CHAPTER II

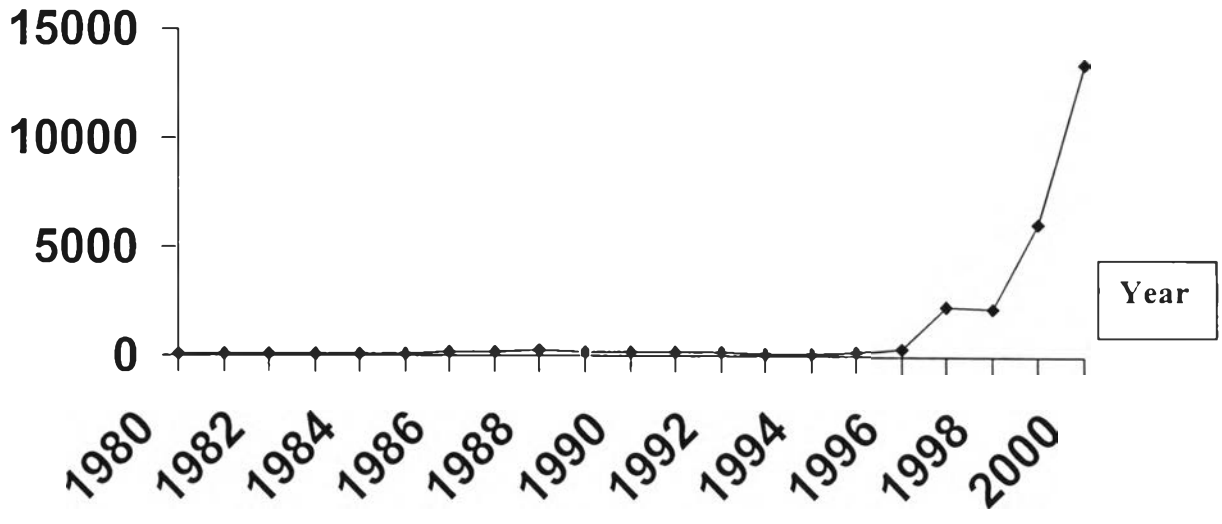
Leptospirosis: Emerging health problem in Thailand

2.1 Introduction:

2.1.1 What are the Leptospirosis problem and its situation in Thailand?

Leptospirosis has become an important public health problem in Thailand because of the increasing number of cases since 1996. In 1999, 6,080 notifications were reported with 266 deaths representing the morbidity rate 9.89 per 100,000 population. The case fatality rate was 4.7%. This was approximately 42-times increase in case comparing to the year 1995 (143 cases). In 2000, 13,461 notifications with 365 deaths were reported. (Figure 2.1) The clinical pattern of leptospirosis cases also changes and the severity increases. The causes of the epidemic have not yet been discovered. The actual burden of disease and the confirmed cases could not been estimated because of the limitation in laboratory facilities. Application of the standard test (Microscopic Agglutination Test or MAT) is limited mainly to the National Institute of Health. Thus, there are many questions on diagnosis and the actual burden of leptospirosis cases. The difficulties to design the prevention and control strategies are also the problem.

Figure 2.1 Number of Leptospirosis cases, Thailand 1980-2000



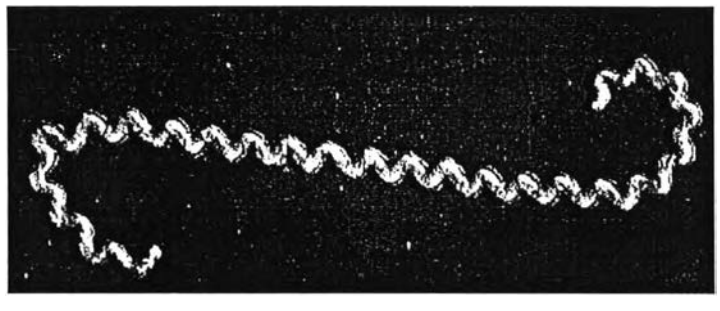
Source; 506 Disease Notification Report, Ministry of Public Health, Thailand

2.1.2 What is leptospirosis?

Leptospirosis is caused by pathogenic spirochetes of the genus Leptospira (1,2). Over 200 hundred pathogenic strains belonging to different serovars have been described. *Leptospires* have been isolated from almost all types of warm-blooded vertebrates. Different leptospiral strains have different host ranges and different geographic distribution. Feral and domestic animal species may serve as sources of infection in man. The disease is found throughout the world, but occurs mainly in countries with a warm and wet climate where the disease often is endemic. Epidemic outbreaks of leptospirosis have been reported frequently (3,4).

The disease is primarily transmitted through the urine of infected animals. *Leptospires* enter the human host through skin abrasions, conjunctiva, or mucus membranes. Consumption of contaminated water and food products, contact with contaminated surface water and contact with contaminated soil or plants are major causes of infections. Infestation by infected rodents and contact with infected live stock and pets all promote infection. Thus,leptospirosis is also an occupational disease. In rural areas, cattle farmers and rice and sugarcane workers are at risk of attracting the disease. Leptospirosis is caused by poor sanitation and can be promoted by flooding in urban areas. In urban areas, sewage system workers and garbage collectors in particular are at risk. Persons engaged in recreational activities such as swimming and rafting are also at risk. Leptospiral infection also may cause disease in animals, which can lead to considerable economic losses.

Figure 2.2 ***Leptospires interrogans***



Leptospira interrogans under electron microscope (magnification =15,000)

2.1.3 Group of leptospirosis

There are three groups of leptospirosis;

1). Group 1, the type associated with farming and food production, where the main chance for contact with carrier animals is strictly occupational and the sources are pigs or cattle. Thus the population at risk is an easily defined and well-recognized social group.

2). Group 2, the type associated with growing crops (rice, taro) sugar or other tropical products in wet conditions. Meat production is uncommon. This social organization dictates that the main leptospiral contacts are indirect, via contaminated waters (rice fields, mud, rivers, drains) or in handling foods contaminated by rodent urine.

3). Group 3, the type of recreational activities associate with the disease.

In Thailand, most cases are agricultural workers (Group 2) and getting the disease via indirect contact with contaminated environment.

2.1.4 Clinical manifestations

Signs and symptoms of leptospirosis resemble a multitude of other fibrile and haemorrhagic illnesses including influenza, malaria, dengue, rickettsiosis, typhoid fever, viral hepatitis and enteric diseases. The clinical manifestations also relate to some extent to the serovar of the infecting strain, but any pathogenic strain may cause severe leptospirosis. In most cases the illness presents with a sudden onset of fever with headache, myalgia (especially in 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 oliguria/anuria (Weil's syndrome), meningitis or haemorrhages. Early treatment may reduce the severity of the disease and may prevent the development of complications. Chaifoo and co-workers (5) recommended the use of the following clinical case definition. According to their definition a suspected case patient is a patient with fever, headache and myalgias, and a probable case patient is a case with fever, headache, myalgias and positive albuminuria.

However, the specificity of the definition of the suspected case patient (a patient with fever, headache and myalgia) is only 15%, The sensitivity and specificity of the definition of the probable case patients (a case patients with fever, headache, myalgias and positive albuminuria.) is only 56% and 82%, respectively. The poor diagnostic values of these definitions illustrate the need for the use of appropriate laboratory support.

2.1.5 Laboratory methods for leptospirosis

The incubation period of leptospirosis is about 2 to 30 days. The usual range is 5 – 14 days. (1) After infection *leptospires* circulate through blood stream and penetrate into organs of the patient approximately 10 days after onset of the disease. After about a week perhaps even later, the *leptospires* appear in the patient's urine and in the first 5 – 10 days' *leptospires* can be found in cerebrospinal fluid. About 7 days, sometimes 10 days or longer, after the onset of disease antibodies can be

detected in the patient's blood. (Table1) Therefore, laboratory investigation for diagnosis of leptospirosis can be performed by the demonstration of *leptospires* and by the demonstration of antibodies to *leptospires*.

Demonstration of *leptospires* can be performed by culturing and direct examination. Culture from blood should be taken during the first week of the disease. Urine should be taken a week after the onset of the illness from midstream urine and filtered through a 0.22-micron disposable filter before inoculated into selective media to suppress contaminants. Cerebrospinal fluid should be taken in the first 5 to 10 days of the disease. Dark-field microscopy or silver staining can be used to perform direct examination. However, these methods often lead to mistake due to artifacts.

The polymerase chain reaction (PCR) can also demonstrate leptospiral DNA in clinical samples. A pair of primer, short DNA sequence that are specific to leptospiral DNA in combination with the heat stable DNA polymerase in the presence of deoxynucleotide, A T G C base and other important salts with optimal concentration are mixed in micro tube. The mixture of those elements are subjected to 3 phases of optimal temperature cycles; denaturation, annealing and extension, lead to a specific amplification of a stretch of leptospiral DNA. The amplified DNA can be detected by gel electrophoresis and with subsequent hybridization using a specific probe after transfer of the amplification to nitrocellulose membranes. PCR has been increasingly used for the diagnosis of infectious diseases caused by slowly growing or fastidious organisms for which culture and serological methods are difficult. PCR has been successfully applied to the detection of *leptospires* in a variety of specimens. The power of PCR is highly sensitive, specific, reliable and rapid.

Although PCR has been proved to provide a useful addition and to be alternative in the diagnosis or characterization of leptospiral strain, PCR along with all post amplification detection procedures such as agarose gel electrophoresis and DNA hybridization which are very complicated, laborious, time consuming limits its use to routine diagnosis. The need of special equipment, well-trained technicians and sophisticated laboratories makes this technique extremely expensive and is rarely used in the developing countries. Besides, PCR itself has problems caused from its power of nucleic acid amplification and high sensitivity. False positive reaction caused by contaminating nucleic acid may occur. The false positive and contamination are significant problems that make in-house developed PCR difficult to introduce into the clinical microbiology laboratory.

Serological diagnosis of leptospirosis is an alternative and essential way to diagnosis leptospirosis. The main antibodies against *leptospires* have 2 different classes, IgM antibodies are usually formed first and IgG antibodies later. IgM antibody level drop off rather quickly by months while IgG antibody level drop off slowly by years.(Table 2.1) Antibodies to *leptospries* can be detected late in the first week after onset of the illness. Thus, most serological test for leptospirosis needs four-fold rise in titer in paired serum to be considered as being suggestive of current infection. To collect paired serum in cases makes diagnosis for leptospirosis very difficult.

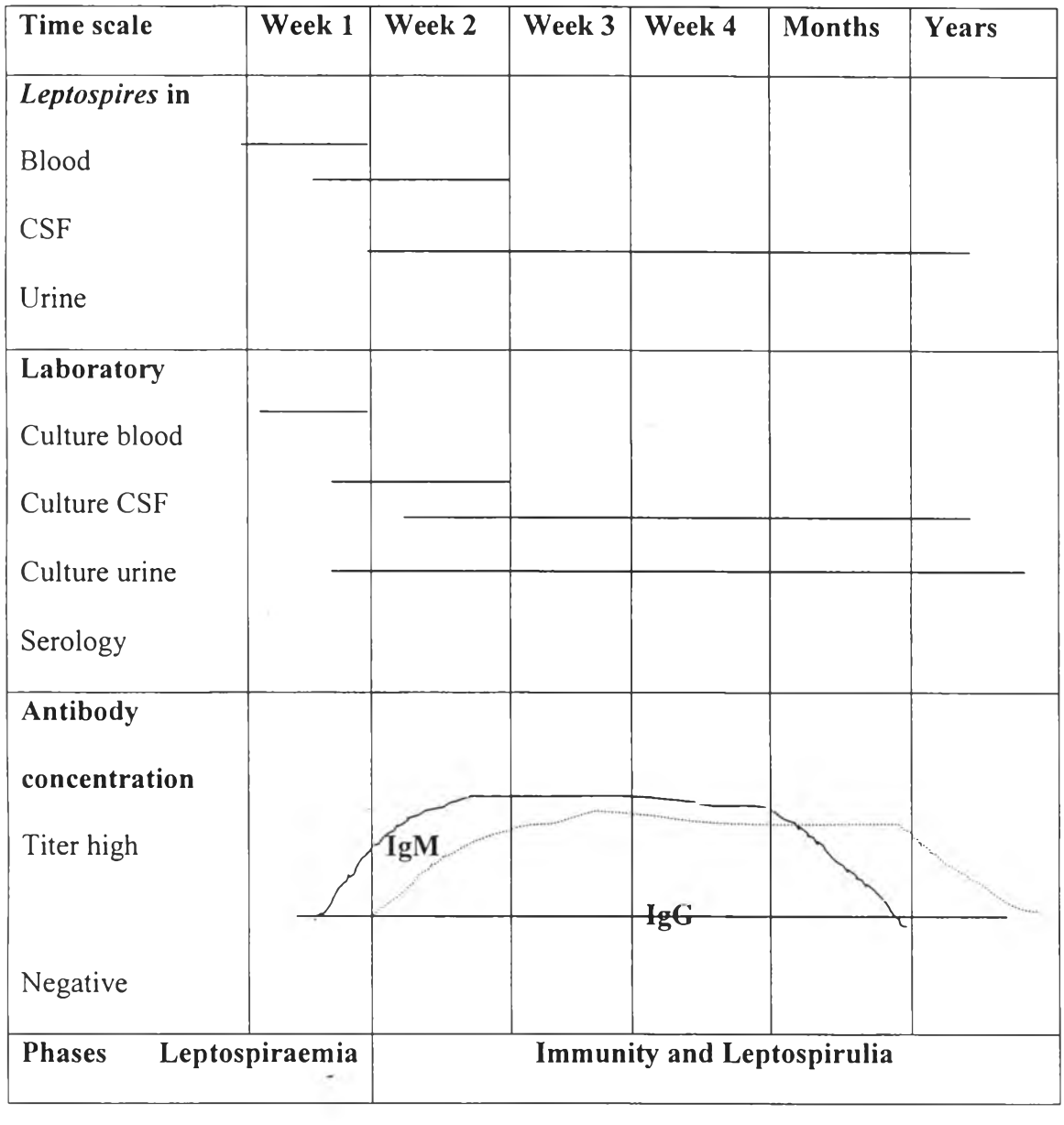
In conclusion, laboratory tests which are considered as gold standard test: including culture, the microscopic agglutination test (MAT) (6), and the enzyme linked immunosorbent assay (ELISA) (7-10) are time consuming, complicated and expensive to perform, and hence in general are not routinely available. The

availability of simple laboratory tests such as the haemagglutination assay (11) or the dipstick assay (12-14) that can be applied in health posts and district hospitals is needed to make a correct and quick diagnosis, thus enabling a prompt and proper treatment. The availability of simple and rapid tests also may help to recognise outbreaks at an early stage, and to distinguish between outbreaks of other infectious disease like dengue haemorrhagic fever (15).

2.2 Why is Leptospirosis epidemic important?

After knowing the epidemic of leptospirosis and understanding the disease, we have to consider why Leptospirosis epidemic is important, and what consequences are coming out from this epidemic. Leptospirosis epidemic is important because the numbers of cases have drastically increased over last few years along with (Figure 2.1) the severity of disease. The difficulty to diagnosis the disease and design the prevention and control strategies are also the reasons for its important.

Table 2.1 Period of *leptospires* in clinical samples and laboratory investigation



2.3. Epidemiology of leptospirosis in Thailand

Leptospirosis was first reported in Thailand in 1942 by Yunibandhu and coworkers (16). During the following decades leptospirosis was reported only sporadically with a few outbreaks during floods. (Figure 2.1). It has become the

emerging health problem since the beginning of the epidemic in 1996. Thus, the epidemiology in Thailand, it can be divided into 2 periods as before and after the 1996 epidemic.

2.3.1. Characteristics of leptospirosis in Thailand: 1942 – 1995 (Before leptospirosis epidemic)

Leptospirosis cases reported from 1942 to 1995 were mostly confined to rural areas and were strongly associated with agricultural occupations and exposure to flood water. The disease showed seasonal variation with most cases occurring during (June to September) and shortly after (October to December) the rainy season with a peak in October. Most leptospirosis cases were male farmers and zewers aged fifteen to forty five years. Males were more likely of getting the disease than females. Fever (88.8% - 100%), myalgias (30.7% - 100%), headache (53.8% - 100%), conjunctival suffusion (61.5% - 100%), meningism (11% - 23%), pulmonary manifestation (10% - 30.7%) and diarrhoea (7% - 25%) were observed (Table 2.2). The most common complications were jaundice (7.6% - 70%) and renal impairment (40% - 100%). Pulmonary haemorrhage was less common. The average case fatality rate was 10% (Ref. 17 - 22).

The most predominant serovars causing illness in different years were bataviae (61.5%-95% of total) and icterohaemorrhagiae (5% - 56% of total). Histopathological studies of the liver in mild leptospirosis cases have indicated that changes observed in patients suffering from a bataviae infection were more marked than in those infected with the serovar javanica (23,24). The predominance of

different serovars in different years suggests that different sources of infections were involved in different epidemics. Seroprevalence surveys performed among the general population indicated a relatively high prevalence of 7.5% - 62.5% (25-27).

The high percentage of antibody in general population revealed the common of leptospirosis infection on those areas. (Table 2.3). The seroprevalence gives an indication of the degree of exposure in the past to infection and possible development of disease in the population. The high seroprevalence suggests that a much higher number of patients may have contracted the disease during previous epidemic than actually had been diagnosed and reported. This suspicion was confirmed by studying patients with fever of unknown origin (FUO). Investigation of serum samples from FUO patients in different hospitals demonstrated that between 4.8% and 35.6% of these patients suffered from leptospirosis (28-32) (Table 2.3).

Seroprevalence studies performed in 1963 and 1964 among rodents captured in rice fields reported prevalence rates in rice field rats (*Bandicota sp.*) as high as 33% in Bangkok province, 26 % in Chiengmai province, and 39.5% in Pitsanuloke province (33) (Table 2.3). The seroprevalence in *Rattus rattus* in these three provinces was 23%, 34.5% and 21.7%, respectively. The seroprevalence in *R. novegicus* trapped in Bangkok province was 40% (26). The prevalence of *leptospires* in *R. novegicus* in Bangkok peaked in November (26). Seroprevalence studies may underestimate the prevalence in rodents. Isolation of *leptospires* from rat's kidney yielded a prevalence of 66.6% compared with a seroprevalence of 36.6% in the same population. Seroprevalence studies suggested that leptospiral infections were not common in house mice (*Rattus exulan*). Serotyping of isolates showed that the serovars most common in rodent were autumnalis, bataviae, javanica and

hebdomadis. These studies indicated that rodents likely are important reservoirs and sources of infections in Thailand. The seroprevalence in dogs captured in Bangkok was 43%. From 8.1% of the dogs *leptospires* (serovars bataviae, javanica, and ballico) could be isolated (36). This shows that dogs could also be important sources of infection.

2.3.2. Characteristics of leptospirosis in Thailand since 1996 (After leptospirosis epidemic): Emerging health problem in Thailand

The epidemic that started after flooding in 1996 in the Nongbunnak district, Nakhon Ratchasima province in northeastern region expanded in 1997 to 15 North-eastern provinces. The highest attack rate was observed in the provinces adjacent to Cambodia that is supplied by Mae Nam Moon River. These provinces receive little rainfall but often are flooded during and after the raining season. The provinces in the highland adjacent to Laos had a lower attack rate (17). In 1998, again the majority of cases occurred in the northeastern region, but there also was an outbreak in northern region. In 1999, 89.7% of the country wide reported cases were from the northeastern region.

From 1996 to1999, flooding was not directly correlated with the epidemics. There was flooding but outbreaks occurred in flooded as well as non-flooded areas and did not happen at the same time as the flooding in some flooded areas (5, 36, 37, 39). During the peak of the outbreaks in 1997 to 1998 the prevalence among suspected cases was as high as 14% to 49.2% in the provinces Udon Thani and Nakhon Ratchasima (36 - 38) (Table 2.3).

Cases of leptospirosis occurred in all age groups except in children 0 to 5 years of age. A peak was observed in the age group of 25 to 54 years, accounting for 80% of all cases. The male per female case ratio was between 7:1 and 9.3:1 in different areas (37). The majority (72.6%) of the cases was farmers in rural areas. The prevalence rate of asymtomatic infection was between 8.4% and 11% (38). The post-epidemic prevalence of *leptospires*-specific antibodies in high-risk groups was 29.6% to 42.9%.

The predominant symptoms were fever (92% -100%) with high grade fever of more than 39 ^{0}C in 46.8 to 57.4% of the cases, headache (77.4% - 100%), myalgia (83.9 - 95.9%) and meningism (4% - 25%) (5, 36 - 38)(Table 2.2). Conjunctival suffusion was reported less than in period before 1995. Diarrhoea was reported often (30.6%- 35%) in 1996 and 1998. Headache was reported to be acute (77.4%) and severe (62.9%). 50% had muscle tenderness. Jaundice was reported less often in 1997 than in 1996 and 1998. Albuminuria was reported in 20% to 46% of the cases. Renal impairment and noticeable pulmonary complication especially pulmonary haemorrhage were reported to be major complications. The case fatality rate was 4% to 5%. (37, 42)

Compared with previous years a change was observed in the commonly occurring serovars. While in previous years the serovars bataviae and icterohaemorrhagiae were most common, icteroheamorrhagiae (41.6%) and ballico (33.3%) were the most common serovars in the 1996 epidemic, bratislava (51.2%) and autumnalis (41.5%) in 1997, and bratislava (57.1%) and sejroe (35.7%) in 1998. Less common serovars detected during the 1996 to 1998 epidemics are akayimo, bangkoki, hebdomadis, hyos and pyrogenes. As strains of each of these different

serovars likely have a different host range this suggests that different animal reservoirs have been involved in each of the outbreaks. Some of the changes in clinical symptoms also could well be related to changes in the predominant serovars.

In 1998, a matched case control study was performed in the northeastern region to investigate the risk of rice field workers (37). The results indicated that some, but not all; rice field activities were associated with leptospirosis infection. Wading through stagnant water (OR 4.8, 95% CI 1.7 - 13.7), applying fertiliser in wet fields for more than 6 hours a day (OR 2.7, 95% CI 1.1 - 6.6), plowing in wet fields for more than 6 hours a day (OR 3.5, 95% CI 1.1 - 11.6), and pulling out rice plant sprouts in wet fields for more than 6 hours a day (OR 4.4, 95% CI 1.7 - 11.3) all were identified as risk factors. (37) Rice field workers form a major occupational risk group. Plowing, pulling out sprouts and fertilising activities were associated with skin cuts and abrasions more than other rice farming activities. It is supposed that skin wounds increase the risk of infection when exposed to contaminated water.

However other agricultural activities like keeping live stock carry risks as well. The diversity of the risk activities is illustrated by the following example. The Buri Rum province which bordering Cambodia in the south of the northeastern region is endemic for leptospirosis. In 1999, 422 cases with 30 deaths were reported. The attack rate was 27.9 per 100,000. In September 1999 out of 500 villagers involved in removing weeds and water hyacinths from a 50 years abandoned pond 115 presented at the hospital with suspicion of leptospirosis. Serological investigation demonstrated the presence of *Leptospires*-specific antibodies in 43 out of 104 (41%) patients. In this outbreak-infected rodent likely had contaminated the pond. Wearing any type of shoes

at all time during the activities was identified as a protective factor (OR 0.4, 95% CI 0.1 - 0.9)(39).

During the 1998 outbreak in Nakhon Ratchasima a study was conducted to determine whether rodent density and infection of rodents with *leptospires* correlated with the occurrence of the epidemic. Rats were trapped in rice fields in epidemic and non-epidemic areas and the isolation of *leptospires* from rats was attempted (40). Water in the rice fields in both epidemic and non-epidemic provinces had pH levels (mean 7.8, median 7.6, and range 6.7-8.5) and temperature levels (mean 34.2, median 34.5, and range 30.0-37.0) that were suitable environments for *Leptospires* survival. The water depth was between 5 and 10 centimetres. The total number of rats in the epidemic area was two times greater than in the non-epidemic area. In the case of the rice-field rat, *Bandicota indica* there were 2.5 times more rats in epidemic areas compared to non-epidemic areas. *Leptospires* were isolated from 41.4% of the rice field rat, *B. indica* trapped from the epidemic area. Rats trapped in the non-epidemic area were not infected.

Serological investigation failed to identify the isolates and the possibility of a new serovar should be considered. During the outbreak in Udon Thani in 1997 the prevalence of *leptospires* in dog was determined to be 4.6%. The serovars pomona, canicola and grippotyphosa were detected. Four species of live stock from all over country were serologically examined between 1997 and 1998. The prevalence of leptospiral antibodies in cattle, buffalo, swine, sheep and goat were 29.8%, 19.0%, 4.3% and 26.7%, respectively. Icterohaemorrhagiae was the predominant serovar in sheep and goat (94.0%). The serovars wolffi, pomona, javanica, pyrogenase, hebdomadis and hyos all were frequently observed in cattle (41) (Table 2.4). The

serovars wolffi, hyos and javanica were common in buffalo, and the serovars ballico, canicola, icteroheamorrhagiae and bataviae were common in swine. A serological survey performed following an outbreak in the Buri Rum province in North-eastern Thailand showed however that bratislava and sejroe were the most common serovars in cattle and that the serovar pyrogenes was endemic in rats. Epidemiological linkage indicated that the major reservoir for infection in man during this outbreak in Buri Rum was cattle followed by rat (42).

2.3.3. Consequences of leptospirosis epidemic

Due to leptospirosis diagnosis needs laboratory facilities to confirm the clinical diagnosis. In Thailand laboratory facilities are not available in every hospital. Thus, physicians can not use the laboratory to confirm diagnosis. They have to diagnose on clinical manifestation which are very varies widely. The consequences of differential diagnosis among physicians are overdiagnosis and late diagnosis and treatment. The result of overdiagnosis is the increasing of reported cases, which leads to increase burden of the disease. The results of late diagnosis and treatment are high case fatality rate and increasing awareness of the community due to death of their people. Thus, the risk group in community seek more health care which also leads to increase burden of the disease. However, actual burden of leptospirosis can not estimate and its consequences are the confusion among physicians in every hospital level and the confusion among health workers and communities. Therefore, problem in diagnosis needs to be improved by using a guideline, which appropriates to Thailand.

Figure 2.3 Causes and consequences of leptospirosis epidemic in Thailand

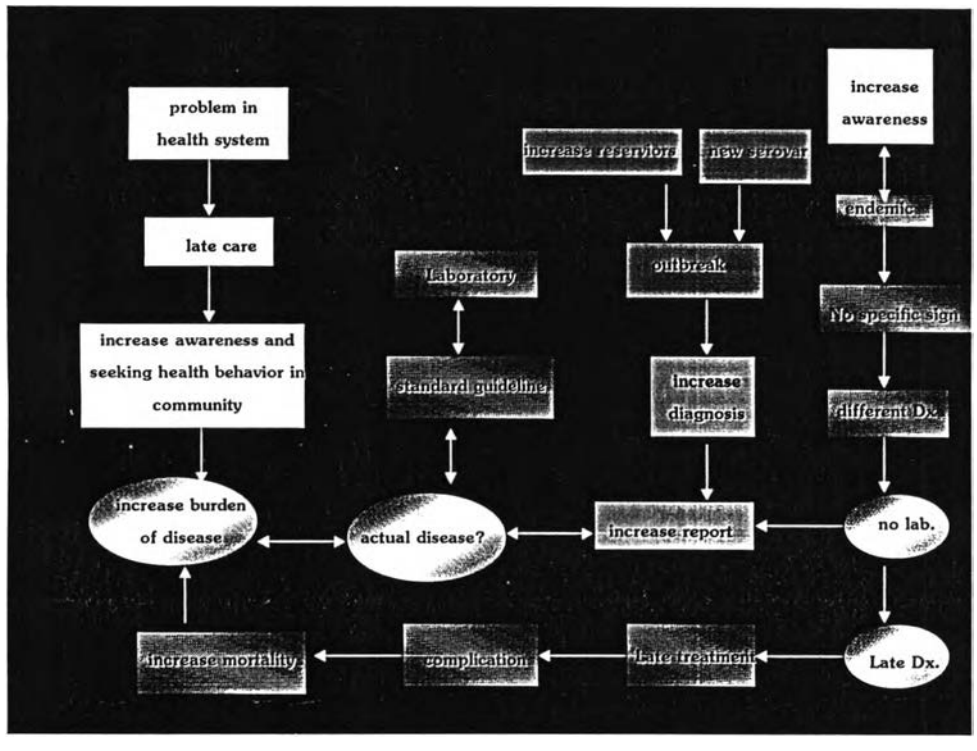


Table 2.2 Clinical manifestation of leptospirosis cases in Thailand, 1948 - 1998

Year	Hospital (city, region)	Percentage of patients with the following clinical symptoms: Fever	Headache	Conjunctival suffusion	Jaundice	Myalgias	Renal impairment	Pulmonary manifestations	Meningism	Diarrhea	Number of patients	Serovar (%)
1948/1950	Chulalongkorn (Bangkok)	98.0	76.9	100	57.7	82.6	61.5	-	-	-	52	bataviae (90%)
1960	Budhachinaraj (Pitsanuloke)	100	100	100	70.0	100	100	10.0	-	25.0	20	icterohcamorrhagiae, ebdomadis, bataviae
1962	Chiengmai (Chiengmai)	95.0	66.0	74.0	37.0	76.0	70.0	10.0	-	8.0	100	icterohcamorrhagiae (56.8%)
1962	Chulalongkorn (Bangkok)	88.8	88.4	94.4	52.0	90.7	64.8	37.0	11.1	-	54	bataviae (95%), icterohcamorrhagiae (5%)
1963/1964	Sirijai (Bankok)	100	85.5	85.5	52.8	80.0	40.0	20.0	12.7	18.1	55	bataviae (76.6%), canicola (10.9%) javanica (7.3%)
1978/1981	Children (Bangkok)	100	53.8	61.5	7.6	30.7	81.8	30.7	23.1	7.0	13	bataviae (61.5%)
1996	Nongbunnak (Nakhon Ratchasrima)	100	100	44.9	65.3	95.9	100	12.2	16.3	30.6	49	icterohaemorrhagiae (41.6%) ballico (33.3%), akiyama (8.3%) hebdomadis (8.3%), hyos (8.3%)
1997	Udon Thani (Udornthani)	100	95.0	16.0	10.0	95.0	43.2	-	4.0	-	74	bratislava (51.2%) autumnalis (41.5%)
1998	Regional 5	92.0	87.0	45.0	45.0	81.0	-	14.0	20.0	35	262	NA
1998	Nakhon Ratchasrima	100	77.4	41.9	64.5	83.9	44.5	-	25.8	-	62	bratislava (57.1%), sejroe (35.7%) bangkoki (3.5%), pyrogenes (3.5%)

Table 2.3 Prevalence of Leptospiral antibodies in General population, Fever of Unknown Origin Cases (FUO) and Suspected Leptospirosis cases in Thailand, 1965 - 1998.

Subject	Year	Province	Prevalence	Male to female ratio	Percentage of subjects with the following serovar
General population					
	1965	Bangkok	14.9 (104/107)	1.2:1	bataviae: 51% (53/100)
	1965	Chiengmai	37.0 (53/143)	2.5:1	icteroheamorrhagiae: 28.3 (15/53)
	1965	Umong Canton	65.2 (45/69)	0.9:1	grippotyphosa: 40% (18/45)
	1991	Chiengmai, Chiengrai and Hong Son	7.5 (45/598)	0.75:1	bataviae: 28.9% (13/45)
Fever of unknown origin					
	1966	Ubon Ratchathani	2.2	-	-
	1966	Nakhon Ratchathani	5.8	-	-
	1976	Bangkok	14.9 (34/228)	-	-
	1983	Prachin Buri	18.9 (7/37)	-	bataviae: 57.0% (4/7)
	1983	Bangkok	35.6 (26/73)	-	bataviae: 92.3% (7/24)
	1991-1993	7 provinces[1]	4.8 (24/500)	0.7:1	pyrogenis: 29.1% (7/24)
	1996	8 provinces[2]	9.8 (41/415)	19:1	icteroheamorrhagiae: 31.7% (13/41)
Suspects					
	1996	Nakhon Ratchasrima	23.8 (20/84)	15:1	ballico: 40.0% (8/20)
	1997	Udon Thani	14.1 (12/85)	4:1	ballico
	1998	Nakhon Ratchasrima	49.2 (129/262)	7:1	-

[1] Sonkha, Surin, Nakhon Ratchasrima, Chin Buri, Chaiyaphum, Ayutthaya and Lampung.
[2] Surin, Nakhon Ratchasrima, kalsin, Chayaphum, Roi Et, Buri Ram, Khin Kean and Ubon Ratchatani.

Table 2.4. Prevalence of leptospiral antibodies and serotypes in animal reservoirs in Thailand, 1964 - 1998.

Year	Province	Animal	% positive (No. positive/No. tested)	Serovar (percentage positive) (isolates or serotype)
1964	Chiengmai	Banicota spp.	26 (13/50)	javanica (96%) and
		R. rattus	34.5 (19/55)	hebodomadis (4%)
		house mice	0 (0/18)	(total 25 isolates)
1964	Pitsanuloke	Banicota spp.	39.5 (17/43)	autumnalis (88%) and
		R. rattus	21.7 (10/46)	javanica (12%)
		housemice	0 (0/25)	(total 25 isolates)
1963	Bangkok	Banicota spp.	33.0 (18/55)	bataviae (68%) and
		R. rattus	23.0 (9/39)	javanica (32%)
		R. norvigius	40.0 (63/160)	(total 73 isolates)
		House mice	3.0 (2/57)	
1995	Bangkok	dog	8.1 (13/163)	bataviae, javanica, ballico (total 13 isolates)

		dog	43.0 (146/333)	bataviae, javanica, canicola, akiyama A, pyrogenes (serology)
1997	Udon Thani	dog	4.6 (3/65)	pomona, canicola and grippotyphosa
1998	Nakhon Ratchasrima	Bandicota indicata	41.4 (12/29)	new serovar (12 isolate)
1997-1998	country wide	cattle	29.8 (741/2.488)	wolffi (48.2%), pomona (45.5%), javanica (36.0%), pyrogenes (30.1%), hebdomadis (21.9%) and hyos (21.6%) (serology)
		buffalo	19 (40/211)	wolffi (40%), hyos (35%) and javanica (25%)
		swine	4.3 (37/857)	ballico (45.9%), canicola (45.9%), icterohaemorrhagiae (37.8%) and bataviae (32.4%)(serology)
		sheep and goat	26.7 (67/251)	icterohaemorrhagiae (94.0%)(serology)

2.3.4. Economic impact of leptospirosis

The economic impact of leptospirosis has not yet been studied in great detail. Based on estimates of costs made in a community hospital the medical care cost for a mild case is 1,800 bahts (US$50) per case, to which costs of at least 10,000 bahts (US$278) should be added for a severe case for the cost of dialysis (36). Other costs such as loss of labour days, costs of transportation are not included in this estimate. The high prevalence of the disease among domestic animals suggests that it may have a considerable economic impact. From 1996 to 1999, 174,600 - 274,508 (FUO) cases were reported annually. From the previous studies in 1966, the proportion of leptospirosis infection in FUO cases in the northeastern region was 2.2 to 9.8%. In 1999, 174,600 FUO cases were reported. Assuming an average proportion of 5% of leptospirosis cases among FUO this would mean 8,700 leptospirosis cases in addition to the 6,080 reported cases. The estimated direct medical cost for mild leptospirosis case in 1999 is 739,000 dollars (29.5 million bahts).

Rodents and live stock play an important role in the transmission of the disease. Thailand has implemented field rat control since 1998. In 1998, 5,1000,000 rats were destroyed. In 1999, extensive field rat control was done and 7,912,947 rats in 19 epidemic provinces were killed. The total budget for field rat control in 1999 was 677,778 dollars (27.1 million bahts). However, it is too early to evaluate the impact of this effort.

2.4.Why does leptospirosis epidemic exist / happen?

Many factors or combination of factors can contribute to leptospirosis emergence. The reason might be the changing of living or working in changing ecological conditions that increase human exposure to animal reservoirs, increasing in rodent population or environmental sources of novel pathogens. Presently, we could not explain the real cause of the epidemic. The new serovar was identified in field rodent in the first epidemic area. New serovar may emerge from genetic changes in existing organisms. Exposure to the new serovar could be involved in the changing clinical pattern. But the cause of the new serovar could not determined.

2.5.What are the priority problems?

While the identification of problems surfaced in many areas, the prioritization is needed for intervention. The high priorities of problem are standardizing the clinical diagnosis of leptospirosis and the using of screening test to confirm clinical diagnosis. Leptospirosis probably is overlooked and underreported in many countries. The deceiving clinical symptoms, the difficult laboratory diagnosis, and the lack of awareness among clinicians and other health care workers all contribute to the fact that the disease is often not diagnosed. These also emphasize the necessary for using the screening test. The difficulty to diagnosis is true in areas where leptospirosis is a public health problem. Lack of information to alert the general public and risk groups about the disease and about ways to protect themselves from infection further

contributes to the spread of the disease and the occurrence of outbreaks. Hence the disease continues to cause disease in sometimes alarming proportions. Reduction of morbidity and mortality due to leptospirosis requires an increased awareness and better knowledge among health care workers to recognise signs and symptoms of the disease. Thus, the first point that should be improvement is the diagnosis of human leptospirosis.

2.6. What could we do to improve problem situation?

We should use the standard guideline for leptospirois diagnosis. However. WHO standard guideline uses MAT to confirm diagnosis and the risk factors are not specific for Thailand. Due to limitation of MAT facility an alternative screening test is needed to confirm diagnosis. We also should use the specific risk factors, which were identified in Thailand in the diagnostic criteria. The appropriate guideline for leptospirosis diagnosis will reveal the actual burden of the leptospirosis epidemic. Confirmed cases will provide better understanding of the disease and also prevention and control strategies.

The existing surveillance system does not provide means for the identification of the source and mode of transmission during an outbreak. The passive surveillance system based on clinical criteria likely detects only a small proportion of infections. The system allows early detection of outbreaks only if data are properly analysed on a weekly basis. In case of delayed analysis the system can not be used as an early warning for epidemic transmission. From reviewing outbreak investigations, an

outbreak is suspected when at least two suspected leptospirosis cases with a recent history of exposure to infected animals or an environment contaminated with animal urine (e.g. wading in flood water or rice field activities) are reported from the same area. Therefore it is recommended that in outbreak investigation, possible sources of infection should be traced and animal serum should be collected and processed immediately for laboratory investigation. To reduce the risk of outbreaks continuous sentinel serosurveys of animal populations should be done. To detect infection in cows, anti-leptospiral antibodies in tank milk should be monitored.

In case of an outbreak notification a response team needs to arrive on site within 24 hours and to start immediate case finding, to start educational activities in the community and to facilitate prompt referring for diagnosis and treatment. Immediate action is required as early treatment reduces morbidity. In order to prevent outbreaks it is recommended that active surveillance should take place in the general hospital during the inter epidemic period. The active surveillance should consist of laboratory testing of all patients with: 1) any haemorrhagic manifestation especially pulmonary haemorrhage, and 2) an admission diagnosis of fever with jaundice, renal failure or aseptic meningitis.

The passive surveillance system should be strengthened. Strategies for active surveillance in animals and human should be developed and implemented. Control measures such as vaccination and treatment of live stock could be considered. Education programs should be developed and used to increase sanitation and altering human behaviour in order to avoid risk activities or to take preventive measure when engaged in such activities. Vaccination and prophylactic treatment of risk groups is

another option that could be considered. Weaknesses and constraints should be identified and used to improve prevention and control strategies. Given the complex epidemiology of leptospirosis tailor made solutions likely are needed to cope with the leptospirosis problem in different situations. In designing strategies to prevent infection wearing shoes may be most effective in the short term. Wearing shoes is a simple measure that can protect people from infection through contact with a contaminated environment and that helps to reduce the number of new open wounds. However, it is not easy for people to wear shoes all the time to avoiding contact to water during their daily work in the rice field.

2.7. Standard guideline for diagnosis of leptospirosis

Standard guideline for leptospirosis diagnosis is designed for those who deal directly with the patient (Table 5). To use the list, note the main clinical features listed, mark the box "Yes" or "No" and write the appropriate score in the right-hand column.

A presumptive diagnosis of leptospirosis may be made if: Part A, or Parts A and B score 26 or more ; 2) Part A, B and C totals 25 or more. A score between 20 and 25 suggests leptospirosis as a possible but unconfirmed diagnosis.

Table 2.5. World Health Organization standard guideline for diagnosis of leptospirosis

Question	Answer	Score
A: Has the patient:		
Headache of sudden onset?	Yes No	2 0
Fever?	Yes No	2 0
If "Yes", Is the temperature 39^0C or more?	Yes No	2 0
Conjunctival Suffusion? *	Yes No	4 0
Meningism? *	Yes No	4 0
Muscle pains (especially calf muscles)? *	Yes No	4 0
* Are all 3 features (conjunctival suffusion, muscle pains and meningism) present together?	Yes No	10 0
Jaundice?	Yes No	1 0
Albuminuria or nitrogen retention?	Yes No	2 0

Total score of part A		
B: Epidemiological factors:		
Has there been contact with animals at home, work, leisure, or in travel, or contact with known (or possibly) contaminated water?	Yes No	10 0
C. Bacteriological laboratory findings: Isolation of *leptospires* in culture- diagnosis certain		
Positive serology-leptospirosis endemic:		
Single positive, low titer	Yes No	2 0
Single positive, high titer	Yes No	10 0
Paired sera, rising titer	Yes No	25 0
Positive serology-leptospirosis not endemic:		
Single positive, low titer	Yes No	5 0
Single positive, high titer	Yes No	15 0
Paired sera, rising titer	Yes No	25 0
Total score (A+B+C)		

2.8. Types of serodiagnosis for leptospirosis.

2.8.1 Standard test: Microscopic Agglutination Test (MAT)

MAT is the basic serological diagnosis for leptospirosis and also is the "gold" standard for serology. The usual method is to mix equal volumes of a series of serum dilutions and leptospiral culture in a microtiter plate or test tube. The serum antigen mixtures are allowed to react for 2 – 4 hours at room temperature. The degree of agglutination and the end point titers are determined by examine a sample of mixture by dark-field microscopy. The reaction may be difficult to interpret if a strain causing the illness is not present in the diagnostic panel, the diagnosis may be missed because agglutination may not be observed. Therefore, a panel of live *leptospires* used for antigens should represent the serovars that occurred in the area where the patient become infected. Moreover, MAT cannot be standardized because live *leptospires* are used as antigen. The age and the density of the antigen are variable, which may affect the reproducibility of the result. Furthermore, it is difficult to maintain live strains, difficult to prepare culture medium and saprophyte or other bacteria easily contaminate cultures.

The MAT is highly sensitive and specific but the assay can be performed only in few specialised and well-equipped laboratories staffed by trained personnel capable of maintaining cultures of leptospiral strains that are needed as live antigens. The MAT often is serovar specific, which means that agglutination only is seen with an antigen belonging to the same serovar as the infecting strain. The serovar specificity

of the MAT is a disadvantage when the assay is used for diagnostic purposes as in order to ensure a high diagnostic sensitivity the assay should be performed using a battery of strains that is representative for all commonly occurring serovars in a certain area. Detailed epidemiological and serological knowledge of the causative strains is thus needed in order to make a rational selection of strains for use as antigen. The serovar specificity of the MAT however makes the assay 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.

2.8.2 Screening test

2.8.2.1 Enzyme linked Immunosorbent Assay (ELISA)

ELISA has been used successfully to detect human and animal antibodies to *leptospires*. The class of the patient's antibodies can be determined IgM and IgG. ELISAs come in a wide variety. Various antigenic preparations can be made and bound to the polystyrene in microtiter plates. Using different enzyme conjugates. After adding substrate, enzyme substrate reaction resulting in color change indicates a positive reaction. The success of the ELISA is probably the method providing highly useful information on class specific antibodies, which is clinically important. Detection of specific anti-*leptospira* IgM antibodies in the single serum specimen indicates current leptospirosis. (Cut-off values depend on the level of persisting

antibodies in general population). However, the use of broadly reactive antigen does not allow differentiation between causative serovars. Generally, the ELISA is positive somewhat earlier in the course of illness than the MAT but the test is slightly less specific. Their advantages in comparison with the MAT are probably the stability of antigenic preparation, their genus specificity and relative simplicity.

The IgM ELISA has been developed and evaluated in Thailand by Bencha Petchchai and others in 1990(43). They used a surface antigen from *L. interrogans* serovar bataviae, pyrogenase and iccterrohemorrhagiae. The study found that IgM ELISA using antigen prepared from serovar bataviae showed highest sensitivity with 98.06% from 103 sera positive by MAT. IgM specificity and the sensitivity of ELISA combined with the board specificity of surface antigen make earlier serodiagnosis of leptospirosis possible. IgM and IgG ELISA, the commercial test kits are now available.

2.8.2.2 Dot ELISA

This technique is similar to the ELISA, but the antigens are dispensed as dots on a solid membrane instead of at the bottom of the microtiter plates to make the interpretation of the result easier than ELISA. From the study of Pappas and other (1985), IgM specific dot-ELISA for diagnosis of human leptospirosis had sensitivity of 91.2% and a specificity of 81% (44). From the study of Silva and others (1997), sensitivity was 98% and the specificity was 100% (45). The dot –ELISA is more advantagous than other techniques because of the possibility of visual reading,

reliable results that do not require special equipment and the possibility of carrying out the test at room temperature. All those advantages facilitate its use in the field and in less equipped laboratories. Presently, this technique is refined into a commercial test kit for diagnosis of leptospirosis named Lepto-Dipstick assay.

2.8.2.3. Indirect fluorescent antibody test (IFAT)

Indirect fluorescent antibody test has mostly been developed for use with both human and animal sera. The IFA is comparable with the ELISA in being broadly reactive but is more subjective. Leptospires can coat onto microscopic slides. Serum dilutions are applied on the antigen spot. Bound anti-*leptospira* antibody is detected with a fluorescein-conjugated class specific antibody under the fluorescence microscope. This technique has been developed and used for diagnosis of human leptospirosis in Thailand by Appassakij and others in 1995 (46). In the study, *leptospires interrogans* serovar bataviae was used as a source of antigen because this serovar was the most common infective serovar, broadly specific and did not compromise the genus specific nature of the test. They found that the IFA titer of equal or greater than 1:100 was high specific but moderately sensitive on acute sera testing. However, the technique has been proved to be a fast and reliable mean to determine the level of antibodies to *leptospires*. Mostly, the result of the IFA is corresponding to the result with the ELISA.

2.8.2.4. Indirect Haemagglutination Test (IHA)

Sheep or human group O erythrocytes coated with genus – specific leptospiral antigen reacted with positive sera which caused agglutination of the cells for detecting antibodies to *leptospires*.

2.8.2.5. Latex Agglutination Test (LA)

The sensitization of commercially available latex particles with genus specific leptospiral antigens is used to react with antiserum to cause agglutination of the particles. This assay is rapid, requires less than 5 minutes to perform and requires no special equipment. It is recommended that the use of boardly reactive antigen of pathogenic *leptospires* for coating of latex particles ensure the efficient detection of a wide spectrum of leptospiral infection.

The two methods, IHA and LA, have been developed and evaluated by Bencha Petchclai and others, a study group at Ramathipbodi hospital in 1990 (43). Using an antigen prepared from *L.interrogans* serovar bataviae, the results showed high sensitivity in control positive sera with cut off titer greater than 1:80. This study has proved that IHA and LA are more sensitive than the MAT in early infection. However, with the assay itself false positive and non-specific reactions are possible depending on antigen used and preparation.

2.8.2.6 . Macroscopic Slide Agglutination Test (MSAT)

The test is performed in a similar manner to other well-known slide tests such as for salmonellosis and brucellusis. Concentrated killed antigen (Patoc I and locally prevalent strains) and patient's serum are mixed on plate, slide or card. The presence of agglutination is determined by naked eye. This method is simple and quick screening test but less specific than the MAT and does not discriminate between antibodies caused by current infections and residual antibodies of past infections.

2.9 Comparison of serodiagnosis

The advantages and disadvantages of serological assay used for diagnosis of leptospirosis is shown in table 2.9. The Dot-ELISA, IHA and LA can be used in every hospital. Moreover, Dot-ELISA has the benefit of detect IgM antibodies, which can provide the current infection.

Table 2.6. Comparison of serological assays used for diagnosis of leptospirosis.

Assays	Application	Advantages	Disadvantages
MAT	Clinical, research	Specific, serovar differentiated	complicated
IFA	Clinical, research	Sensitive, specific and simple	Subjective, FA microscope
IgM ELISA	Clinical, research	Sensitive, specific and commercial	equipment
Dot ELISA	Clinical, research	Sensitive, specific, commercial and simple	subjective
IHA and LA	Clinical	Simple and commercial	subjective
MSAT	Clinical	Simple, rapid and commercial	Not sensitive and not specific

2.10 How do these solutions work?

2.10.1 Physician training

Physician knowledge is the most important to improve the quality of leptospirosis diagnosis. To solve the problem of over or under diagnosis from

surveillance data, following the standard guideline for leptospirosis diagnosis is important. Then, data from surveillance system will provide the actual number of leptospirosis cases. These confirmed cases' data will give a good epidemiological picture of cases such as; age, gender, occupation of cases, clinical picture, risk activities and risk areas etc. The problem of treatment and also health system facilities may be revealed. A better epidemiological understanding is needed to issue appropriate measures to reduce risks of exposure and to prevent transmission of the disease. The control and eventually eradication of the disease is even more complicated as it requires tracing of the source of infection and determination of the mode of transmission.

2.10.2 Using laboratory results to confirm diagnosis

Serodiagnosis is essential for the diagnosis and treatment. It also will help physicians to confirm their diagnosis and also improve their treatment. Hence, the reliable and available commercial test kits for rapid diagnoses are too expensive afforded by the patient of leptospirosis who are mostly in the low socioeconomic status. The in-house developed serodiagnosis tests, which have been evaluated, should be improved and considered to be used as screening test for leptospirosis in the hospital. In-house screening test will solve the problem of limit MAT test. The MAT, which is the only one assay for diagnosis of causative serovar, should be combined with other screening to reveal the strains or serovars that is important to

epidemiologic surveillance. For this reason, MAT should use in research and only to confirm the positive sample from screening test.

2.10.3 Development of control and prevention strategies.

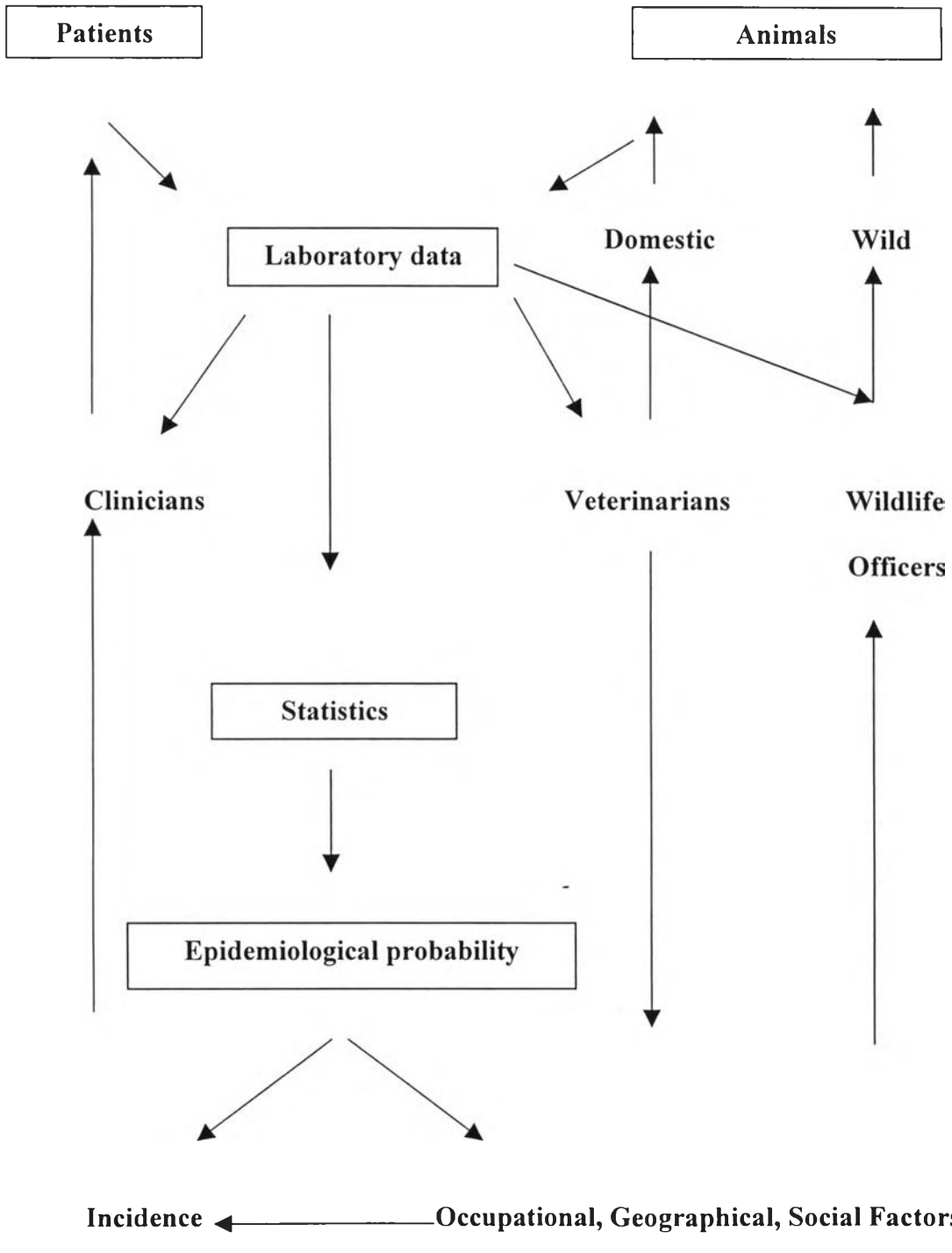
As *leptospires* may have different animal reservoirs it is often very difficult to identify the source of infection and to design and implement strategies to break the transmission cycle. Once the source and mode of transmission have been identified several control measures can be taken. Risk group and risk activities can be identified. The public can be informed which preventive measures to take and how to avoid infection. Measures that may be appropriate are measures to improve sanitation and to control rodents. For instance traps can be placed, and rodents can be prevented access to food and drinking water supplies. It can be decided to vaccinate and treat life stock and pet. It can be advised to wear protective clothing during occupational risk activities.

2.11. Which intervention should we make?

We should adapt the standard guideline for diagnosis leptospirosis by using the specific risk factors, which are appropriate to Thailand. The research on the efficiency of screening test should be performed. For surveillance system, case identification, initially based on clinical criteria but should be confirmed by laboratory findings. Collating information from human and animal sources, and linking with

frequency data and serological surveys of antibody prevalence are needed to establish endemically rates and epidemic parameters. Contemporaneous incidence and prevalence rates in local animals are likely to be the source of the leptospirosis in humans. (Figure 2.4)

Figure 2.4 Interactions and interdependence of sources of information available for ascertaining the origins of infections with leptospirosis, and for epidemiological control.



(Modified and reproduced with permission from Faine, S., ed., Guildline for the Control of Leptospirosis, WHO Offset Publication No. 67, Geneva, World Health organization, 1982).

2.11 Recommendations for Prevention and Control of Leptospirosis

1. Active case surveillance in endemic and epidemic areas should be conducted.
2. Health care practitioners should be made aware of the need for early recognition and of the wide range of symptoms associated with leptospirosis.
3. Laboratory testing should be implemented at health post and district hospital level.
4. Implementation of rodents' control in and around rice fields is imperative.
5. Laboratory surveillance of potential animal reservoirs
6. Designing strategies to reduce infection in live stock and pets.

Educate the agricultural workers about the dangers of contaminated water with emphasise the need to wear boots when farming in wet environments.

2.12 Conclusion

Leptospirosis was first diagnosed in Thailand 58 years ago. Since then the disease has been neglected for many decades. Awareness has improved in recent years but the disease probably still is underreported and misdiagnosed as is evident from the yearly increase in the number of reported cases during the past 5 years.

The climatological conditions favour the transmission of the disease during and after the raining season and during floods. The peak incidence is after the raining season and most cases occur in rural areas in the North-eastern region, a region with relatively little rainfall but with annual flooding High number of leptospirosis cases however have been reported from provinces in all regions. A diverse of rodents, different species of live stock and pets likely are important reservoirs. Different rat species, dogs, cows, buffalo, pigs, coats and sheep all showed a high degree of infection and potentially all could play a role in outbreaks. Flooding, rodent density and specific human activities including specific rice farming activities, close contact with live stock and wading in stagnant water all play an important role in the development of epidemics. During different epidemics illness was caused by infection with different serovars. Different animal reservoirs and different modes of transmission likely have been involved during different epidemics.

Renal impairment and pulmonary hemorrhages are major complications of leptospirosis in Thailand. Although the severity of disease has increased, mortality has decreased. Exposure to different reservoirs or a new serovar could be involved in the changing clinical pattern. The reason for the reduction in mortality might be that the awareness and the ability of the doctors to recognise and treat the disease and its complications have improved. Still the actual burden of disease is probably greater than the reported level.

To solve the leptospirosis epidemic, First of all, standard guideline and screening test to diagnose leptospirosis should be used. Improving laboratory facilities for diagnosis could further help to reduce the morbidity and mortality by allowing the

making a better and earlier diagnosis and thus offering better treatment. The introduction of rapid tests at different levels of the healthcare system including health posts and district hospitals is recommended in order to allow active surveillance to take place effectively.

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