AN OUTBREAK OF LEPTOSPIROSIS, THAILAND- THE IMPORTANCE OF THE LABORATORY

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Abstract. The reported incidence of leptospirosis increased 30-fold in Thailand between 1995 and 2000. Despite many hypotheses to explain the increase, the true etiology remains unknown. We conducted a review of the national surveillance system for leptospirosis, examining the reporting practices, system attributes, and utilization of laboratory confirmation in two northeastern provinces. Using standard guidelines for evaluation of public health surveillance systems, we assessed the timeliness, completeness, and accuracy of data; the sensitivity and specificity of case ascertainment; and the overall usefulness of the Thai leptospirosis surveillance system. Physicians were interviewed to assess compliance and understanding of the case definition. Capacity for confirmation of leptospirosis by a Thai latex agglutination test was assessed. Completeness for variables critical for linking epidemiologic and laboratory data for leptospirosis was 69%. Twenty-eight percent of 208 provincial surveillance reports were considered timely. Interviewed physicians indicated that the national case definition was difficult to understand and apply, and that laboratory confirmation was infrequently used. Compared to a standardized microscopic agglutination test (MAT) panel, the Thai test was specific, but relatively insensitive. We found that a lack of a standardized case definition for leptospirosis, the infrequent use of confirmatory laboratory testing, and the inability to link clinical, epidemiologic, and laboratory data hindered system utility. This surveillance system for leptospirosis highlights difficulties with surveillance of febrile illnesses in general, and the importance of laboratory confirmation for infections that are difficult to diagnose clinically.

INTRODUCTION

Leptospirosis is a zoonotic infection of worldwide distribution. Humans acquire disease through contact with the organism in contaminated soil or water, and present with an acute febrile illness with a 5-10% incidence of serious sequelae (Tappero *et al*, 1998; Trevejo *et al*, 1998; Faine *et al*, 1999). The protean nature of the clinical manifestations of leptospirosis makes laboratory confirmation of suspected cases crucial to assess the true burden of disease.

Leptospirosis is one of 58 reportable infectious diseases in Thailand. Surveillance for leptospirosis is accomplished through a passive surveillance system coordinated centrally by the Bureau of Epidemiology (BOE), Ministry of Public Health, Nonthaburi, Thailand. The BOE requests that a standardized form (506 surveillance form) that includes information on demographics, date of illness onset, and occupation be completed by health officials on every reported case of leptospirosis. '506' forms are generated at various levels of the Thai health care system, but are reported monthly to district health offices, provincial health offices, and eventually to

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the BOE where national data are assimilated. Private hospitals are independent of the state-run Thai health care system, but are required to submit 506 forms directly to the regional or national offices.

Laboratory testing for leptospirosis occurs at multiple levels within this system. A latex agglutination assay for detection of anti-leptospiral antibodies has been developed as a screening assay for leptospirosis by the Thailand National Institute of Health (NIH) (Naigowit *et al*, 2001), the agency responsible for national infectious disease laboratory testing. In addition, several other commercial rapid assays are available. The frequency of use of these various screening assays among practitioners in Thailand has not been previously assessed. Suspected cases may be confirmed serologically by indirect immunofluorescence (IFA) or by microscopic agglutination test (MAT) at one of 12 regional public health laboratories, or at the Thai NIH.

Historically, the annual incidence of leptospirosis in Thailand has been reported as 0.3/100,000 population, using data from national passive surveillance (Sundharagiati and Harinasuta, 1964; Aiumskul and Chutipong, 1992; Tangkanakul and Kingnate, 1998). However, beginning in 1996, Thailand reported a dramatic increase in the number of reported cases (Tangkanakul and Kingnate, 1998; Tangkanakul, 2000), and by 2000, the estimated annual incidence was 23/100,000 population (Fig 1). While the basic demographics of cases were unchanged, geographic expansion in the distribution of cases was reported (Tangkanakul, 2000; Tangkanakul *et al*, 2000a; Ratanasang *et al*, 2001). Although many hypotheses have been generated to explain the increase (Poonsuksombat *et al*, 1999; Suwancheroen *et al*, 2000; Tangkanakul *et al*, 2000b; Sinives, 2001), including an emerging epidemic of leptospirosis, the true explanation remains unknown. We evaluated the national surveillance system for leptospirosis in Thailand to assess current reporting practices, investigate possible reporting or other artifacts, evaluate the geographic distribution of reported cases, and assess the utilization of confirmatory laboratory testing for leptospirosis in Thailand.

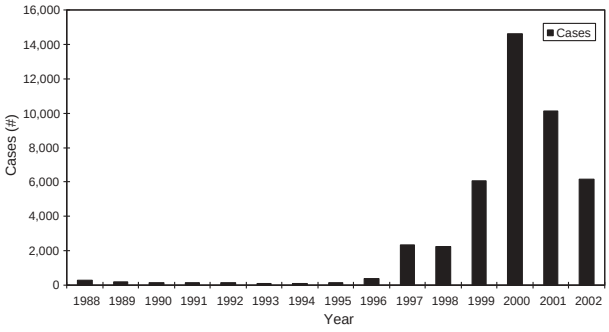

Fig 1–Number of reported cases of leptospirosis per year, 1988-2002, Thailand.

METHODS

Using standardized guidelines for the evaluation of public health surveillance systems (Klaucke *et al*, 1988), the national surveillance system for leptospirosis was assessed for its timeliness, completeness, and accuracy of data, the sensitivity and specificity of case ascertainment, and the overall usefulness of the system. In evaluating the sensitivity of the leptospirosis surveillance system, both the sensitivity of the overall system and the sensitivity of individual case criteria used were assessed. According to MOPH guidelines, a clinical case of possible or probable leptospirosis was defined based on the World Health Organization (WHO) point scale criteria (Faine *et al*, 1999) (Table 1).

The primary surveillance system review was conducted during a 4-week period during February and March, 2001 at the Provincial Health Office (PHO) of Mahasarakam Province (2000 population: 940,000), an area of northeastern Thailand where leptospirosis is reported to be endemic, and which reported an increase in cases of leptospirosis from 1996 to 2000, rising from 12 cases (annual incidence: 1/100,000) in 1996 to 392 cases (41/100,000) in 2000.

We reviewed all reported records for leptospirosis from the 506 database of the PHO for the 13 districts in Mahasarakham in 2000, and correlated these records with those in the national database of the BOE. Surveillance data were also collected from Hospital A, a single 120-bed district hospital within this province serving

Table 1
World Health Organization standard guidelines for diagnosis of leptospirosis.

Question	Answer	Score
A: Has the patient:		
Headache of sudden onset?	Yes	2
	No	0
Fever?	Yes	2
	No	0
If 'Yes', Is the temperature 39ºC or more?	Yes	2
	No	0
Conjunctival suffusion?[a]	Yes	4
	No	0
Meningism?[a]	Yes	4
	No	0
Muscle pains (especially calf muscles)[a]	Yes	4
	No	0
[a]Are all 3 features (conjunctival suffusion, muscle pains and meningism)	Yes	10
present together?	No	0
Jaundice?	Yes	1
	No	0
Albuminuria or nitrogen retention?	Yes	2
	No	0
Total score of part A		
B: Epidemiological factors:		
Has there been contact with animals at home, work, leisure,	Yes	10
or in travel, or contact with known (or possibly) contaminated water?	No	0
C. Bacteriological laboratory findings:		
Isolation of leptospires in culture- diagnosis certain		
Positive serology-leptospirosis endemic:		
Single positive, low titer	Yes	2
	No	0
Single positive, high titer	Yes	10
	No	0
Paired sera, rising titer	Yes	25
	No	0
Positive serology-leptospirosis not endemic:		
Single positive, low titer	Yes	5
	No	0
Single positive, high titer	Yes	15
	No	0
Paired sera, rising titer	Yes	25
	No	0
Total score (A+B+C)[b]		

[b]A total score of >25 from A, B, and C, or a total score of >26 from A or A and B indicates probable leptospirosis infection. A total score of 20-25 from A, B, and C indicates possible leptospirosis

a population of approximately 118,000 in a rural setting. From this hospital, we reviewed 48 leptospirosis case report forms from 1999 and 2000, and compared them with in-patient hospital records, to assess completeness and accuracy of reporting at the district and provincial levels. In addition, an observational study of surveillance practices of a convenience sample of local practicing physicians was conducted in this district hospital over a 4-day period, and an

open-ended knowledge, attitudes, and practices interview was completed by one physician at Hospital A and two physicians at two neighboring district hospitals regarding knowledge of the leptospirosis case definition, utilization of laboratory confirmation, and reporting practices.

We next sought to review of the utilization of laboratory confirmation by assessing data from a leptospirosis outbreak investigation conducted in 2000 in Sakon Nakhon Province (2000 population: 1.1 million), also located in a reportedly endemic region. From June through August, 2000, 100 patients with clinically suspected leptospirosis were investigated and underwent laboratory testing. Laboratory confirmation of leptospirosis was obtained from an independent laboratory (Armed Forces Research Institute for Medical Sciences, Bangkok, Thailand) by MAT using a battery of 22 serogroups (30 serovars). Data on laboratory-confirmed cases was compared to the central BOE database. The BOE database was searched for the presence of the serologically confirmed cases of leptospirosis from the Sakon Nakhon study. Two staff members of the Thai NIH were interviewed regarding laboratory confirmation practices for leptospirosis at a regional and national level.

The accuracy of the Thai NIH MAT for serologic confirmation of leptospirosis was assessed by comparison of MAT results for a battery of 20 serum specimens from 11 leptospirosis cases and 3 non-cases provided by the US Centers for Disease Control and Prevention (CDC). In addition, the performance of the widely distributed Thai latex agglutination test was assessed by comparison to CDC's MAT results for this same set of serum specimens. Sensitivity, specificity, and positive and negative predictive values were calculated using standard methodologies.

RESULTS

Maha Sarakham Province in 2000 reported 392 cases of leptospirosis to BOE. For these cases, we assessed the completeness of the variable 'Hospital (patient) Number' for any diagnosis, including leptospirosis, a critical variable for linking epidemiologic data within the database. This variable was found to be 69% complete (32/46) at the district hospital (Hospital A) level, and 56% complete (221/392) at the PHO, for hospitals throughout the province. Of 32 cases of leptospirosis reported from Hospital A in 2000, 31 (97%) were also recorded in the provincial database. Further, review of hospital medical records showed that no cases of suspected leptospirosis were unreported from the hospital to the PHO in 2000.

Timeliness of reporting among the 13 districts in Maha Sarakham Province was assessed for the month of January 2000, the only month with data available for review; of the 208 reports for leptospirosis generated among the 13 district hospitals in the province, 53 (26%) were received within 3 days, the interval considered timely by the MOPH. Seven district hospitals had no data to report; the range of percentages of reports submitted within 3 days ranged from 2% to 100% among those hospitals reporting data.

Discussion with three physicians in Maha Sarakam Province indicated that the current WHO case definition suggested by the Ministry of Public Health's Leptospirosis Control Office was not used for the diagnosis of leptospirosis; instead, they relied on their own personal clinical impression, based on symptoms recognized from cases they had seen in the past, results of nonspecific screening tests performed on all patients with febrile illness, or a combination of these approaches. Reasons offered for not using the case definition included unfamiliarity with the case definition, and the impression that the case definition was too cumbersome and complicated for practical use in the clinical setting.

During the 2000 Sakon Nakhon study, all suspected cases had undergone confirmatory laboratory testing with MAT; of 100 total suspect cases tested, 45 (45%) were serologically confirmed by MAT. Using the variable 'Hospital Number' as the identifier, the surveillance database of the PHO was assessed for inclusion of these patients; of the 45 cases identified in the MAT database as seropositive, none were included in the provincial surveillance database.

The ability of the Thai NIH laboratory to serologically confirm cases of leptospirosis was assessed by comparing the results of the Thai MAT and the Thai latex agglutination test to the

MAT results obtained by CDC for the same battery of 20 cases and non-case specimens. The Thai and CDC MAT results were concurrent in 14 (70%) sera, and for 6 specimens (30%) from known cases, the MAT results were discrepant, with a negative result in the Thai MAT and a positive result in the CDC MAT [Thai MAT sensitivity and positive predictive value (PPV): 54%; specificity and negative predictive value (NPV): 100%]. The Thai latex agglutination test was concordant with the CDC MAT for 18 (90%) samples; in one sample, the Thai latex agglutination test was positive whereas the CDC MAT was negative, and in another, the Thai latex test was negative whereas the CDC MAT was positive (Thai latex agglutination test sensitivity and PPV: 92%; specificity and NPV: 86%).

Because there was no system in place for the linkage of laboratory confirmed cases of leptospirosis with those detected through the surveillance system, the specificities of the case definition and the overall surveillance system could not be determined.

DISCUSSION

Our review of the current surveillance system for leptospirosis in Thailand highlights several issues regarding passive surveillance for febrile diseases, including the need for a standardized, consistently applied case definition; the utility of specific guidelines for diagnosis, reporting, and testing; and, most importantly, the need for linkage of epidemiologic and laboratory data. It also emphasizes the need for confirmatory laboratory testing as an integral part of surveillance for these illnesses. Because the signs and symptoms of leptospirosis, including fever, chills, and myalgias, are nonspecific, the differential diagnosis for such symptoms in the tropics is large, and includes such illnesses as dengue, rickettsial infection, influenza, hepatitis, and malaria (Tappero *et al*, 1998; Faine *et al*, 1999). Differentiating between these illnesses is critical for choosing treatment and determining prognosis.

The lack of awareness among physicians of a standardized case definition for leptospirosis would represent a serious barrier to the ascertainment of accurate surveillance information. While the current WHO case definition for the diagnosis of leptospirosis does include laboratory data, a case may still meet the criteria on a clinical basis, and be defined by symptoms and signs alone. Based on limited interviews with practitioners, and discussions with others at the national, provincial, and district level, the current case definition suggested by the MOPH for a reportable case of leptospirosis is either unknown, or is simply not used. As a result, a variety of criteria are used by health providers and district and provincial health officials to determine which cases to report. Discrepancies in numbers of reported cases between geographic regions may in part be reflective of differing clinical criteria used by a variety of physicians recording leptospirosis as the cause of illness at hospital discharge, with possible over- or underrepresentation of true cases of leptospirosis in certain areas, depending on the diagnosing and reporting practices of health providers. In addition, the current heterogeneity of case definitions may in part explain the large discrepancies in various surveillance parameters, including completeness and reporting compliance, observed among hospitals, districts, and provinces. Additional work is needed to evaluate these hypotheses and identify case definitions that can be implemented in the field.

Laboratory confirmation of at least a subset of suspected cases of leptospirosis is vital. Although laboratory screening of every patient suspected of having the disease may not be feasible, an inability to confirm infection in at least a subgroup of suspected cases in the setting of a suspected outbreak may lead to misclassification and over-reporting. This could have ramifications, including misdirected efforts at prevention, control, and treatment of leptospirosis or illness due to another etiology. Finally, the laboratory confirmation of cases and the ability to link these cases to epidemiologic information is critical in identifying modifiable risk factors and developing educational, control, and prevention strategies. Coordination of epidemiologic data with laboratory results using a unique identifier common to all reporting forms and laboratory data generated at the district, regional, and na-

tional level would enable linkage of epidemiologic data with the results of confirmatory testing for leptospirosis and other diseases within a central database. This would allow for a more accurate estimate of the true burden of disease, the determination of demographic, risk factor, and clinical presentation characteristics associated with confirmed cases, and the development of appropriate prevention and control strategies.

Historically, laboratory confirmation of leptospirosis has been difficult and labor-intensive due to the challenges of culturing the organism and the difficulty, complexity, and expense of confirmatory MAT testing. The recent introduction of a number of rapid diagnostic tests (Smits *et al*, 1999; Yersin *et al*, 1999; Levett *et al*, 2001) has greatly simplified the serologic diagnosis of leptospirosis compared to the MAT. Development of such assays and comprehensive validations of them should be encouraged. However, MAT remains the gold standard for diagnosis, and is a critical component of laboratory confirmation of leptospirosis cases. An independent assessment of the Thai NIH performance of MAT indicated that, although the MAT method used by the Thai NIH laboratory allowed for specificity in testing for leptospirosis in clinical samples, the sensitivity was low compared to the CDC MAT, most likely due to differences in methodology and inadequate controls for the antigens used by the Thai NIH. The addition of appropriate controls for performing the MAT and the development of an updated panel of antigens that is more reflective of locally prevalent serovars would be important steps in increasing the ability to perform confirmatory testing at the national level and allow for better estimates of disease burden.

This surveillance analysis had a number of limitations. It was restricted to two provinces within Thailand, and the observational portion of the study was conducted over a limited time period. Our ability to generalize these conclusions to the surveillance for leptospirosis on a national level in Thailand is limited, although we have no reason to believe that surveillance in Maha Sarakam is unique. The various important attributes of the leptospirosis surveillance system in Maha Sarakam Province, such as timeliness, completeness of reporting, and acceptability, displayed substantial heterogeneity across districts that is likely to be present in other provinces and, therefore, at the national level.

The current surveillance system in Thailand benefits from its strong, organized infrastructure, and the dedication and motivation of its personnel. Several ongoing surveillance projects in Thailand affirm the overall strength of nationwide surveillance in general (Phonboon *et al*, 1986; Mills *et al*, 1997). While there are particular weaknesses in the current leptospirosis surveillance system in Thailand, the current system appears capable of addressing these difficulties through a combination of training, improved diagnostics and linkage of laboratory to epidemiologic data.

Overall, the difficulties of leptospirosis surveillance in Thailand serve to reinforce the challenges of passive surveillance for febrile illnesses in many countries; Thailand is certainly not unique in facing these difficulties. The accurate detection of cases in a timely fashion has important public health ramifications for identifying risk factors and guiding prevention strategies. By more closely integrating laboratory confirmation into the overall surveillance for leptospirosis, Thailand can serve as a model for the difficult task of improving surveillance for leptospirosis in endemic areas of the world.

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