CHAPTER III

Proposal: The prevalence and risk ratio of patients suspected of having leptospirosis

3.1 Introduction:

Leptospirosis is a disease that can be transmitted to people by vertebrate animals of worldwide distribution. It is caused by spirochetes of the genus *Leptospira*. Leptospirosis leads to an acute febrile illness that may be followed by more severe, potentially fatal, condition (1). Human exposure occurs either directly through contact with the urine of infected animals or indirectly through a urine-contaminated environment (1). Modes of entry of *leptospires* into the body include cuts, abrasions, waterlogged skin, mucous membranes, conjunctiva, and possibly ingestion.

Certain occupational groups have also been shown to be more vulnerable to leptospirosis infection due to exposure to animals, animal products, or contaminated soil or water. These include rice-farmers, fishermen, sugarcane workers, sewer workers, and military personnel (1). The disease is endemic in countries with a humid subtropical or climates such as Thailand, but reliable incidence figures are not available due to nonspecific clinical presentations and the prevalence of misdiagnosis. (1)

There are many possible clinical presentations and courses for leptospirosis. None of the presenting features of leptospirosis is pathognomonic signs whether of the mild or of the severe form. Thus, leptospirosis is difficult to diagnose clinically.

the mild or of the severe form. Thus, leptospirosis is difficult to diagnose clinically. Laboratory confirmation is necessary for leptospirosis diagnosis. At the time of the initially diagnosis, it is not possible to tell clinically for the courses of the disease whether the patients will develop complications or not. The delay treatment will increase the case fatality rate and complication especially more than 4 days after the onset of symptoms. Therefore, early diagnosis and treatment are very important.

World Health Organization (WHO) has recommended the standard guideline for leptospirosis diagnosis. The standard guideline composes of three parts 1) Part A clinical manifestation 2) Part B risk factors associated with leptospirosis infection and 3) Part C, the standard test results (Microscopic Agglutination Test or MAT). WHO guideline uses very wide risk factors that are not country specific. This guideline is also not appropriate in Thailand because of the limited of MAT. In Thailand, the MAT can be performed only in a specialised and well-equipped laboratories staffed by trained personnel capable to maintain cultures of leptospiral strains that are needed as the live antigen. Thus, it can only be performed in the National Institute of Health. The MAT is a disadvantage when the assay is used for diagnostic purposes because of it should be performed using a battery of strains that is representative for all in a certain area commonly occurring serovars. However, the serovar specificity of the MAT is an important epidemiological tool as it can be used for serological typing of the infecting strain. Knowledge of the serovar of the infecting strain can be essential for tracing the source of infection.

This proposal aims to adapt the WHO standard guideline tailored to specific situation in Thailand. The study adapts especially on Part B and Part C. This involves defining the specific risk factors, which had been identified in Thailand by

Tangkanakul et al, 1998 (2) and development of simple screening test (Lepto-Dipstick assay) that can be used in the field study.

3.2 Problem statements and Rationale

Epidemic of leptospirosis has occurred in Thailand since 1996. Sign and symptoms of the cases present with a sudden onset of fever with headache, myalgia (especially of calf muscles) and prostration with or without any of the following: conjunctival suffusion, meningeal irritation, anuria/oliguria and/or proteinuria, jaundice and hemorrhages of the intestines or the lungs. Ninety percent of the infected persons have mild influenza like symptoms. Ten percent develop severe disease such as jaundice, bleeding and oligouria/anuria (Weil's syndrome), meningitis or haemorrhages. Moreover, most cases present many forms and severity as mention above. Thus, an initial clinical diagnosis is not enough to diagnose the disease. Due to unavailable laboratory facilities, Thai physicians must rely on their own experiences to diagnose this disease based solely on clinical manifestations resulting in differential diagnosis.

The consequences of differential diagnosis among physicians are both overdiagnosis and late diagnosis and treatment. The result of overdiagnosis is the increasing of reported cases, which leads to increase burden of the disease. Early treatment may reduce the severity of the disease and may prevent the development of complications. Thus, the results of late diagnosis and treatment are increasing severe complication and high case fatality rate. However, actual burden of leptospirosis can not estimate and its consequences are the confusion among physicians in every

hospital level and the indirection among health workers and communities to provide the occurrence of leptospirosis.

Therefore, problem in diagnosis needs to be improved by using a standard guideline, which tailored to Thailand. With the appropriate guideline, the actual number of leptospirosis cases (actual burden of the disease) can be estimated. These confirmed cases' data may give a good epidemiological picture of cases such as; age, gender, occupation of cases, clinical picture, risk activities and risk areas etc. Case fatality rate and complication among these cases also reveal treatment and health system facilities. The control and eventually eradication of the disease strategies can develop. These benefits also emphasize the usefulness of having the standard guideline, which tailored to Thailand.

3.3 Lepto Dipstick Assay

Due to the disadvantage of the gold standard test (Microscopic Agglutination Test or MAT), the Lepto-Dipstick assays is used to measure the recent leptospirosis infection in this study. The Lepto-Dipstick developed by Royal Tropical Institute, Amsterdam, The Netherlands. It is a packaged kit (Figure 3.1) and requires no specialized equipment. Its ingredients are highly stable and can be stored at room temperature (20 ^{o}C-25 ^{o}C). No need for "live" stock 2 years shelf life at room temperature and 1 month's shelf life at temperature up to 37 ^{o}C. The assay is based on the binding of human *leptospira*-specific IgM antibodies to the *leptospira* antigen (appear 7-10 days after infection).The broadly reactive *leptospira* antigen ensures the efficient detection of a wide spectrum of *leptospira* infections. Bound IgM antibodies

are specifically detected with an anti-human IgM dye conjugate. It is an inexpensive, rapid and easy to use.

3.3.1 Sensitivity and specificity of Lepto-dipstick assay

Lepto Dipstick Assay reveals evidence of anti-*leptospiral* antibody result, but serovar identification cannot be achieved through this method alone. However, rapid diagnosis of an acute leptospirosis infection can be determined. Sensitivity and specificity of this assay are similar to those of ELISA for the detection of *leptospira*-specific IgM antibodies. IgM ELISA is accepted for being the gold standard test for detection of *leptospira* – specific IgM antibody (3). In a previous study, the Panbio *Leptospira* IgM ELISA kit has shown that the sensitivity is 100% and specificity is 93%, when compared to MAT. This study found that the IgM ELISA was more sensitive than MAT because cases were detected in fewer days after infection, than the MAT (4).

The dipstick method is the most practical test to perform in a field laboratory setting because the samples can be tested individually, and the results are easily interpreted. In the previous study, sensitivity and specificity of this test are as follows.

1). Gussenhoven et al. found in a study on Dutch patients that when compared to the MAT, the sensitivity of the dipstick assay was 86.8% and the specificity was 92.7% (5).

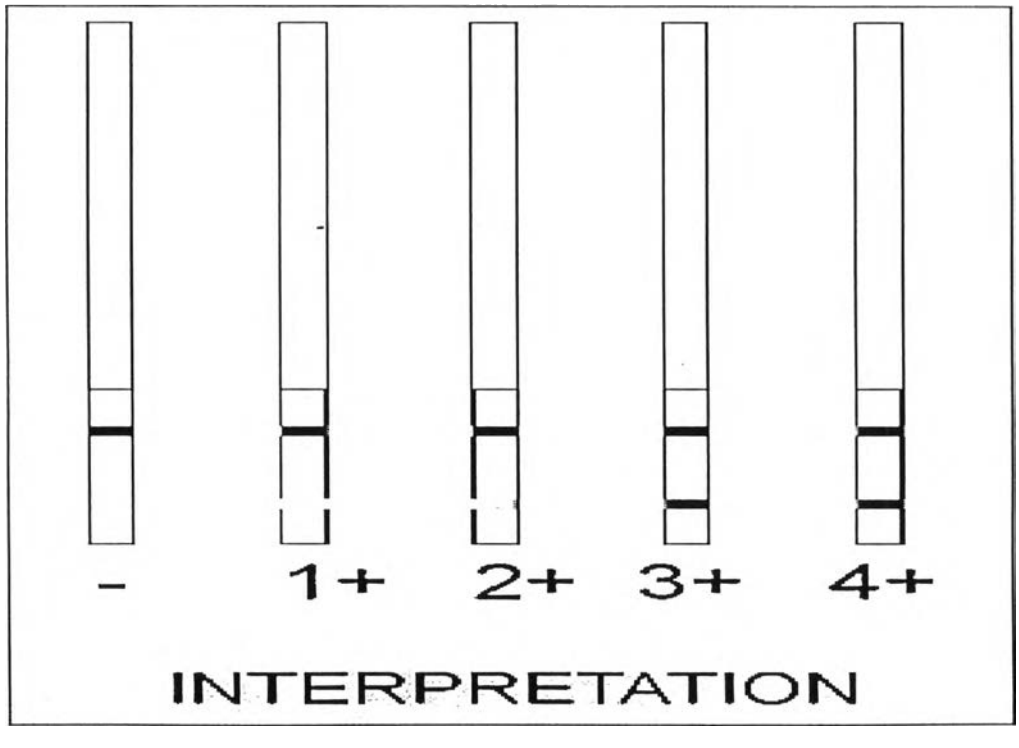
2). It has been reported, IgM specific dot-ELISA (Lepto-dipstick assay) for diagnosis of human leptospirosis had sensitivity of 91.2% and a specificity of 81% (6).

3). From the study of Silva and others (1997), sensitivity of Lepto-dipstick assay was 98% and the specificity was 100% (7). Thus, it appears to be a suitable screening test for the rapid diagnosis of leptospirosis in many clinical settings.

3.3.2 Method of Lepto-dipstick assay

Making a 1:50 dilution of serum in the detection reagent and incubating a wetted dipstick in this solution (3 hours) performs the assay. Staining of the antigen band reveals the presence of *leptospira*-specific IgM antibodies in the serum sample. A staining intensity of 2 or greater infers positively. The strength of the staining is important in the interpretation of the test results. The provided, colored reference strip is used to compare the staining intensity. Internal Control also presents.

Figure 3.1 Interpretation for Lepto Dipstick Assay



3.4 Objectives

3.4.1 General objectives

To identify and measure the effects of specific risk factors that had been identified in Thailand for leptospirosis diagnosis among patient suspected leptospirosis.

3.4.2 Specific objectives

1. To test an association between leptospirosis and specific risk factors in-patients presenting with signs and symptoms suspected leptospirosis.
2. To conduct a field test of the Lepto dipstick assay in a rural endemic tropical region.

3.4.3 Hypothesis

Prevalence of leptospirosis in-patient suspected leptotospirosis who had risk factors is more than who did not have risk factors.

3.5 Methodology

3.5.1 Study design

This study design aims to examine associations of specific risk factors in-patient suspected leptospirosis as they exist in a defined population at one particular time (an analytic cross sectional study design) (8). The respondents are classified according to present or absence specific risk factors. The risk positive patient is defined as an inpatient who presented with fever and at least one of myalgia or headache, and had engaged in at least one of the following risk factors for more than 6 hours a day during the 15 days before illness: plowing, pulling out sprouts, fertilizing and caring, or walking through water. The risk negative patient is defined as an inpatient that presented with fever and at least one of myalgia or headache but did not have the specified risk factors. Blood serum drawn from the patients are tested for IgM antibodies to leptospirosis infection with the Lepto-dipstick assay.

3.5.2 Study population and study area

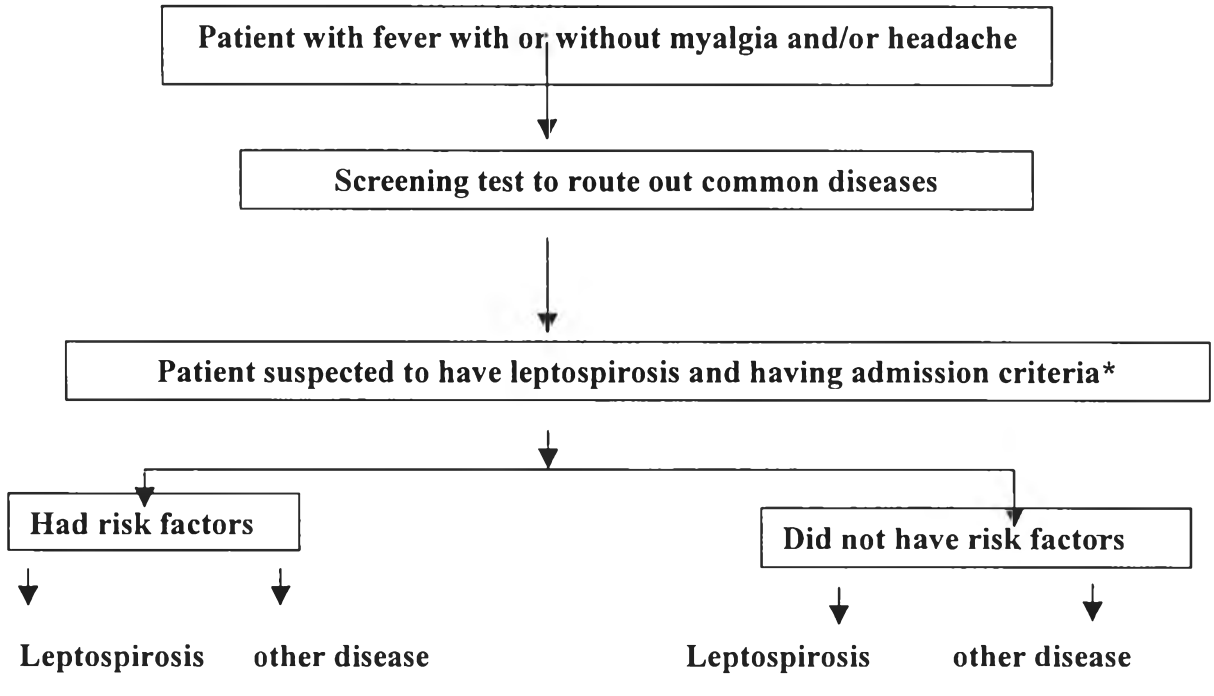
The study is conducted in 23 community hospitals in Nakorn Ratchasima province. This province is selected because its high incidence and high morbidity rate (15.76/100,000 population or 397 cases) of Leptospirosis is found in 1999. Moreover, there is a complete infrastructure of public heath system, which can support the study and make it possible to follow up all participants. Twenty-three community hospitals are chosen to ensure enough patient suspected cases during the of leptospirosis.

Nakorn Ratchasima is the largest province in the northeastern region, and it is divided into 24 districts with 2.5 millions population. It is 256 kilometers from Bangkok. About 67% of the population has agriculture occupation.

3.5.3 Direction of the study

Physicians of the hospital are informed about the study and asked to assist in collecting information from patients who present with fever and at least one of myalgia or headache. The questionnaire is used to assess the patient's exposure to the following risk factors for more than 6 hours a day during the 15 days before illness: plowing, pulling out sprouts, fertilizing and caring, or walking through water as well as demographic information. Those patients who have engaged in at least one of the activities are assigned to be the risk positive patients, while the others are assigned to be the risk negative patients. A blood sample is obtained 2 times from participants, first time on the date of admission and second time 2 weeks after admission. The Lepto-dipstick assay is performed on these samples as a screening test to determine leptospirosis infection status. Informed consent is obtained from all subjects.

Figure 3.2 Direction of the study



* = The admission criteria are the patients who have exhaustion, vital sign instability (T more than 39 centigrade, blood pressure less than 90/60 mmHg, pulse rate mare than 80 and respiratory rate more than 20), severe headache, conjunctival suffusion (red eyes) or having jaundice or renal insufficiency.

3.5.4 Sample Size

Sample size formula

$N1 = (Zalpha + Zbeta)^2 x\ PQ\ x\ (r+1)/\ (P_1 - P_0)^2$

$P = P1 + r\ P_0/\ 1+r$ and $r = n_0/n_1$

n_1 = number of exposed group (risk group)

n_0 = number of non-exposed group (control group)

r = proportion of nonexposed : exposed

P = proportion of average outcome among two group, Q=1-P

P1= proportion of exposed that has outcome

P0 = proportion of non exposed that has outcome

Based on a prevalence rate of 5% (9,10) among fever cases which could not identify source of infection (Fever of unknown origin or FUO) in the northeastern region (Table 2.3) and an odds ratio value of 4 derived from Tangkanakul et al.'s study, the sample size calculation is as follows.

$N1 = (Zalpha + Zbeta)^2 x\ PQ\ x\ (r+1)/\ (P_1 - P_0)^2$

where zalpha is 1.96

Zbeta is 1.28 (90% power)

$(Zalpha + Zbeta)^2 = (1.96 + 1.28)^2 = 10.5$

$P = P1 + r P_0 / 1 + r$ and $r = n_0/n_1$

$P = 0.2 + (0.66 \times 0.05 / 1 + 0.66) = 0.22$

(r = 40/60, 60% of population are agricultural = 0.66)

P1= proportion of exposed that has outcome = 0.05 x 4 = 0.2

P0 = proportion of non exposed that has outcome = 0.05

and P1 – P0 = (.20 -.05) = 0.15

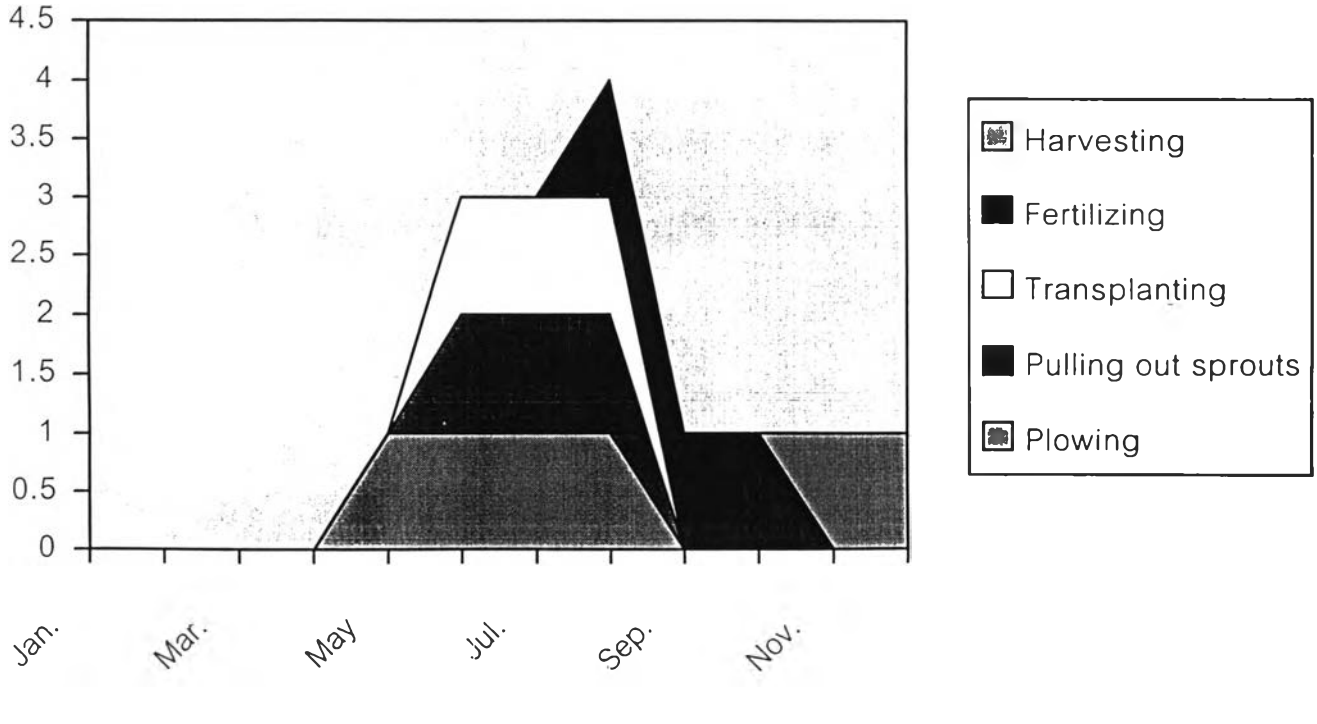
N1 = (10.5) x 0.22 x 0.78 x (0.66 +1)/ (0.15) 2 = 133, N0 = 133 x 2/3 = 89

Total sample size = 222 However, the estimate sample size is 250 (10% plus) for sparing of loss follow up cases.

3.5.5 Study period

The study conducts in the period of rice farming activities (May – December).

Figure 3.3 Period of rice farming activities



3.5.6. Laboratory Methods

Antibodies to *leptospries* can be detected late of the first week after onset of the illness but patient always seeks for treatment in the first week of illness. Thus, most serological test for leptospirosis needs four-fold rise in titer in paired serum considers being suggestion of current infection. In this study, the dipstick test is performed on the paired samples obtained from each patient. Three separate readers independently read assay results for trying to eliminate reader bias. The range of values for the test bands are –, 1+, 2+, 3+, and 4+. Serums from patients with results graded 2+ or higher are considered positive for recent leptospirosis infection.

3.5.7. Outcome variables

The outcome measure intends to compare the prevalence of leptospirosis between risk positive patients and risk negative patients.

a. Prevalence of leptospirosis in risk positive patients who have IgM positive by using lepto-dipstick assay, in all number of patients who have risk during the study period.
b. Prevalence of leptospirosis in risk negative patients who have IgM positive by using lepto-dipstick assay, in all number of patients who do not have risk during the study period.
c. Prevalence ratio is the ratio of prevalence among risk positive patients and risk negative patients.

d. Prevalence difference is the difference of prevalence among risk positive patients and risk negative patients.

3.5.8. Data collection method and instrument

The standardized questionnaire, which composes of 3, parts (Appendix 1): Part (A) asking about characteristics of the respondents; part (B) filling by the physicians and nurses whom incharges of the patients, asking about the specific risk factors. Part (C) filling by the technician whom performs lepto-dipstick assay.

3.5.9. Analysis of data

After data collection, data entry is done with Epi Info software (version 6.02, CDC, Atlanta, GA). Using lepto-dipstick assay identifies the prevalence of leptospirosis cases in risk positive and risk negative patients. Then, the prevalence ratio (risk ratio) and the prevalence differences (risk differences) are calculated. Univariate descriptive statistics and odds ratios are also calculated using EpiInfo. Logistic regression is conducted to determine independent risk.

3.5.10. Expected outcomes and benefits

a. The outcome of this study determines whether the prevalence of positive leptospirosis serum tests is higher in-patients who have risk factors versus patients who do not have risk factors. The prevalence ratio and prevalence difference

among these risks positive and risk negative patients leads to identify these specific risk factors as part of the guideline to diagnose leptospirosis in Thailand.

b. The study improves the awareness and surveillance system.

3.5.11. Potential problems

The potential problem can occur in situation of flooding, which all patients contact, to the contaminated water. Thus, the risk factors can not been identified. Some selection bias may be present which may give a higher than expected prevalence. The reasons are some patients die before admission and some patients do not come to the hospital. Because antibodies to *leptospiraes* usually reach high levels about 7-10 days after illness, tests for leptospirosis often fail to detect the disease during the early acute phase of the illness. Paired serum samples are needed to reliably make a negative diagnosis. The success of the study depends on the collection of second blood serum of cases. Furthermore, recall bias may be a factor among the patients when considering their responses to the questionnaires. This may have affected the data on days from disease onset and length of risk factor exposure. Thus, there may be incorrect identification of risk positive patients and risk negative patients.

3.6 Activities

1. Proposal submission and approval; the proposal is submitted to the Leptospirosis Control Office. After approval, the following activities are planned and carried out.
2. After approval of the proposal, the Leptospirosis Control Office initiates and coordinates the Nakorn Ratchasrima provincial health office and sets the data collection group, supervisory group and laboratory group for testing Lepto-dipstick assay.
3. Communication and inform all of the responsible doctors and nurses in each community hospital. Formation of the process to achieve the study.
4. Training the technicians for testing Lepto-dipstick assay and also the data collection group to use the questionnaire.
5. Pre-testing and revision of the questionnaire. The pre- testing of the questionnaire prior to data collection should be done in the study population, in different area. Practicability and understandability of the questionnaire should be tested and modified as necessary. It takes a week and the 2-4 activities can be carried out within the same period.
6. The study is implemented after revision of the questionnaire.
7. Data collection and evaluating of the progress of the project is done and supervised by supervisory group from Leptospirosis control office twice a month in the first three months and then once a month. The supervisory group does the logbook to access the progress of the study, the amount of second serum of the patient and also the complete of questionnaires.

8. Data entry and Data analysis; Data entry is done every month by the supervisory group. Data analysis is done after finishing the study. It takes 2 weeks for analysis of data.
9. Report writing; the researcher in supervisory group writes final report to submit to the Leptospirosis Control Office. Not only the results but also discussion and the suggestion on advantages and disadvantages for using the specific risk factors and Lepto-dipstick assay in diagnosis of leptospirosis. It takes a month.
10. Submission of the report to the Leptospirosis control office.

Table 3.1 Gantt chart showing the activities and time schedule for the study

	Activities	May (week)				Jun. (week)				Jul. (week)				Aug. (week)				Sep. (week)				Oct. (week)				Nov. (week)				Dec. (week)				Jan.	
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	2	4
1.	Proposal submission and approval																																		
2.	Formation of supervisory group, data collection group and laboratory group																																		
3.	Communication and inform all doctors and nurses in community hospital																																		
4.	Training the technician for testing Lepto-dipstick assay																																		
5.	Pre-testing and revision of questionnaire																																		
6.	Implementation of the study																																		
7.	Data collection and evaluating of the progress of the project																																		
8.	Data entry																																		
9.	Data analysis																																		
10.	Report writing																																		
11.	Submission of the final report																																		

3.7 Ethical issue

There are no serious ethical problems in the study. The only issue is that the taking the serum from the patient which may need prior inform consent of the patient.

3.8 Manpower requirement

a. Supervisory group

For the study following personnel are required for the program operation and supervision

1.	Representative from Leptospirosis control office	2
2.	Representative from National Institute of health	1
3.	Representative from Nakorn Ratchasima provincial health office	1
4.	Representative from northeastern epidemiological center	1
5.	Representative from northeastern regional laboratory center	1
6.	Total	6

b.The data collection group.

The data collection group undertakes the data collection by using the standardized questionnaire and serum collection. .

1.	One doctor from each community hospital (23 hospitals)	23
2.	One nurse from each community hospital (23 hospitals)	23
3.	One researcher from each community hospital (23 hospitals)	23
4.	Total	69

c. Laboratory group

The technicians in this group tests the serum by using the Lepto-dipstick assay and making the report for the results

1.	One technician from each community hospital (23 hospitals)	23
2.	Total	23

d. Total man power for the whole research group

1.	Supervisory group	6
2.	Data collection group	69
3.	Laboratory group	23
4.	Total	98

3.9 Budget requirement

The budget for the study is sponsored by Leptospirosis Control Office, Communicable Disease Department, and Ministry of Public Public Health, Thailand.

No	Activities	cost (bahts)
a.	**Data collection expenses**	
1.	Communication and Inform 23 doctors, 23 nurses and 6 supervisors 52 persons x 100 bahts (2 coffee break+lunch) x 2 times (before and after)	10,400
2.	Preparation and revise questionnaire by supervisors group 6 persons x 1000 bahts (residential and transportation) x 1 days	6,000
3.	Training expenses for the 23 technicians by 2 supervisors	

	25 persons x 100 bahts (2 coffee break+lunch) x 1 times	2,500
4.	Evaluation of the progress by 6 supervisors	
	6 persons x 1000 bahts (residential and transportation) x 15 times	90,000
4.	Perdium for field research	
	23 persons x 500 bath/month x 8 months	92,000
5.	Data entry and data analysis	
	Stationers and computer processing	20,000
5.	Report writing	10,000
	Total cost for data collection	230,900
b.	**Equipment and Lepto-dipstick assay**	
1.	500 Unit (10 CC syringe and needle) x 30 baht	15,000
2.	250 test for first serum and 250 test for second serum x 130 baht	65,000
	Total cost for equipment and Lepto-dipstick assay	80,000
c.	**Transportation cost for supervisors**	
1.	Central supervisors 3 persons x 15 times x 1,500 bahts	67,500
2.	Gasoline for supervisory group in field work	20,000
	Total cost for transportation	87,500
d.	**General** -	10,000
e.	**Grand total cost** 9,281 US dollars	408,400
	Official exchange rate 1 dollar = 44 baht	

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