RISK FACTORS FOR SEVERE LEPTOSPIROSIS OF KHON KAEN PROVINCE: A CASE-CONTROL STUDY

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ABSTRACT: This unmatched case-control study aimed to identify risk factors for severe leptospirosis (compared to non-severe leptospirosis) among leptospirosis cases who lived in urban and rural settings in Khon Kaen Province, Thailand. The study population included all reported leptospirosis cases from passive surveillance system in Khon Kaen Province during January 1999 to December 2000. These years were selected because the province encountered important leptospirosis outbreaks which could arise initiation of setting up better leptospirosis surveillance system, improvement on clinical, and laboratory diagnosis. The results showed that among the 2,188 serologically confirmed cases, 658 (30.1%) were classified as severe. Those who resided in rural areas were protected against severe disease (OR = 0.67, 95% CI 0.47 – 0.96, p < 0.029). Those who started treatment late (> 2 days) and were farmers had 5.36 times higher odds than those who started treatment early (≤ 2 days) (95% CI: 3.10 – 9.27, p < 0.0001). Those who started treatment late (> 2 days) and were not farmers were at 2.53 times higher odds than those who started treatment early (≤ 2 days) (95% CI: 2.06 – 3.11, p < 0.0001). Those aged > 36 years were at 1.27 times odds higher than those aged ≤ 36 years (95% CI: 1.05 – 1.54, p < 0.015). These could be concluded that persons more frequently exposed to leptospirosis may develop some protection against severe forms of the infection. As per results, we are able to recommend to have emphasized on the importance of early diagnosis and prompt treatment in order to prevent the development of the severe manifestations, especially at areas that have same epidemiological and environmental contexts. Further studies on potential risk environmental factors in order to reduce the number of reservoirs and occurrences of the disease in both mild and severe forms should be carried on, especially study on specific serovars that maybe affected to severe manifestations of the disease.

Keywords: Leptospirosis, Severe manifestation, Case-control, Risk factor, Khon Kaen

INTRODUCTION

Leptospirosis, a bacterial zoonosis that occurs worldwide, is caused by spirochetes of the genus *Leptospira*. It is considered a re-emerging communicable disease of potentially major global concern. Its epidemiology can be affected by social and economic developments in several tropical countries. The world-wide incidence of infection varies from sporadic cases in temperate areas, to > 80/100,000 population per year in the tropics, including some parts of the United States, the Caribbean, and some Southeast Asian countries [1, 2]. In Thailand, the incidence of reported cases of leptospirosis has been increasing at an alarming rate [3, 4]. Known risk factors include type of occupation (farmers, ranchers, abattoir workers, trappers, etc.), recreational activities, and poor sanitation. Environments with periodic flooding may constitute special risks [5].

The early detection of leptospirosis, and the institution of appropriate antibiotic treatment, is critical for those patients who might develop, or have developed, more severe signs and symptoms [6]. While it is known that only 10% of leptospirosis cases develop severe disease (including renal, hepatic, and pulmonary disease), the epidemiological and environmental risk factors

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of severe disease are still not clearly defined especially in Thailand. Knowledge of the risk factors of severe disease could assist in guiding aggressive therapy for patients at risk of severe leptospirosis and would prevent more cases by giving immediate communication intervention for prevention and control measures of the disease. To identify the risk factors of severe leptospirosis, we conducted an unmated case-control study, comparing severe leptospirosis cases with non-severe cases reported to Khon Kaen Provincial Health Office, Ministry of Public Health, Thailand, between January 1999 and December 2000 since both years are considered as important leptospirosis outbreak events in Khon Kaen province and these years have been initiating improvement passive surveillance reporting system in both clinical and laboratory approaches.

MATERIALS AND METHODS

Setting

This unmatched case-control study was conducted in Khon Kaen Province. This province is one of northeastern provinces in Thailand that has an estimated population about 1.8 millions.

Study subjects

The study subjects included all reported leptospirosis cases from the passive surveillance system; at epidemiology section of Khon Kaen Provincial Health Office (KKPHO), Ministry of Public Health (MOPH), Thailand, during January 1999 to December 2000. All cases were residing in Khon Kaen Province at least a month before contacting with leptospirosis.

Definition of severe leptospirosis case

A case of leptospirosis was defined as a resident of Khon Kaen Province who had developed febrile illness between January 1999 and December 2000, consistent with the WHO case definition of leptospirosis [7], with at least one of positive laboratory tests for anti-leptospiral antibodies. A severe case of leptospirosis was defined as any case of leptospirosis reported during the study period, with one or more of the following--jaundice, hemorrhagic manifestations, renal dysfunction, leukocytosis, cardiac involvement, pulmonary involvement, and death. The criteria for severity were based on the previously published literatures [3, 8-15]. Hemorrhagic manifestations included hemoptysis, pulmonary hemorrhage, subarachnoid hemorrhage, any gastrointestinal bleeding, and hematuria. Renal dysfunction was defined as renal failure, oliguria (urine output $\leq$ 0.5 L per 24 hours), anuria, or having undergone dialysis. Leukocytosis was defined as > 12,500 cells/mm^3. Cardiac involvement was defined as the presence of any abnormal electrocardiogram (EKG). Pulmonary involvement was defined as any evidence of pneumonia or pulmonary edema.

Definition of control

The controls in this study were confirmed cases of leptospirosis who did not develop any of the symptoms of severe leptospirosis listed above. They were designated as non-severe cases of leptospirosis.

Study variables

Dependent variable

The dependent variable was persons having the severe form of leptospirosis, the diagnosis of which was based on the presence of manifestations that mentioned on case definition part.

Independent variables

Five independent variables were:

- Residence

It was the place where subjects stayed at least a month before being diagnosed with leptospirosis. This was categorized into rural area, including district (Amphur) and sub-district areas (Tambon) and urban area, including municipality and sanitary areas within Khon Kaen Province. This classification was abstracted from the 506 Surveillance System Reports at epidemiology section, Khon Kaen Provincial Health Office, Thailand.

- Accessibility of health facility

Health facility was defined as any government health facilities in Khon Kaen Province, which is under the control of the Khon Kaen Provincial Health Office and Ministry of Public Health from the year 1999 to 2000. The accessibility measurement was based on the distance between house of the subject and the consulted health facility; GIS method was used to calculate this distance. Thirty kilometers was used as the cut-off point of the distance, based on the study on referral system on health service in Thailand. More than thirty kilometers was considered as "far" and the rest was considered as "near" ($\leq$ 30 kilometers) from their consulted health facility.

- History of diabetes mellitus and/or hypertension

It was defined as presence or absence of diabetes mellitus and / or hypertension before onset date of leptospirosis, based on the records. It was categorized into two groups as "with" and

"without" history of diabetes mellitus and/or hypertension in their medical records.

- Antibiotic use

It was defined as taking any appropriate antibiotics against leptospirosis. The appropriate antibiotic treatment means that subjects were given or have taken any of the following: (1) doxycycline, (2) penicillin G, (3) tetracycline, or any of their commercial preparations during the time of the illness. It could be classified into two groups: "with" and "without" proper antibiotic use.

- Initiation of treatment

It was defined as the number of days between onset of disease and first use of appropriate antibiotic treatment. Two days was used as the cut-off point of the initiation of the treatment. It was classified into two groups: early initiation of treatment (≤ 2 days) and delayed initiation of treatment (> 2 days) from the onset of the disease.

Socio-demographic variables

Four socio-demographic variables were:

- Age

The age in years of subjects that was stated in the 506 Surveillance System Reports at the time of the illness. It was categorized into two age groups as age > 36 years old and ≤ 36 years old, based on the Brazil Study [14].

- Gender

The gender was categorized as male and female.

- Marital status

The marital status of subjects was classified into two groups as married and non-married which includes single, widowed, separated and divorced.

- Occupation

The occupations of the subjects as recorded in the 506 Surveillance System Report were classified into two groups as farmer, defined as any subject who farm rice, corn and/or perform any other field procedures at the time of the illness and non-farmer, that defined as subjects who did not farm rice, corn nor perform any other field procedures.

Data collection procedure

Check-list form was designed to gather interested information. The data were collected form non-hospitalized and hospitalized cases which were reported to Khon Kaen Provincial Health Office passive surveillance system by health facilities within the province. The reports included 506 and 507 Surveillance System reporting forms, individual and outbreak investigation reports as well as hospital and/or health facilities records from 22 government health facilities within the province during year 1999 and 2000.

Ethical consideration

This study was approved to conduct this study by Khon Kaen Regional Hospital Ethical Committees.

Data analysis

Descriptive analysis consisted of determining the frequency and percentage distribution of subjects according to the variables of interest. Inferential analysis consisted of univariate, stratified and multiple logistic regression analyses, utilizing STATA version 7 computer software (Reference: serial 197045144).

Crude odds ratios (OR) , 95% confidence intervals (95% CI), and p-values for associations between independent variables and severe leptospirosis were computed using the chi-square test (x^2) or Fisher's exact test. Variables with p-values ≤ 0.25 were included in stratified and multiple logistic regression analyses, based on Hosmer and Lemeshow 's algorithm [16]. Stratified analysis was used to determine potential confounders and effect measure modifiers in the association between selected variables and severe leptospirosis. Finally, multiple logistic regression analysis was used to determine the association between the severe form of leptospirosis and independent variables, controlling for selected potential confounders, and the effect modifiers identified from univariate and stratified analyses. Independent variables, whose interaction terms had p-values < 0.10 from stratified analysis, or which resulted in stratum-specific risk ratios that greatly varied from each other, were incorporated into the model. The interaction terms were added into the first main effects full model by fitting one model at a time, and LR-test results were assessed for significance of the interaction term added. Wald's tests for each of the interaction terms were also evaluated. A full model was again fitted with the main effects and significant interaction terms, which then underwent backward elimination. The variables with the p-value > 0.05 were deleted from the full model. The importance of each variable included in the model was verified by: a) examination of the Wald statistic for each variable and LR-test, to compare the full model with the reduced model. If the difference in the odds ratios from the more complete model to the reduced model was > 10%, then the factor was considered a confounder and was not deleted from the model.

Table 1 Univariate analysis of factors of severe forms of leptospirosis cases in Khon Kaen province, Thailand during January 1999 - December 2000

Variables	Categories	Severe leptospirosis n (%)	Non-severe leptospirosis n (%)	Crude Odds Ratio (95% CI)	P-value
Residence	Rural	602 (91.5%)	1433 (93.6%)	0.73 (0.52 – 1.03)	0.069
	Urban	56 (8.5%)	97 (6.4%)	1.00	
Age	> 36 yrs old	360 (54.7%)	772 (50.5%)	1.19 (0.99 – 1.42)	0.068
	≤ 36 yrs old	298 (45.3%)	758 (49.5%)	1.00	
Gender*	Male	485 (73.7 %)	1144 (74.8%)	0.95 (0.77 – 1.17)	0.601
	Female	173 (26.3%)	386 (25.2%)	1.00	
Initiation of treatment	> 2 days	428 (65.0%)	618 (40.4%)	2.75 (2.26 – 3.34)	<0.001
	≤ 2 days	230 (35.0%)	912 (59.6%)	1.00	
Occupation	Non-farmers	103 (15.6%)	173 (11.3%)	1.46 (1.12 – 1.89)	0.005
	Farmer	555 (84.4%)	1357 (88.7%)	1.00	
Accessibility of health facility*	> 30 kms	103 (16.3%)	242 (17.0%)	0.95 (0.74 – 1.23)	0.717
	≤ 30 kms	528 (83.7%)	1184 (83.0 %)	1.00	
History of diabetes and /or hypertension*	With	13 (2.0%)	3 (0.2%)	10.26 (2.91 – 36.12)	<0.001
	Without	645 (98.0%)	1527 (99.8%)	1.00	
Antibiotic use*	Without	1 (0.2%)	0.00 (0.00%)	-	-
	With	657 (99.8%)	1530 (100.00%)	1.00	
Marital Status*	Not married	79 (12.0%)	95 (6.2%)	2.06 (1.50 – 2.82)	<0.001
	Married	579 (88.0%)	1435 (93.8%)	1.00	

* Not entered in multiple logistic regression model

RESULTS

In the period January 1999 to December 2000, 2188 cases met the case definition for leptospirosis. Among these, 658 (30%) were severe cases of leptospirosis, while 1530 (69.9%) were non-severe. Among the severe cases, 121 (18.4%) had jaundice, 312 (47.4%) had hemorrhagic manifestations, 135 (20.5%) had renal dysfunction, 249 (11.4%) had leukocytosis, 39 (5.9%) had heart involvement, 10 (1.5%) had pulmonary involvement, and 60 (9.1%) died from leptospirosis. In addition, 2 cases had concurrent HIV infection; one case had tuberculosis, and another Stephen Johnson Syndrome (SJS; a form of toxic epidermal necrolysis). The estimated rate of severe leptospirosis in Khon Kaen Province was 10.84 cases / 100,000 population / year.

In Table 1 showed univariate analysis results that living in the rural areas seemed to present an apparent protective effect with odds ratio of 0.73 (95% CI: 0.52 – 1.03). However, there was no significant association between residence and severe leptospirosis (p 0.069). Cases with a history of either diabetes mellitus or hypertension had odds 10.26 times of severe leptospirosis ($p < 0.001$). Older cases (> 36 years old) were more likely to have severe leptospirosis than older cases (though not significantly, p 0.07). Gender was not associated with severity. Farming was protective against severe leptospirosis (p 0.01). Only 0.2% of those with severe leptospirosis failed to receive appropriate antibiotic treatment and all non-severe cases received antibiotic treatment. We were, therefore, unable to assess whether the provision or lack of antibiotic treatment had any effect on severity of disease.

In the multiple logistic regression analysis, five selected variables were entered in the full model. They were residence, distance to health facility, day of initiation of treatment, age, sex, occupation, and the interaction term of initiation of treatment and occupation. They were not entered because very few leptospirosis cases had history of diabetes mellitus and/or hypertension, and almost all leptospirosis patients used antibiotics. Moreover, marital status was not included because of biological implausibility even it was shown statistically significant in univariate analysis steps. In opposite, accessibility of health facility and gender were included in the stratified and multiple logistic regression analyses based on *a priori* knowledge despite not being statistically significant in univariate analysis (Table 1).

Tables 2 and 3 show results of multivariable analysis. Those who resided in rural areas were protected against severe disease (OR = 0.67, 95% CI 0.47 – 0.96, $p < 0.029$). Farmers who started treatment late (> 2 days) had odds of severe leptospirosis 2.53 times higher than in farmers who started treatment early (≤ 2 days) (95% CI: 2.06 – 3.11, $p < 0.001$). Non-farmers who started treatment late had odds of severe leptospirosis 5.36 times higher than in non-farmers who started

Table 2 Multiple logistic regression model: modeled odds ratios, 95% confidence intervals, and p-values of independent variables for severe vs. non-severe leptospirosis

Variables	Adjusted OR (95% CI)	P-value
Residence (urban vs. rural)	0.67 (0.47 – 0.96)	0.029
Age (>36 years vs. ≤36 years)	1.27 (1.05 – 1.54)	0.015
Late initiation of treatment (>2 days vs. ≤2 days after onset)	5.36 (3.10 – 9.27)	< 0.001
Occupation (non-farmers vs. farmers)	1.09 (0.69 – 1.74)	0.701
Interaction Term (Initiation of Treatment x Occupation)	0.47 (0.26 – 0.85)	0.012

Table 3 Modeled odds ratios and 95% confidence intervals of late initiation of treatment for severe leptospirosis in non-farmers and farmers in Khon Kaen Province, Thailand during January 1999 - December 2000

Variables	Categories	Adjusted OR (95% CI)	P-value
Non-farmer	Initiation of treatment ≤ 2 days	1.00	<0.001
	Initiation of treatment > 2 days	5.36 (3.10 – 9.27)	
Farmer	Initiation of treatment ≤ 2 days	1.00	<0.001
	Initiation of treatment > 2 days	2.53 (2.06 – 3.11)	

treatment early (95% CI: 3.10 – 9.27, p < 0.0001). Those aged > 36 years were had odds of severe leptospirosis 1.27 times higher than in those aged ≤ 36 years (95% CI: 1.05 – 1.54, p < 0.015).

DISCUSSION

The overall rate of severe leptospirosis in Khon Kaen Province was lower than those noted by other studies [8, 10, 12, 15-18]. This might be because the criteria for defining severe cases in Khon Kaen were somewhat different from those used in these studies [14,15,17-19], which included the additional criteria of severe anemia, electrolyte imbalance, and hemodynamic disturbance (shock).

The association of urban living with severe leptospirosis might have several explanations deserving further study. First, the frequency of exposure might be higher in rural than urban areas, providing more protection against severe leptospirosis. An association of leptospirosis with rural households has been previously reported [14]. The farming occupation was also reported as associated with leptospirosis [3,4]. This association might suggest a higher rate of infection, and concomitant potential protection against severe disease, among farmers. Second, serovars in urban areas may differ from rural ones, and may be associated with more severe disease.

The association of severe leptospirosis with increased distance from a healthcare facility has not been previously reported. This may be due to delays in care-seeking because of the distance to be traveled, or may be a marker for another leptospirosis complicating factor. Regardless of the explanation, those who live far from health facilities should be educated to seek early diagnosis and care for severe febrile illness.

We found that severe leptospirosis was associated with urban residence, increased distance of household from healthcare facility (> 30 kilometers), and initiation of treatment at > 2 days of illness. Farming appeared to be protective. These results re-emphasize the importance of early diagnosis and treatment in the prevention of severe leptospirosis. Public-health prevention programs should include this information in physician education efforts. The findings also suggested that acquired immunity may be important in preventing severe leptospirosis. Information dissemination on environmental sanitation in order to reduce the number of reservoirs of the disease should be provided to communities. More suggestion as derived from the experience of the investigation during data collection, these could be improvement in manpower resources to improve and / or sustain the surveillance system.

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DECLARATION OF CONFLICTING INTERESTS

No financial or other conflicts of interest exist.

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