Journal of Infection and Public Health 12 (2019) 705–711

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: http://www.elsevier.com/locate/jiph

Prevention and control of leptospirosis in people and surveillance of the pathogenic *Leptospira* in rats and in surface water found at villages

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ARTICLE INFO

Article history:
Received 12 October 2018
Received in revised form 10 January 2019
Accepted 24 March 2019

Keywords:
Perception
Behavior prevention
Leptospira
Leptospirosis

ABSTRACT

Background: Leptospirosis is a major public health problem in Thailand. This disease is caused by a *Leptospira* infection. *Leptospira* is found in rats and other animals that can contaminate soil and water resources. This research aimed to (1) study the perceptions and preventive measures taken to control leptospirosis among local populations and (2) to detect *Leptospira* in rats and natural surface water.
Methods: This cross-sectional study was performed in both villages with and without case histories of leptospirosis. The research procedures were divided into 2 parts. First, the perceptions and preventive measures for leptospirosis used data from 108 sampled subjects that were recruited in May 2015. Second, the rats and surface water samples were collected. DNA was extracted from collected samples and then specific genes specific to *Leptospira* were detected using PCR technique.
Results: The awareness of leptospirosis of samples in two villages was at high level with the same percentage being at 91.6%. However, the preventive behaviors to leptospirosis of participants from both villages were found to be at only the moderate level. *Leptospira* detection in rats and surface water used a PCR technique. There was no *Leptospira* found in any of the 270 rat samples and 100 surface water samples taken from both villages.
Conclusion: Regardless, perception and preventive behaviors for dealing with leptospirosis should be continuously encouraged even when its presence is not detected. Clearly, people have to practice good behaviors for the prevention of this pathogen to be safe.

Introduction

Leptospirosis is a major public health problem with outbreaks occurring around the world, including Thailand. This disease is caused by an infection from the pathogen *Leptospira* and can be transmitted from animals to humans. *Leptospira* can infect hosts such as rats, cattle, dogs, goats, sheep, and pigs and can contaminate soil and water resources through urine from infected animals. *Leptospira* can survive in water or soil for weeks to months [1,2]. Temperature, humidity, and rainfall are important factors for the survival of *Leptospira* [3]. River resource with pH levels of between 6.7 and 7.3 and with a dissolved solid salt content of between 3.78% and 3.85% were optimal for *Leptospira* growth [4]. The number of cases of leptospirosis tends to increase in the rainy season. In some areas, such as parts of Asia, outbreaks most often occur after flooding, as happened in the Philippines in November 2009 [5].

Human can become infected through the contact to either urine of infected animals or urine contaminated soil and water [6]. Moreover, the bacteria can enter through a scratch on the skin, a wound, or through mucous membranes found in the mouth, eyes, and nose [1,7]. People engaging in outdoor activities and agricultural workers are high risk of getting a leptospirosis disease infection during work and activities that exposes them to water for a long time [5]. Thus, the best prevention and control of leptospirosis should be to give more information and knowledge to people in local areas about this threat. The people have to engage in activities or behaviors that likely can prevent *Leptospira*. We have studied this matter using a design method and questionnaire for local people to assess their daily activities and the frequency of participation in them. Rats in paddy fields are a weighted priority factor for outbreaks of leptospirosis because local people like to eat and sell rats from paddy fields. Moreover, people still walk bare foot when walking around in paddy fields and who engage in activities such as fishing and swimming in local water sources. In this way, they are at risk for other infectious diseases, as well. These problems are a particular concern in rural areas. Therefore, the purpose of this study was to

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https://doi.org/10.1016/j.jiph.2019.03.019

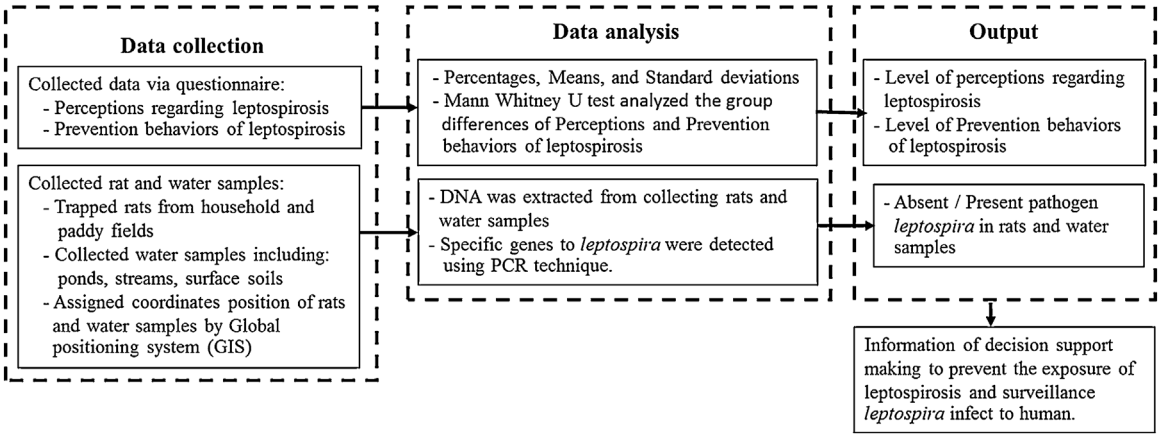

Fig. 1. Research framework.

survey perceptions and preventive behaviors of leptospirosis and to detect *Leptospira* in rats and natural surface water using a Polymerase Chain Reaction (PCR) technique. This effort will hopefully prevent the exposure of leptospirosis for local populations and can detect any *Leptospira* that could potentially infect any humans.

Methodology

Study areas

The study was conducted at the Nong Chang Sub-district, Muang Samsib District, Ubon Ratchathani Province of Thailand. It could be reached by traveling to the northeast which is from Bangkok for about 640 km. In addition, it is surrounded by a paddy fields and water resources. The study areas included two villages based on the cases of reported leptospirosis outbreaks occurring between January 2011 and December 2013. First village with case histories of leptospirosis in past three years was chosen. It is located at 15°33′ 43.49″ north and 104°47′ 48.12″ eastern with 139 households and 530 persons. Second, the village which is located at 15°32′ 59.20″ north and 104°49′ 54.54″ eastern without a history of leptospirosis cases, consisted of 148 households and 661 persons. The following data was provided by The Ubon Ratchathani Provincial Health Office.

Study design

The cross-sectional descriptive study was planned for developing strategies of leptospirosis prevention and control. The data set was composed of three subjects that included information regarding the participants in the survey and information about rats collected in paddy fields and on the surfaces of water. This research had the full cooperation of public health volunteers form The Pharoj Sub-district Health Promoting Hospital who surveyed and collected data. The research framework information is provided in Fig. 1.

Perceptions and preventive behavior of Leptospirosis

Participants

The 130 households were calculated by a probability formula appropriate for the sample size (Eq. (1)) [8], whereas a total of 108 questionnaires were returned. The heads or representatives of households were randomly interviews. Sixty samples were obtained in the village having case histories and forty-eight samples were obtained from the village without case histories. Participants have been living in the village more than five months and were more than eighteen years old. The structured questionnaire was designed for community health prevention of leptospirosis had been developed by Wongbutdee et al. [9] (see Supplementary data). All data was completely collected by questionnaire in May 2015.

$$\frac{NZ^2_{\frac{\alpha}{2}}P(1-P)}{[d^2(N-1)]+[Z^2_{\frac{\alpha}{2}}p(1-P)]} \quad (1)$$

N: the total number of households = 287
Z: confidence level at 95%
P: the expected population proportion of prevention behavior of
leptospirosis = 0.66 [9]
d: the margin of error in estimating p (acceptable error = 0.06)

Measurements

Perceptions regarding leptospirosis (PRL) were measured by nominal scales that were divided into either “Agree” or “Disagree” categories for assessing knowledge and to obtain information about leptospirosis-related diseases. The questions composed of positive and negative meaning items. The PRL sections contained 16 items. If participants responded that they agree, they were given a score of 1. In contrast, if they responded that they disagreed or chose unknown, they were given a score of 0. Three levels of PRL were interpreted based on a total score of 16 points by equal interval. High levels of PRL were given a score between 11–16 points, with 6–10 being considered to be at a moderate level. Thus, it was considered to be a low level if it were less than 6.

Preventative behaviors of leptospirosis (PBL) were measured by ordinal scales from “always” to “never.” If participants had continually been in an activity related to leptospirosis behavior prevention throughout the week, the response received a score of 4. For frequency activities that were marked “often” (4–6 times/week) they received a score of 3. If “sometimes” (1–3 times/week) was selected, a score of 2 was given; for “never,” a score of 1 was given. On the other hand, negative items were rated from “Always” (1), “Often” (2), “Sometimes” (3), and “Never” (4). Three levels of PBL were interpreted based on a total score (64 points) by equal interval. The high levels, moderate levels, and low levels of PBL were 43–61 points, 22–42 points, and 1–21 points, respectively.

Trapping of rats and surface water collection

A sample size of rats were determined by a probability formula following equation 2 [8], where Z is the confidence level at 95% (1.96), p is the expected population proportion of presence of *Leptospira* in rat (8.7%) [10], and d is the margin of error in estimating p (acceptable error = 0.04). A total of 270 rats were trapped from

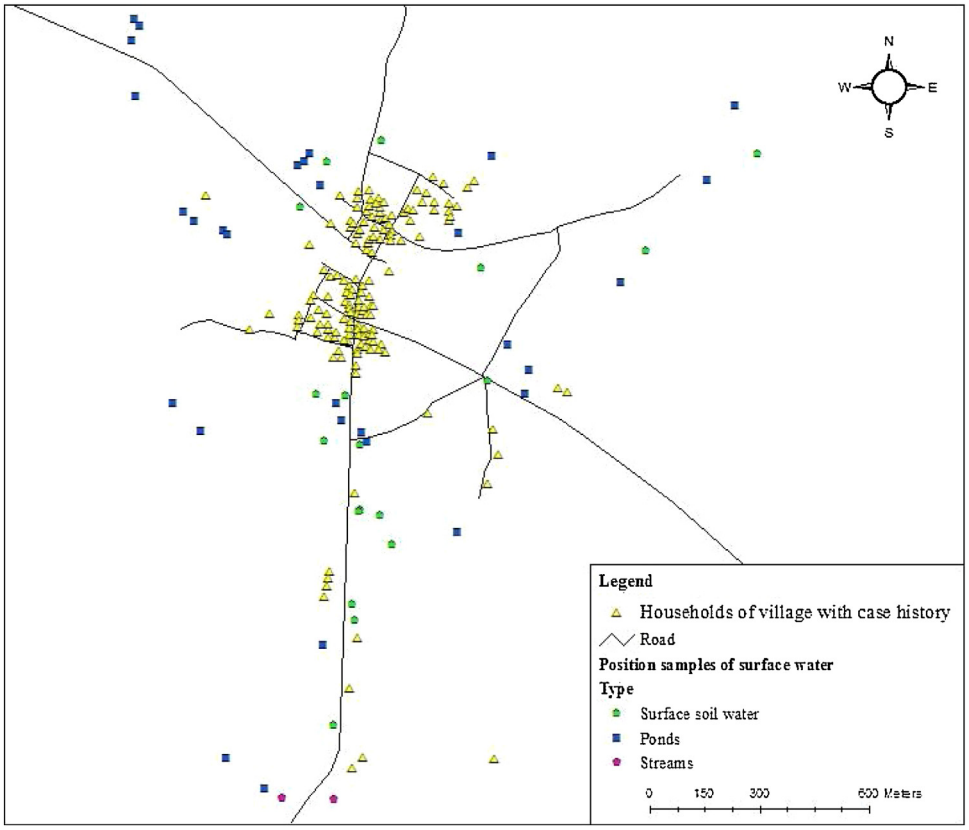

Fig. 2. The position of 51 water samples from natural resources including: ponds, streams, and surface soil water in village with case history.

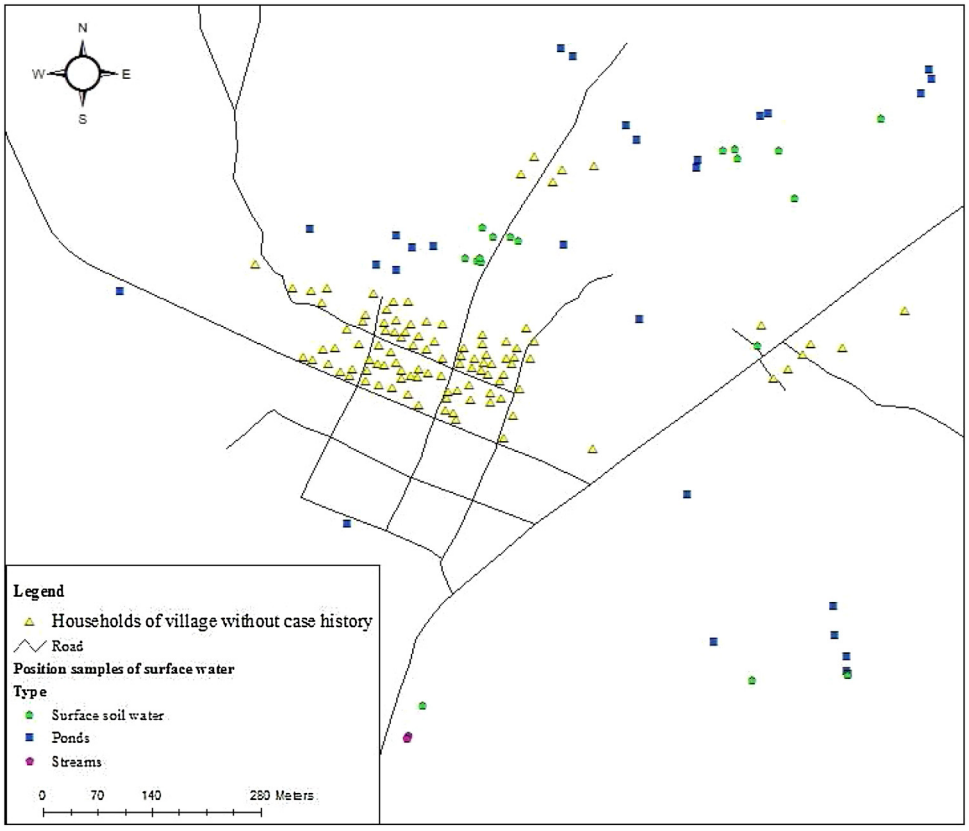

Fig. 3. The position of 49 water samples from natural resources including: ponds, streams, and surface soil water in village without case history.

households and paddy fields between December 2014 to February 2015. 178 rats were captured from villages with case histories of leptospirosis (72 rats from households and 106 rats from paddy fields), whereas 92 rats were obtained from villages without case histories (51 rats from households and 41 from paddy fields). The 100 water samples were collected from natural resources between September and October 2015, which 51 samples including: ponds (27 sample), streams (3 samples), and surface soil water (21 samples), were obtained from villages with case histories (Fig. 2). This area was plot base on 2.25 km^2 (1.5 km × 1.5 km) gird covering. Subsequently, the villages without any case histories collected the 49 samples (27 sample from ponds, 3 samples from streams, and 21 samples from surface soil water), by square grid cell of 1 km × 1 km (Fig. 3). All sample positions were assigned coordinates on the earth's surface by a global positioning system (Garmin eTrex Legend HCX).

$$n = z^2p(1-p)/d^2 \quad (2)$$

Leptospira detection in rats and surface water samples DNA extraction

All rats were euthanized with isoflurane. Animal protocols were approved by The Ubon Ratchathani University-Animal Care and Use Committee (ID#5/2555/research). The rats' kidneys were collected and then were homogenized through a sterilized mesh. Next, 1 ml of each kidney homogenate was centrifuged at 800 × *g* for 1 min. The kidney supernatants were collected and centrifuged at 13,000 × *g* for 5 min. The sample pellets were used for DNA extraction using QIAamp® DNA Mini (Qiagen, Valencia, USA). For DNA extraction in water samples, 50 ml of each sample was centrifuged at 10,000 × *g* for 20 min. The pellets were extracted with the same rats' kidney DNA extraction kit. DNA samples were stored at −20 °C until ready for use.

Leptospira detection

The *16s rRNA* and *LipL32* genes specific to *Leptospira* were the target gene for *Leptospira* detection in rats' kidney and water samples using PCR technique. The *16s rRNA* primers were reported by Merien et al. [11] (16s rRNA-F: 5′-GGCGGCGCGTCTTAAACATG-3′ and 16s rRNA-R: 5′-TTCCCCCCATTGAGCAAGATT-3′), while *LipL32* primers specific for pathogenic *Leptospira* (LipL32-F: 5′-GGACGGTTTAGTCGATGGAA-3′ and LipL32-R: 5′-GCATAATCGCCGACATTCTT-3′) were reported [10]. The PCR reaction mixture was prepared following a protocol by Jittimanee and Wongbutdee [10]. DNA amplification was performed under the following conditions: pre-amplification for 1 cycle at 95 °C for 1 min, and PCR amplification for 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s. *Leptospira interrogans* serovar Pomona DNA was used as a positive control, while the *GAPDH* gene specific to rat were amplified in all samples to ensure that DNA had been properly extracted from rats' kidney. The PCR amplification products (8 μl) were analyzed using 2% agarose gel electrophoresis.

Statistical analyses

Data was analyzed by using frequencies, percentages, means, and standard deviations.

The PRL and PBL were determined a nominal distribution, which were rejected assumption by Kolmogorov–Smirnov test. Therefore, the non-parametric statistic was used to investigate the group differences. The Mann Whitney U test analyzed the total score of PRL and PBL between village present case reports and village absent case reports with the significance at level 0.05.

Table 1
Numbers and percentages of socio-demographic information of participants from village with/without history of leptospirosis.

Social demographic information	With case history		Without case history	
	Number (n)	%	Number (n)	%
Gender				
Male	8	13.3	16	33.3
Female	52	86.7	32	66.7
Status				
Single	10	16.6	5	10.4
Married	43	71.7	36	75.0
Divorced/widowed	7	11.7	7	14.6
Education				
Elementary and lower	47	78.3	32	66.7
Secondary school	7	11.7	4	8.3
High school	3	5.0	9	18.8
Diploma	–	–	2	4.2
Bachelor	3	5.0	–	–
Monthly income (1 bath = 32.20 USD)				
less than 5000 Bath (155.28 USD)	38	63.3	27	56.3
5000–10,000 bath (155.28–310.55 USD)	17	28.3	14	29.2
10,000–20,000 bath (310.55–621.11 USD)	4	6.7	5	10.4
More than 20,000 bath (621.11 USD	1	1.7	2	4.1
Careers				
Farmer	50	83.3	38	79.2
General hired hand	–	–	3	2.1
Merchant	4	6.7	3	6.3
Government officer	1	1.7	1	2.1
Other	5	8.3	3	6.3
Perceptions of information about leptospirosis				
Got information from external sources	1	1.6	5	10.4
Did not get information from external sources	59	26.7	43	89.6
Animal husbandry				
I breed animals such as dogs, cats, buffalos, and pigs	44	73.3	29	60.4
I do not breed animals	16	26.7	19	39.6
Time per day for farming/plowing/sowing without shoes				
Less than 6 h	8	13.3	23	51.1
More than 6 h	52	86.7	22	48.9

Results

In 2015, the socio-demographic information of village case report showed that 86.7% were female and 71.7% were married. 78.3% had completed elementary school and lower. The percentages of monthly revenues that were less than 5000 baht (155.28 USD) were 63.3%, and 83.3% were engaged in farming. The information in village non-case reports showed that 66.7% were female and 75.0% were married. 66.7% had completed elementary school and lower. The percentage of monthly revenues that were less than 5000 baht (155.28 USD) were 56.3%, and 77.1% were engaged in farming (Table 1).

Perceptions regarding leptospirosis

Leptospirosis perception of volunteers is shown in Table 2. In village case reports, most subjects agreed that leptospirosis is deadly to humans (98.3%). Fever, severe headache, and severe muscle pain, especially in the calf and femur were the main symptoms of leptospirosis (98.3%). Wearing boots and gloves can prevent leptospirosis (98.3%). However, there was still some (78.3%) of subjects that disagreed with the statement that "The incubation period of leptospirosis is 60 days", and 51.7% disagreed that only farmers can be infected with leptospirosis. All participants of village without history of leptospirosis strongly agreed that wearing boots and

Table 2
Perceptions regarding leptospirosis of participants from village with/without history of leptospirosis in 2015.

Perceptions regarding leptospirosis	With case history		Without case history	
	Agree N (%)	Disagree N (%)	Agree N (%)	Disagree N (%)
1. Leptospirosis is caused by bacteria in rat's urine	53 (88.3)	7 (11.7)	46 (95.8)	2 (4.2)
2. Leptospirosis is caused by bacteria in the urine of pigs, cattle, and dogs	40 (66.7)	20 (33.3)	33 (68.7)	15 (31.3)
3. The cause of Leptospirosis is *Leptospira*	30 (50.0)	30 (50.0)	35 (72.9)	13 (27.1)
4. Leptospirosis occurs in the rainy season	58 (96.7)	2 (3.3)	46 (95.8)	2 (4.2)
5. The animals that spread the disease are rat, pigs, cattle, and dogs	50 (83.3)	10 (16.7)	42 (87.5)	6 (12.5)
6. Leptospirosis exists in water sources and canals	56 (93.3)	4 (6.7)	47 (97.2)	1 (2.1)
7. Wet soil is a source of Leptospirosis	57 (95.0)	3 (5.0)	47 (97.2)	1 (2.1)
8. Leptospirosis infection can be caused by contact with the urine of infected animals and contaminated water	58 (96.7)	2 (3.3)	44 (91.7)	4 (8.3)
9. Leptospirosis infection is caused by inhalation	43 (71.7)	17 (28.3)	28 (58.3)	20 (41.7)
10. Leptospirosis can only infect farmers[a]	29 (48.3)	31 (51.7)	23 (47.9)	25 (52.1)
11. The incubation period of Leptospirosis is 60 days[a]	13 (21.7)	47 (78.3)	10 (20.8)	38 (79.2)
12. Leptospirosis can cause death	59 (98.3)	1 (1.7)	46 (95.8)	2 (4.2)
13. The main symptoms of Leptospirosis include fever, severe headache, and severe muscle pain, especially in the calf and femur	59 (98.3)	1 (1.7)	46 (95.8)	2 (4.2)
14. Patients with severe Leptospirosis have such symptoms as jaundice, acute liver failure, and acute kidney failure	49 (81.7)	11 (18.3)	44 (91.7)	4 (8.3)
15. Wearing of boots and gloves can prevent Leptospirosis	59 (98.3)	1 (1.7)	48(100.0)	–
16. Dead animals from areas affected by Leptospirosis or with unknown causes of death should not be consumed	56 (93.3)	4 (6.7)	44 (91.7)	4 (8.3)

[a] Remarks: the questions are negative meaning item.

Table 3
Perception level (s) of leptospirosis of participants from village with/without history of leptospirosis.

Leptospirosis perception level	With case history		Without case history	
	Number	Percent	Number	Percent
High level	55	91.6	44	91.6
Middle level	4	6.7	3	6.3
Low level	1	1.7	1	2.1
Total	60	100	48	100

Mean = 13.55, SD = 3.37.
Z of Mann–Whitney U = −1.025, p-value = 0.306.

gloves could prevent leptospirosis infection. They also agreed that fever, severe headache, and severe muscle pain, especially in the calf and femur were the main symptoms of leptospirosis (95.8%) and that leptospirosis is deadly to humans (95.8%). However, there was still some (79.2%) of subjects that disagreed with the statement "The incubation period of leptospirosis is 60 days", and 52.1% disagreed that only farmers can be infected with leptospirosis.

The perception level of leptospirosis was shown to be high in both villages with 91.6% showing a basic knowledge of leptospirosis (Table 3). The statistical analysis of perception level of leptospirosis in both villages showed no significant difference (p-value 0.306).

Leptospirosis preventive behavior

Leptospirosis preventive behavior from questionnaires is shown in Table 4. Most subjects washed their hands before and after eating (91.7%) and drank clean water (boiled water, tap water, and bottled water) (83.3%). However, most subjects had incorrect

Table 4
Preventive behaviors in relation to leptospirosis of participants from village with/without history of leptospirosis.

Leptospirosis preventive behavior	With case history				Without case history			
	Always N (%)	Often N (%)	Sometimes N (%)	Never N (%)	Always N (%)	Often N (%)	Sometimes N (%)	Never N (%)
1. You bring animals, such as cattle, pigs, dogs, and cats, to breed in your home[a]	27 (45.0)	3 (5.0)	–	30 (50.0)	22 (45.8)	5 (10.4)	1 (2.1)	20 (41.7)
2. You get rid of animal feces from the animal husbandry area	26 (43.3)	9 (15.0)	9 (15.0)	16 (26.7)	22 (45.9)	4 (8.3)	5 (10.4)	17 (35.4)
3. You drain waste water from stalls or house	42 (70.0)	13 (21.7)	1 (1.6)	4 (6.7)	35 (72.8)	3 (6.3)	3 (6.3)	7 (14.6)
4. You wash your hands every time before and after eating	55 (91.7)	4 (6.7)	1 (1.6)	–	43 (89.6)	1 (2.1)	1 (2.1)	3 (6.2)
5. You always eat clean and freshly cooked food	45 (75.1)	11 (18.3)	2 (3.3)	2 (3.3)	43 (89.5)	1 (2.1)	1 (2.1)	3 (6.3)
6. You eat half-cooked meat or entrails of animals[a]	36 (60.0)	20 (33.3)	1 (1.7)	3 (5.0)	24 (50.0)	17 (35.4)	1 (2.1)	6 (12.5)
7. You always drink clean water (boiled water, tap water, bottled water)	50 (83.3)	9 (15.0)	–	1 (1.7)	42 (87.5)	–	1 (2.1)	5 (10.4)
8. You bathe or soak in water sources, such as canals, swamps, and creeks[a]	39 (65.0)	17 (28.3)	3 (5.0)	1 (1.7)	37 (77.0)	7 (14.6)	1 (2.1)	3 (6.3)
9. You trample in mud barefooted while having wounds/scratches on your legs and/or feet[a]	33 (55.0)	22 (36.8)	3 (5.0)	2 (3.2)	37 (77.0)	7 (14.6)	1 (2.1)	3 (6.3)
10. You do not wear boots while walking in water while farming and/or gardening[a]	24 (40.0)	14 (23.3)	9 (15.0)	13 (21.7)	23 (47.9)	11 (22.9)	–	14 (29.2)
11. You are in contact urine of cattle, rats, and/or pigs every day[a]	48 (80.0)	6 (10.0)	3 (5.0)	3 (5.0)	35 (72.9)	6 (12.5)	2 (4.2)	5 (10.4)
12. You wash your body with clean water and soap immediately after walking in water, mud, and/or fields	42 (70.0)	8 (13.3)	10 (16.7)	–	42 (87.5)	–	2 (4.2)	4 (8.3)
13. You dissect animals while wearing gloves	5 (8.3)	10 (16.7)	4 (6.7)	41 (68.3)	6 (12.5)	4 (8.3)	2 (4.2)	36 (75.0)
14. You wash your hands with clean water and soap after touching dead bodies of animals	32 (53.3)	14 (23.4)	8 (13.3)	6 (10.0)	40 (83.3)	–	3 (6.3)	5 (10.4)
15. You control and eliminate rats in and around the house areas through the use of cages, rat traps, and/or rat glue	14 (23.3)	9 (15.1)	11 (18.3)	26 (43.3)	10 (20.8)	9 (18.8)	7 (14.6)	22 (45.8)
16. You clean rubbish from the area around your house[a]	5 (8.3)	13 (21.7)	13 (21.7)	29 (48.3)	3 (6.3)	8 (16.7)	4 (8.3)	33 (68.7)

[a] Remarks: the questions are negatively contrasted.

Table 5
Leptospirosis preventive behavior of participants from village with/without history of leptospirosis.

Leptospirosis preventive behavior	With case history		Without case history	
	Number	Percent	Number	Percent
High level	0	0	2	1.9
Middle level	58	96.7	40	90.7
Low level	2	3.3	6	7.4
Total	60	100	48	100

Mean = 28.90, SD = 6.11.
Z of Mann–Whitney U = −1.575, p-value = 0.115.

preventive behaviors in village case reports. For example, the participants came in direct contact with urine of cattle, rats, and/or pigs every day (80.0%). Most subjects did not wear gloves while dissecting animals (68.3%) and had bathed or had soaked in water sources such as canals, swamps, and creeks (65.0%). In village non-case reports, leptospirosis preventive behaviors involved the participants washing hands before and after eating (89.6%) and ate half-cooked meat or entrails of animals (89.5%). However, most subjects bathed or soaked in water sources such as canals, swamps, and creeks and trampled barefoot in mud while having leg or feet wounds/scratches (77.0%).

The preventive behaviors related to leptospirosis were at middle levels in both villages. There were practiced in 96.7% of subjects in villages with case history and in 90.7% in villages without case history (Table 5). The statistical analysis of the leptospirosis preventive behavior in both villages was no significant different (p-value 0.115).

Leptospira *detection in rats and water samples*

The PCR technique was used to detect *Leptospira* in rats and surface water samples. The *16s rRNA* and *LipL32* genes were a target to diagnose *Leptospira* in rats' kidneys. These genes could not be detected in all 270 rats' kidney DNA samples taken from villages with/without case history, as well as all 100 surface water samples showed no detection of *Leptospira*.

Discussion

This study revealed that most participants agreed with the notion that leptospirosis can causes death. Wearing boots or/and gloves can prevent leptospirosis. The main symptoms of leptospirosis were fever, severe headache, and severe muscle pain, especially in the calf and femur. Leptospirosis outbreaks occur in the rainy season and *Leptospira* can survive in the wetlands. Leptospirosis infection can be caused by contact with the urine of infected animals and contaminated water. The Ministry of Public Health, Thailand has been running the policy initiatives for prevention and is key in the control and surveillance of leptospirosis in risky areas. They suggest that people should be educated on how a person can contact the disease. This means avoiding swimming, soaking, or bathing in water that may be contaminated with urine infection, and always wear boots and/or gloves around potentially contaminated water. Workers who are at risk for leptospirosis should always be informed to wear rubber gloves and boots. If an infected animal is found, people must separate the animal to prevent transmission to other animals. In addition, village people should jointly control rodent rats in particular habitats where human contact is likely such as use of any poison, closure of rodent access to house, and use of rat traps [12]. This is particularly true in rural areas, residential areas, work places, and tourist attractions. Despite the results showing that the participants' perceptions regarding leptospirosis were at a high level (91.6%), there were some false perceptions held by respondents. Remarkably, some people still believe that only farmers are affected by the disease (48.3% in village case reports and 47.9% in village non-case reports). These results are very similar to an earlier study done in 2013 [9]. In addition, the participants in local areas had not received information regarding leptospirosis from external sources. This includes information from television, public health volunteers, hospital staff, and related agencies. Thus, the researchers cooperated with hospital and public health volunteers to provide campaign activities and interventions that provided knowledge about leptospirosis to the local villagers.

For determining the levels of leptospirosis preventive behavior, the questionnaires were designed for measuring the preventive behaviors that had been practiced every day of the week. This study showed this level to be only at a moderate level of 96.7% in villages with case histories, and at 90.7% in villages without case histories. These people demonstrated good behaviors such as washing hands each time before and after eating. They also always drank clean water by boiling it, drinking tap water, and drank bottled water. Similarly, previous studies indicated that washing hands and drinking clean water could prevent exposure to the pathogen *Leptosprira* [13]. Nevertheless, there are still concerns about people living in rural areas since most people did not wear boots for activities involved with growing and harvesting rice. If the skin is broken from a cut, scratch, or canker sore the bacteria *Leptospira* can enter the body through the skin [1,9,14,15]. Sakundarno et al. [16] found that people that had not used personal protective equipment were at significant risk for leptospirosis infection. Additionally, some researchers reported that prolonged barefooted exposure to water leads to high risk for contracting leptospirosis [17–19]. In addition, most people had still contacts with the urine of cattle, rats, and pigs through bad behaviors for prevention (Table 4). Thus, our prevention campaigns included brochures and posters, role playing, and games that demonstrated to participants how to enhance effective leptospirosis surveillance and control. The strategies of prevention and control of leptospirosis play a major role in reducing risks. It should be continuously promoted for all people engaged in high risk activities.

Although, *Leptospira* could not be detected in trapped rats in villages which had case reported histories of leptospirosis patients; or in villages without case history; there was still reports obtained from The Pharoj Sub-district Health Promoting Hospital that found leptospirosis patients in village case reports filed during 2014–2015. The *Leptospira* infection in this village may have been caused by other *Leptospira* reservoir hosts such as cattle, dogs, and pigs like in other previous studies [4,18,20]. Using poor protection behaviors such as being less concerned about wearing boots during farming and gardening activity can increase the risk to contact to *Leptospira* contaminated water and soil. In our study, we could not detect *Leptospira* in the surface water samples that were randomly collected from villages with case histories. Although leptospirosis patients were found in this area, the details regarding the specific areas and the timing of infection of these leptospirosis patients were not available. This data can be benefit to survey *Leptospira* in environment as shown in other research, the exactly date and suspect area of infection can help the researcher for seeking *Leptospira* in water and soil and assessing the environmental risk [21]. There also should be similar attempts to focus on other water sources from nearby residential areas that have case histories of leptospirosis patient [22].

Conclusions

The perception level of leptospirosis in both village respondents (with/without leptospirosis patients) was not different, with both having a high level of knowledge. However preventive behaviors in these villages were only at a moderate level. Appropriate per-

ceptions and preventative behaviors regarding leptospirosis play a major role in monitoring and controlling the disease. Consequently, health care staff and relevant agencies must promote activities that lead to correct perceptions and reduce risky behaviors that pave the way for healthy lifestyles that minimize the risk of leptospirosis. Although *Leptospira* had not been detected in rats and surface water samples used in this study, our research survey of *Leptospira* in the environment is still beneficial for better assessing the environmental risk for leptospirosis, and for improving the perceptions and positive behaviors necessary for prevention and control of leptospirosis.

Funding

This research was supported by Grants from The Office of Research Administration and Academic Service Ubon Ratchathani University, Thailand.

Competing interests

None declared.

Ethical approval

Animal protocols were approved by The Ubon Ratchathani University-Animal Care and Use Committee (ID#5/2555/research).

Human protocols were approved by The Ubon Ratchathani University-Human Ethic Committee (ID# UBU-EC-2/2557).

Acknowledgements

We would also like to express thanks to Mr. Wacharapong Saengnill at College of Medicine and Public Health, Ubon Ratchathani University for assistant research and technical support. Finally, appreciation is expressed to The Phraroj Tambon Health Promoting Hospital, Ubon Ratchathani Province for its collaboration in this study.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jiph.2019.03.019.

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