Environmental and Travel Factors Related to Leptospirosis in Thailand

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Abstract

To identify potential environmental and travel factors related to leptospirosis, we conducted an unmatched case controlled study and household assessment of cases and controls in Nakhon Ratchasima province (north-eastern, Thailand) from August to December, 1998. Fifty-six cases and 145 controls were included in the study. Cases were hospitalized patients who had been diagnosed with leptospirosis and tested positive for anti-leptospiral IgM antibody using the Panbio ELISA (Panbio Inc, Brisbane, Australia). Controls were the neighbors of cases who had tested negative. Standardized questionnaires and household assessments were used to collect information on demographics, number of animals kept, evidence of rats in the home, presence of rat food inside the home, road characteristics, awareness of leptospirosis disease, environment, and travel history. Multivariant, unconditional logistic regression demonstrated that travel on potholed roads was independently associated with leptospirosis infection (OR 5.0; 95%CI 1.2-20.2) and traveling by car was a protective factor (OR 0.2; 95%CI 0.06-0.9).

Key word : Leptospirosis, Travel, Risks Factor, Thailand, Environment

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Leptospirosis in Thailand has been primarily related to indirect occupational exposure to contaminated water. Before 1996, leptospirosis occurred sporadically throughout the country with outbreaks usually associated with flooding. In 1997, a large epidemic occurred in 15 northeastern provinces and spread to other regions of the country in 1998. Although large in scope, its epidemiological pattern was similar to earlier outbreaks. Most of the affected people were male farmers of working age (15-54 years), and peak incidence was in October(1). New and greater numbers of serovars have been recognized(2). However, flooding could not be explained as the important factor in the ongoing epidemic. The identified risk factors were related to rice activities and walking through water(3). Field rodents (*Bandicota indica*) were identified as a major reservoir(4).

Leptospires need many factors to survive in the environment and maintain a life cycle in its reservoirs. The disease is recognized as a public health problem when human leptospirosis cases are present. However, enzootic or epizootic outbreaks may occur when the factors of agent and reservoir get together. This can be called the silent zone of leptospirosis. Therefore, the potential for a human leptospirosis epidemic can occur when the environment changes to increase the opportunities for humans to contact *leptospires.*

This study aimed to address the variations of animal exposure and the factors of environment and travel in an effort to understand more about the factors which increase the risk or enhance protection against leptospirosis. Comparisons between the environments of cases and controls, such as the sanitation of housing, the keeping of animals, and differences in travel factors, should be useful for prevention and control of leptospirosis.

MATERIAL AND METHOD

Study site

Nakhon Ratchasima province, the largest province in the northeastern region, is divided into 24 districts and has a population of 2.5 million. It is located 256 kilometers from the capital, Bangkok, and is close to Cambodia. About 67 per cent of the people are farmers. In 1996, an outbreak of leptospirosis was recognized in two districts. By 1997, the morbidity rate for leptospirosis in this province was 16 per 100,000 people.

Definition

Suspected cases were patients who were diagnosed with leptospirosis. Confirmed cases were suspected cases that had a positive ELISA IgM antibody test. Controls were neighbors of the confirmed cases. Controls with a history of illness within 30 days prior to interview, or were positive for IgM anti-leptospiral antibodies were excluded. Controls were identified from the neighboring household of each case-patient from the same village. For the selection of controls from a village, an opposite house was selected.

Unmatched case control study

An unmatched case control study was conducted including a household assessment procedure in the houses of cases and controls from August through December 1998. The interviews and household assessments were performed within seven days after the patients had been clinically diagnosed with leptospirosis. The standardized questionnaires and a household assessment were used to collect information on the demographics of cases and controls, number of animals kept, evidence of rats in the home, evidence of rat food inside the home, characteristics of the roads, awareness of leptospirosis disease, environment, and history of travel. Data from cases that were not confirmed by the laboratory were excluded.

Laboratory studies

Blood was collected from each suspected case and from each control and allowed to clot for at least 3 hours at 10°C. Samples were centrifuged, the serum was separated, and serum samples were kept frozen until tested. Serum samples were evaluated for the presence of anti-*leptospir*a IgM antibodies using the Panbio IgM ELISA (Panbio Inc, Brisbane, Australia) at the provincial hospital laboratory. Serum was diluted 1/100 in diluent provided with the ELISA kit; diluted serum was incubated with antigen-coagmicrowell strips for 20 min at room temperature (100 µl/well). The wells were then washed with phosphate buffered saline containing 0.05 per cent Tween 20, incubated for 20 min with anti-human IgM peroxidase (100 µl/well) and washed again. This was followed by 10-min incubation with tetramethylbenzidine substrate (100 µl/well). The reaction was stopped by the addition of

100 μl of phosphoric acid per well, and the strips were read at 450 nm with a microplate reader. Results were interpreted according to the manufacturer's instructions.

Statistical analysis

Data entry was done with Epi Info software (Version 6.02/World Health Organization). Subjects were sorted based on cases and controls. Chi-square tests were done for keeping animals, and a p-value < 0.05 was considered significant. Univariate descriptive statistics and unmatched odds ratios were also calculated and assessed using Epi Info software. Multivariable, unconditional logistic regression, using the Stata software system (Version 5.0) was performed to determine independent risk factors for disease.

RESULTS

Demographic data

From August to December, 80 suspected cases were enrolled from the Nakhon Ratchasima province. (Nakhon Ratchasima is in the fifth Public Health Region which had the highest attack rate). Of these, 62 cases were confirmed by IgM ELISA. The mean age of these 62 cases was 37 yrs, SD 13.8 (median = 36 yrs, ranged from 15 to 59 yrs). The ratio of males to females was 9.3 to 1 (56:6). Forty-five (72.6%) of the 62 cases were rice farmers and, 6 cases (9.7%) were dry land farmers. Three of the 62 confirmed cases died from severe complications and were excluded (CFR = 4.8%). All cases gave a history of fever at the time of presentation (Fig. 1). The three main symptoms most frequently reported were fever, headache, and myalgia, while 50 per cent had muscle tenderness and 46.8 per cent had fever of more than 39°C. Headaches were reported to be acute and severe. The occurrence of leptospirosis cases was highest in October.

The study enrolled 145 controls. The male: female respondent ratio was 11.1:1 (133:12). The mean age was 36.9, SD 14.1 (median 34, range 15-71). Sixty-seven per cent (98/145) were rice farmers, 10 per cent (15/145) were dry land farmers. There were no significant differences between age, gender, and occupation among cases and controls. Ninety-three per cent of respondents lived in rural areas, 1.8 per cent in suburban and 1.8 per cent in urban areas. Ninety-seven per cent lived in flat areas, 16.1 per cent lived near water reservoirs, and 1.8 per cent lived in mountainous areas. There were no significant differences in the number of house occupants in case homes (mean 4.39, median 4, range 2-10) and the control homes (mean 4.4, median 4, range 1-9).

Unmatched case control study

Domestic animals were kept by 87.5 per cent and 86.2 per cent of the cases and controls, respectively. Seventy per cent of the respondents kept chickens and fifty per cent kept dogs. Controls kept more cats and pigs than the cases, while the cases kept more cows and water buffalo than the controls. However, the number of cows kept by the controls was more than the cases. There were no significant differences in animal type between cases and controls (Table 1).

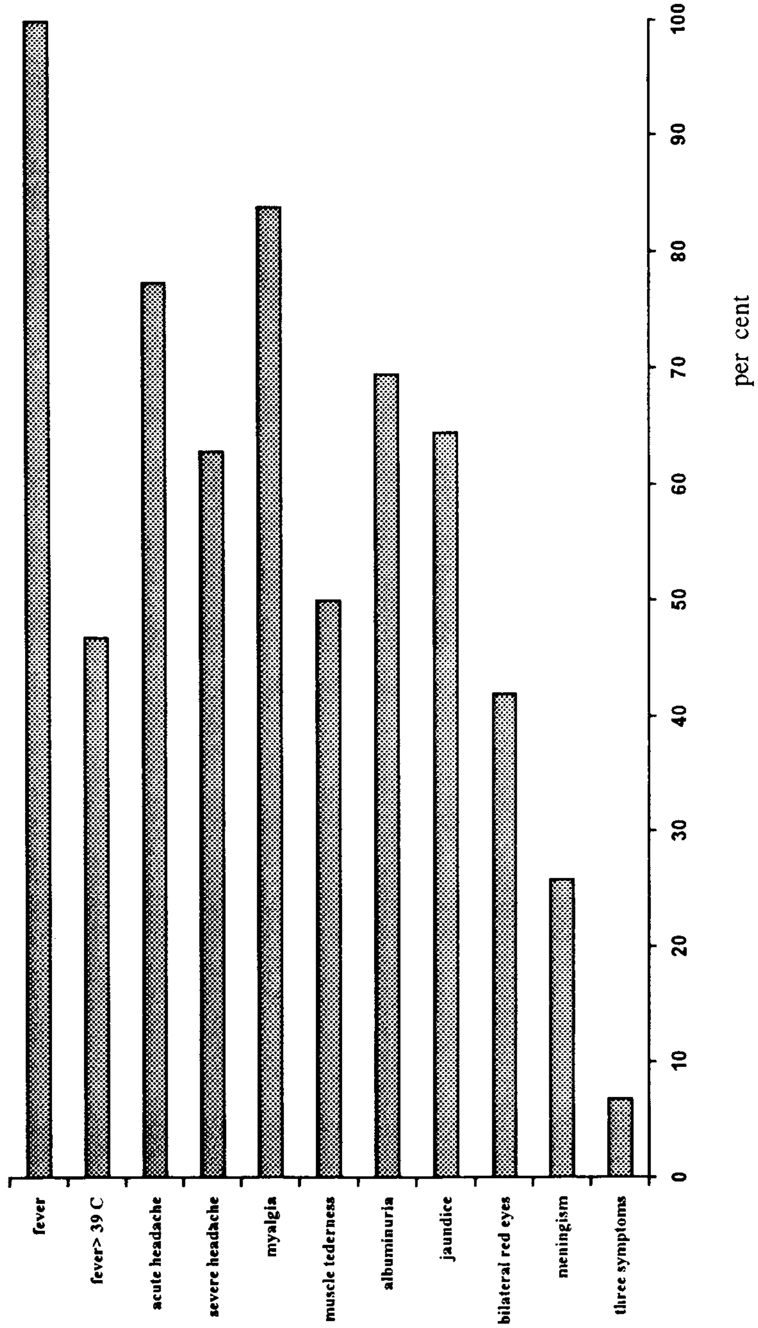
Results of the unmatched, univariate analysis comparing cases and controls are shown in Table 2. Patients were significantly more likely than controls to have reported walking on potholed roads. Controls were significantly more likely than patients to have reported more awareness of leptospirosis and traveled by car on asphalt roads. To control for possible confounding factors and determine independent risk factors for disease, mutivariate unconditional logistic regression was performed (Table 3). The adjusted odd ratios suggested that cases were more likely than controls to have traveled on potholed roads (OR 5.0; 95%CI 1.2-20.2). Traveling by car was identified as a protective factor (OR 0.2; 95%CI 0.06-0.9).

DISCUSSION

Leptospires survive very well in humid areas with warm temperatures (28-32°C) and mild pH (6.2-8.0). A variety of animals can serve as its reservoir. Therefore, animal food sources, habitats,

Table 1 Comparative frequency results for keeping animals.

Type	Case kept %	Control kept %	P-value
Chicken	73.5	70.1	0.8
Dog	53.1	52.0	0.9
Cat	10.2	19.7	0.2
Duck	14.3	10.2	0.6
Cow	8.2	5.6	0.8
Buffalo	6.1	3.1	0.6
Pig	0	6	0.3



3- plus = red eyes, meningism and jaundice

Fig. 1. Signs and symptoms of leptospirosis cases, Nakhon Ratchasima province, August-December, 1998.

Table 2. Potential risk factors among patient-control study participants with associated unmatched odd ratios (ORs) and 95% confidence intervals (95%CIs).

Potential factors	Case	%	Control	%	Univariate OR (95%CI)
Poor sanitation	12.5	7/56	4.8	7/145	2.8 (0.9, 8.4)
Keeping pet dog	53.1	26/49	52.0	66/128	1.1 (0.6, 2.1)
Keeping cat	10.2	5/49	19.7	25/129	0.5 (0.2, 1.3)
Evidence of rats food in house	85.5	47/55	80.0	116/145	1.5 (0.6, 3.5)
Evidence of rats inside home	90.9	50/55	84.2	123/145	1.8 (0.6, 4.9)
Traveling by car	51.8	29/56	81.9	118/144	0.2 (0.1, 0.5)*
Traveling by walk	66.1	37/56	45.1	65/144	2.4 (1.3, 4.5)*
Patholes road	37.5	21/56	17.1	25/144	2.9 (1.5, 5.9)*
Asphalt road	25.0	14/56	44.4	64/144	0.4 (0.2, 0.8)*
Awareness of leptospirosis	16.1	9/56	34.0	49/144	0.4 (0.2, 0.8)*

* = significant

Table 3. Multivariate analysis of potential risk factors among patient-control study participants.

Potential factors	Case	%	Control	%	Univariate OR (95%CI)	Multivariate OR (95%CI)
Poor sanitation	12.5	7/56	4.8	7/145	2.8 (0.9, 8.4)	0.3 (0.02, 4.4)
Keeping pet dog	53.1	26/49	52.0	66/128	1.1 (0.6, 2.1)	0.5 (0.1, 1.9)
Evidence of rats food in house	85.5	47/55	80.0	116/145	1.5 (0.6, 3.5)	1.2 (0.1, 9.8)
Traveling by car	51.8	29/56	81.9	118/144	0.2 (0.1, 0.5)*	0.2 (0.06, 0.9)*
Traveling by walk	66.1	37/56	45.1	65/144	2.4 (1.3, 4.5)*	2.1 (0.5, 8.6)
Patholes road	37.5	21/56	17.1	25/144	2.9 (1.5, 5.9)	5.0 (1.2, 20.2)*
Asphalt road	25.0	14/56	44.4	64/144	0.4 (0.2, 0.8)*	0.4 (0.08, 1.7)

* = significant

behaviors, predators, competitors, and other ecological parameters can determine the occurrence and local spreading of leptospirosis[5]. Cases reported poorer sanitation in their homes than controls even if there were no significant differences. The evidence of rats and their food sources inside the homes of cases supported the assumption that there may be many rats and poor sanitation in the homes of cases. Fifty per cent of respondents kept dogs. Controls were two times more likely to keep cats than were cases. Both dogs and cats can act as predators for rodents and also maintain the life cycle of *leptospires*. Thus, they can be both risk factors and protective factors for leptospirosis. Domestic cats have been reported to be less commonly infected with *leptospires* than dogs. However, there is no evidence to suggest that cats are less capable of transmitting *leptospires*[6].

The number and types of livestock raised are important when evaluating human risk from leptospirosis. Number and types of animals, transportation, and the ability to access health care can also reflect the economic status of people. Therefore, controls might be wealthier than cases because they could keep pigs, more cows, and have better access to health care than cases. Poor awareness of the disease among cases could relate to the problem of access to care or to an issue of perceptions of care. This lack of knowledge could reflect a difference in economic status as well. Differences in travel factors between cases (66%, travel by walking) and controls (82% travel by car) might further support an economic advantage in controls.

Traveling by car can prevent exposure to contaminated water from a potholed road. The potholed road can hold stagnant water, which is the main factor for the survival of *leptospires*. The potholes can also serve as reservoirs of urine on the roads. The concentration of *leptospires* in the potholed roads should be high enough to cause the disease, and walking on potholed roads could increase the risk. However, this study could not prove the presence of the organism in the potholed roads because we were not prepared to culture *leptospires* from water.

The differences in environment such as slope, soil, temperature, and humidity are the geographic limitations of leptospirosis territory for both humans and animals(7). The environments also relate to human behaviors and cultural processes which may affect the distribution of the disease. In this study, controls were the neighbors of cases so that the geographical factors of their land and home were the same. The general demographics of cases and controls were also the same. However, there were areas that deserve attention. Most respondents lived in flat areas, and only 1.6 per cent lived near mountainous areas. One reason that might support this difference in the disease incidence is that, normally, rat populations are lower than in flat areas (5). There was no significant difference in the number of occupants in case and control houses. This means that human population density was the same. Rodent population should depend on poor sanitation, and the behavior of cases and controls should be more than on population density.

Most of the cases were male farmers of working age, and rice field rats (*Bandicota indica*) were identified as a major reservoir(4). Thus, environmental assessment of the work places of cases and controls could be done. Comparisons could also be made on the temperature of the paddy fields, water levels, and the pH levels of water and soil. A clear limitation in the performance of workplace assessments in this study was the long distances between the rice fields and their homes. It was also difficult to measure and limit the borders of their paddy fields.

Many factors still need to be integrated into the understanding of leptospirosis distribution in varying regions, human behaviors, culture, and environmental differences in Thailand. However, we can enhance certain protective factors by modifying the environment, especially by improving potholed roads to prevent water and urine collection. Changing human behavior through the wearing of waterproof footwear when walking outside can decrease leptospirosis infections from contaminated water. Improving sanitation is also an effective way to decrease rodent populations by limiting sources of food and habitat for rats. All of these techniques should decrease infection by *leptospires*.

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สิ่งแวดล้อมและการเดินทางที่มีผลต่อโรคเลปโตสไปโรซีสในประเทศไทย

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การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อหาปัจจัยเสี่ยงที่มีผลต่อการเกิดโรคเลปโตสไปโรซีส เป็นการศึกษาเปรียบเทียบ (unmatched case control study) ที่จังหวัดนครราชสีมา ระหว่างเดือนสิงหาคมถึงธันวาคม ผู้ป่วย 56 ราย และกลุ่มควบคุม 145 ราย เข้าร่วมการศึกษา ผู้ป่วยที่เข้าร่วมการศึกษาเป็นผู้ป่วยในและได้รับการวินิจฉัยว่าเป็นโรคเลปโตสไปโรซีส และตรวจพบแอนติบอดีย์ชนิด IgM ต่อเชื้อ*เลปโตสไปร่า* โดยวิธี Panbio ELISA (Panbio' Inc, Brisbane, Australia) กลุ่มควบคุมเป็นเพื่อนบ้านของผู้ป่วยและไม่มีแอนติบอดีย์ชนิด IgM ต่อเชื้อ*เลปโตสไปร่า* เก็บข้อมูลโดยใช้แบบสอบถามมาตรฐานและการประเมินบ้านผู้เข้าร่วมการศึกษา ข้อมูลที่เก็บได้แก่ ข้อมูลทั่วไปของผู้ป่วย จำนวนสัตว์ที่เลี้ยงไว้ ร่องรอยของหนูและอาหารของหนูภายในบ้าน ลักษณะถนน ความตื่นตัวเกี่ยวกับโรคเลปโตสไปโรซีส สภาวะแวดล้อม และประวัติการเดินทาง วิเคราะห์ข้อมูลโดยใช้ Multivariable, unconditional logistic regression ผลการศึกษาพบว่า การเดินทางบนถนนที่มีหลุม เป็นปัจจัยเสี่ยงต่อการเป็นโรคเลปโตสไปโรซี (OR 5.0; 95%CI 1.2-20.2) และการเดินทางโดยรถยนต์เป็นปัจจัยที่ช่วยป้องกันการเกิดโรค (OR 0.2; 95%CI 0.06-0.9).

คำสำคัญ : โรคเลปโตสไปโรซีส, การเดินทาง, ปัจจัยเสี่ยง, ประเทศไทย, สิ่งแวดล้อม

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