8	-	31	15.7 (10.9-21.5)
Australis	18	9	2	29	14.6 (10.0-20.4)
Lai	13	5	3	21	10.6 (6.7-15.8)
Patoc	9	4	3	16	8.0 (4.7-12.8)
Grippotyphosa	15	-	-	15	7.6 (4.3-12.2)
Hebdomadis	14	1	-	15	7.6 (4.3-12.2)
Javanica	13	2	-	15	7.6 (4.3-12.2)
Bataviae	9	2	-	11	5.6 (2.8-9.7)
Hardjo-bovis	10	-	-	10	5.0 (2.4-9.1)
Pomona	3	7	-	10	5.0 (2.4-9.1)
Celledoni	7	1	-	8	1.8 (1.8-7.8)
Icterohaemorrhagiae	3	4	1	8	1.8 (1.8-7.8)
Pyrogenes	5	1	1	7	1.4 (1.4-7.1)
Copenhageni	3	1	1	5	0.8 (0.8-5.8)

of the seropositive samples varied from 1:100 to 1:400. More samples had a serum dilution of 1:100 (239) compared to other dilutions (1:200 in 70 samples and 1:400 in 18 samples).

RISK FACTORS

Males had a slightly higher but not significant seroprevalence on the MAT (38.4%; 95%CI 27.2-50.5) than females (34.4%; 95%CI 26.1-43.4) (OR 1.12, 95%CI 0.61, 2.04). Overall the seroprevalence (MAT) varied between age groups ((χ^2= 9.38; df=4; P=0.052). People in the 36-45 year age group had the highest seroprevalence (53.6; 95%CI 33.9-72.5) followed by the 18-25 age group (46.7; 95%CI 21.3-73.4). People 34 to 45 years old were 2.45 times (95%CI 1.01-5.91) more likely to have leptospiral antibodies than those older than 55 years of age (Table IV).

People with a history of fever in the last x months were significantly more likely to be seropositive (OR 3.83, 95%CI 2.07-7.07; (χ^2= 19.2; df=1; P=0.001) than those without fever. People who had been treated for fever were also significantly more likely to be seropositive than those not treated (OR 3.39, 95%CI 1.84-6.27; (χ^2= 7.52; df=1; P=0.006).

Overall the seroprevalence (MAT) was not significantly different between the different occupations ((χ^2= 4.12; df=3; P=0.25). However people who were working around the forest (54.5%; 95%CI 23.4-83.3) and national service participants (50%; 95%CI 27.2-72.8) had the highest seroprevalence. Patients with skin wounds also had a significantly higher seroprevalence (58.8%; 95%CI 40.7-75.4) than those without skin wounds (31.1%; 95%CI 24.1-38.8) (OR 5; 95%CI 2.34-10.68; (χ^2= 9.41; df=1; P=0.002). Owning a pet animal, such as a dog or cat, did not increase the likelihood of a seropositive reaction ((χ^2= 1.27; df=1; P=0.25). In contrast contact with farm animals (OR 2.2, 95%CI 1.22-3.99; (χ^2= 6.89; df=1; P=0.009) increased the probability of leptospiral infection. The presence of rats in or near the participant's residence ((χ^2= 60.6; df=1; P=0.001) or the sighting of rats more than 3 times within a day of the survey ((χ^2= 53.0; df=1; P=0.001) increased the probability of seropositivity in the residents.

In the final multivariable logistic regression model the presence of skin wounds (OR 3.05), farm animals (OR 2.51) and rats (OR 11.2) were all significantly associated with seropositivity. The logistic regression model for Leptospira antibodies exhibited a good overall fit (Hosmer and Lemeshow Chi-square value 2.484, P=0.648).

Table IV: Relationship between seropositivity (MAT) to Leptospira spp. and host and environmental factors

Variables	Total	Seropositive	Seroprevalence (95%CI)	OR (95%CI)	P
Locality					
Wind & Fairy Caves	61	25	41.0 (28.6-54.3)	2.08 (0.87-5.02)	0.38
Mt. Singai	70	27	38.6 (27.2-51.0)	1.88 (0.80-4.46)	
Kg. Sebayor	27	9	33.3 (16.5-54.0)	1.50 (0.51-4.39)	
Kg. Etingan	40	10	25.0 (12.7-41.2)	1.00	
Sex					
Male	73	28	38.4 (27.2-50.5)	1.19 (0.65-2.16)	0.575
Female	125	43	34.4 (26.1-43.4)	1.00	
Age					
18-25	15	7	46.7 (21.3-73.4)	1.86 (0.61-5.69)	0.052 [a]
26-35	21	3	14.3 (3.0-36.3)	0.35 (0.10-1.31)	
36-45	28	15	53.6 (33.9-72.5)	2.45 (1.01-5.91)	
46-55	56	21	37.5 (24.9-51.5)	1.27 (0.62-2.61)	
>55	78	25	32.0 (21.9-43.6)	1.00	
Education					
No Schooling	57	21	36.8 (24.4-50.7)	0.78 (0.16-3.82)	0.945
Primary	51	19	37.2 (24.1-51.9)	0.79 (0.16-3.93)	
Secondary	83	28	33.7 (23.7-44.9)	0.68 (0.14-3.25)	
Tertiary	7	3	42.9 (9.9-81.6)	1.00	
Occupation					
Cleaner	46	16	34.8 (21.4-50.2)	1.12 (0.55-2.30)	0.25
Work around forest	11	6	54.5 (23.4-83.3)	2.52 (0.73-8.78)	
National Service	20	10	50.0 (27.2-72.8)	2.10 (0.81-5.47)	
Farmer/Dairy farmer	121	39	32.2 (24.0-41.3)	1.00	
Fever					
Yes	90	47	52.2 (41.4-62.9)	3.83 (2.07-7.07)	0.001
No	108	24	22.2 (14.8-31.2)	1.00	
Skin wounds					
Yes	34	20	58.8 (40.7-75.4)	5.00 (2.34-10.68)	0.002 [a]
No	164	51	31.1 (24.1-38.8)	1.00	
Presence of dog or cat					
Yes	130	43	33.8 (25.1-41.9)	0.71 (0.39-1.29)	0.259
No	68	28	41.2 (29.4-53.8)	1.00	
Presence of Farm animals					
Yes	98	44	44.9 (34.8-55.3)	2.20 (1.22-3.99)	0.009 [a]
No	100	27	27.0 (18.6-36.8)	1.00	
Presence of rats					
Yes	121	69	57.0 (47.7-66.0)	50.8 (11.6-212.0)	0.001 [a]
No	77	2	2.6 (0.3-9.1)	1.00	
Sited rats more than 3 times per day by participants					
Yes	96	59	61.5 (51.0-71.2)	12.0 (7.7-24.8)	0.001
No	102	12	11.8 (6.2-19.6)	1.00	

[a] variables (with P-values<0.20) were offered to the multivariable logistic regression model

Table V: Final logistic regression model

Description of variables	B	SE[a]	Waldb	Sigc	OR[d] (95% CI)
Skin wounds	1.116	0.493	5.132	0.023	3.05 (1.16-8.01)
Presence of farm animals	0.921	0.374	6.056	0.014	2.51 (1.21-5.23)
Presence of rats	3.878	0.747	26.977	0.000	48.3(11.19-208)
Constant	-4.288	0.767	31.216	0.000	

[a]SE=Standard error; [b]Wald=a test that a coefficient is zero based on the Wald statistic; [c]Sig=Significance for the Wald statistic
dOR=Oddratios

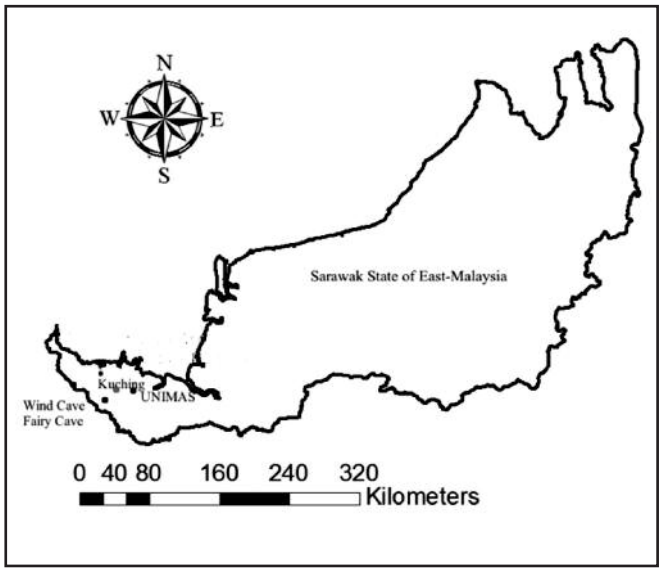

Fig. 1: Sampling sites.

DISCUSSION

In this study over one third of the sampled individuals were seropositive to leptospires (35.9%; 95%CI 29.2-43.0). Other serological surveys of the general population have reported a seroprevalence up to 25%.[14] However the current study targeted a population where it could be expected that the seroprevalence was higher than the overall general population. Another targeted study of residents in the Rajang Basin of Sarawak similarly reported an elevated seroprevalence of 30.6%.[15] Studies in other tropical countries, including Barbados, have reported a seroprevalence of 29.8% in various occupational groups and in 1987 a seroprevalence as high as 25% was reported in patients hospitalised in Pakistan.[16] From a global perspective a seroprevalence that is this high is unusual, even in areas where leptospirosis is endemic, although a seroprevalence of 30 to 50% has been reported in high-risk groups.[16]

In the current study inhabitants residing in the vicinity of the Wind and Fairy Caves had a higher, although not significant, risk of seroconversion to leptospires than residents from the other surveyed locations. Wind and Fairy Caves are surrounded by forest and residents would be expected to more frequently come into contact with wildlife (rats, squirrels and bats) or leptospire-contaminated water or soil than residents from urban areas. In the research of Thayaparan *et al.*, (2015)[17] a high seroprevalence in rats, squirrels and bats from these regions has been shown. The titres detected in the sampled humans could be the result of current infections but also could be the result of previous infections, as titres can remain for up to 20 years. [18]

In this study Serovar Lepto 175 was the dominant strain (31.3%) affecting people. Although its virulence has not been confirmed and it is currently classified as an intermediate strain, it has a high level of 16S rRNA gene sequence similarity to *Leptospira wolffii* sv. Khorat strain Khorat-H2 (99.1%).[19] *Leptospira wolffii* has been linked to clinical leptospirosis and has been isolated from humans and other animals in Thailand, India and Iran[20, 21] and this serovar could potentially cause an outbreak in the future. Serovars Autumnalis (15.7%), Canicola (15.7%) and Lai (10.6%) were also commonly found in this study, with evidence of infection to most serovars present in all four locations.

In this study the seroprevalence in males (37.5%; 95%CI 26.4-49.7) was slightly higher than that in females (34.9%; 95%CI 26.6-43.9). This could be attributed to the fact that most of the work done outside of the home environment in this region was performed by men. Furthermore, jobs that revolves around the forest, such as woodcutting and hunting, tend to be male-dominated positions.

Although in the univariable analyses people between the ages of 36 and 45 and 18 to 25 years were at a greater likelihood of being seropositive compared to people older than 55, age did not remain in the final multivariable logistic regression model. The greater risk found in the univariable analysis for people between 18 and 25 years of age may be associated with this group be dominated by National Service Participants and researchers who would be expected to have greater exposure to the forest and hence wild animals than other groups.

Of 90 respondents who reported to have suffered from a fever within three months of sampling, approximately half (52.2%; 95%CI41.4-62.9) were seropositive to leptospires. Similarly just under half (49.23%) of those who received treatment for fever were also seropositive, while 20 of 34 (58.82%; 95%CI 40.7-75.4) who had skin wounds were seropositive. This supports the importance of infection entering via skin lesions and the presence of fever as a common symptom of leptospiral infection.[22, 23] Individuals with wounds have the potential to be infected if they are involved in activities likely to expose them to leptospires, such as through agricultural pursuits, forestry businesses or wildlife and rodent interactions. In a previous case control study in the Seychelles, leptospirosis was positively associated with activities in forests, which may be related to an increase in environmental exposure to the pathogen.[22]

The presence of domesticated pets did not significantly increase the risk of infection in this study. In contrast exposure to farm animals and rodents presented a major risk of infection. These findings highlight the role of peri-urban rats as the principal source of leptospiral transmission, as has been reported by others.[24-27] However, surprisingly rodent-associated serovars, such as Icterohaemorrhagiae (1.8% positive) and Copenhageni (0.8%), did not appear to be of major importance in this study. In contrast, Canicola (15.7%), which is mostly carried by dogs,[28] presented itself as a major threat in this study.

Overseas travel, involvement in water-related activities and camping around forests (data not shown) were not linked to seropositivity, however the number of residents engaging in these pursuits was small. As has been documented in previous studies, a number of outbreaks in Borneo and around the world have occurred after patients have been exposed to jungle environments or been involved in aquatic sports.[29-32]

The key findings of this study indicate that residents of forested areas having skin wounds, contact with farm animals and the presence of rats are at a greater risk of leptospirosis. This is further compounded if the subjects work with animals or depend on the forest for their livelihood. Skin wounds appear to be the primary route of infection, with fever as the main symptom, meaning anyone with skin-cuts who complains of subsequent fever should be considered as a potential case of leptospirosis. This research also identified the potential role of rats as a source of leptospiral infection for residents, highlighting the need to make rodent control a priority in disease management. In the multivariable logistic model the findings that owning farm animals or the presence of rats in the vicinity of the participant's house, together with the results of other studies,[3,33] highlight the importance of these species in leptospiral infection of humans.

Potential causes of bias and confounding factors in the study

This study was designed to gain information on the seroprevalence to leptospires from a population living and working around wildlife inhabitants. The population was biased, as individuals younger than 18 years of age were not sampled, which may have resulted in overestimation of the seroprevalence by targeting individuals more likely to have exposure to the pathogen through occupational exposure.

CONCLUSIONS

Although the findings of this study indicate that the intermediate strain Lepto 175 Sarawak might be responsible for asymptomatic infections in Sarawak, there is a need for increased awareness of the risks associated with living close to places where wildlife inhabit and measures should be developed to minimise exposure to these animals. The presence of rodents appears to be a strong risk factor in this instance, and it would be pertinent to keep the population of these animals under control in urban and peri-urban areas. Information obtained from this study should be used in the future to design more focused and specific research. Further blood sampling, serology and isolation of the strains from the community and hospital based leptospirosis confirmed cases should be performed to determine whether the infections are current or indicative of previous infection. By doing so, it is also possible to establish, albeit roughly, which particular strains are active in the vicinity. If possible, children less than 18 years should be included in a future study, as they sometimes accompany their parents into the forest and [34] suggested that children as young as seven may be infected with leptospirosis.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest

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