Prevalence of Antibodies to *Leptospira* Serovars in Rodents and Shrews Trapped in Low and High Endemic Areas in Thailand

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Abstract

Objective : To investigate the prevalence of antibodies to *Leptospira* serovars in rodents and shrews trapped in urban and rural areas in low and high endemic areas in Thailand.

Material and Method : A total of 1,664 serum samples were collected from rodents and shrews in areas of low and high endemicity for leptospirosis. Four areas classified by case rates (CR) per 100,000 population of leptospirosis were urban Area I Bangkok (CR = 0.07), rural Area II (CR = 0.24), rural Area III (CR = 1.97) and rural Area IV (CR = 48.20). All serum samples were investigated for antibodies to leptospires by microscopic agglutination test (MAT) using antigens from each of the 22 pathogenic serovars of *Leptospira interrrogans* : australis, autumnalis, ballum, bangkok, bataviae, bratislava, canicola, celledoni, copenhageni, djasiman, grippotyphosa, hardjo, hebdomadis, ictero-haemorrhagiae, javanica, pomona, pyrogenes, rachmati, saigon, sejroe, tarassovi and wolffi and one non-pathogenic strain of *L. biflexa* serovar patoc.

Results : Ninety-four (5.6%) serum samples were positive for *Leptospira* antibodies. The most commonly detected antibodies were to serovars pyrogenes (39.1%), sejroe (19.1%), bataviae (10.0%), pomona (6.4%), autumnalis (5.5%), copenhageni (3.6%) and javanica (3.6%). The positive rates in Area I, II, III and IV were 7.6 per cent, 2.9 per cent, 4.6 per cent and 7.1 per cent, respectively. The seroprevalence in rural areas tended to increase significantly with high endemicity for leptospirosis (Chi-square for trend, p = 0.04). The seropositive rates by animal species were 39/496 (7.9%), 22/322 (6.8%), 23/492 (4.7%), 6/170 (3.5%), 4/175 (2.3%), 0/4 (0%) and 0/5 (0%) in *Rattus norvegicus, Rattus exulans, Rattus rattus, Bandicota indica, Bandicota savilei, Mus musculus* and *Suncus murinus,* respectively. There was a statistical trend between seropositive rates in *R. exulans* and endemicity for leptospirosis (Chi-square for trend, p = 0.04).

Conclusion : The 5.6 per cent of rodents and shrews trapped in urban and rural areas in Thailand were reservoirs of leptospires. The results of high seroprevalence in rats also indicate the high endemicity for leptospirosis.

Key word : Leptospirosis, Seroprevalence, Rodents, Low and High Endemic Areas

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Leptospirosis is a spirochetal zoonosis of worldwide distribution, affecting many species of wild and domestic mammals. Leptospirosis is caused by the genus *Leptospira*[1,2], in which current phylogenetic taxonomic system, *L. interrogans* is one pathogenic member of 17 *Leptospira* species[3]. More than 210 serovars (serotypes) of pathogenic leptospires have been described[1-3]. After acute infection, animals may become chronic carriers harbouring leptospires in their kidneys and genital tracts. Urinary shedding of leptospires can lead to human or animal infections either through direct contact with urine or by contamination of soil and surface water[1,4]. The natural reservoirs for leptospires are rodents, dogs, pigs and cattle.

Leptospirosis is an endemic disease which occurs in all regions of Thailand. People in both urban and rural areas may be exposed to the leptospires, however, highly endemic areas are located in the north-eastern region[5]. The objective of the study was to investigate the prevalence of antibodies to *Leptospira* serovars using a microscopic agglutination test in rodents and shrews trapped in urban and rural areas of low and high endemicity for leptospirosis in Thailand.

MATERIAL AND METHOD

Study population

During the period between October 1998 to April 2000, a total of 1,664 rodents and shrews were trapped alive in four different areas of Thailand. The low and high endemicity for leptospirosis was divided by case rates (CR) per 100,000 population of leptospirosis reported by the Ministry of Public Health (MoPH) as shown in Table 1. The low endemic areas consisted of Area I (urban), Bangkok which is the capital of Thailand, with CR of 0.07 and Area II (rural) with an average CR of 0.24 (range 0-0.47). The averages and ranges in rural high endemic areas of Areas III and IV were CR of 1.97 (1.19-3.44) and 48.20 (15.06-112.71), respectively.

Microscopic agglutination test (MAT)

Serum specimens were tested for the presence of leptospiral antibodies using the following 22 *L. interrogans* (sensu lato) serovars, australis, autumnalis, ballum, bangkok, bataviae, bratislava, canicola, celledoni, copenhageni, djasiman, grippotyphosa, hardjo, hebdomadis, icterohaemorrhagiae, javanica, pomona, pyrogenes, rachmati, saigon, sejroe, tarassovi and wolffi and one non-pathogenic strain of *L. biflexa* serovar patoc, as antigens. The test was performed by using a suspension of 10^8 bacterial cells/ml. A serum which gave a positive result at a screening dilution of 1 : 50 was considered to have leptospiral antibody.

Statistical analysis

Data were analyzed by EPI INFO version 6.0. Comparison of prevalence between low and high

Table 1. Case rates per 100,000 population of leptospirosis in 1999 as reported by MoPH.

Area	City	Region	Case rate
I	Bangkok	Central	0.07
II	Surat Thani	South	0
	Chon Buri	Central	0.47
III	Trang	South	1.19
	Phitsanulok	North	1.38
	Phra Nakhon Si Ayutthaya	Central	1.78
	Chanthaburi	Central	2.06
	Phetchabun	North	3.44
IV	Udon Thani	North-eastern	15.06
	Nakhon Ratchasima	North-eastern	16.83
	Buri Rum	North-eastern	112.71

endemicity for leptospirosis was performed by Chi-square for trend. The level of critical significance was assigned at $p < 0.05$.

RESULTS

A total of 1,664 rodents and shrews were trapped in the present study (Table 2). In Area I, 500 animals were trapped, including *R. norvegicus* (91.6%), *R. rattus* (5.6%), *R. exulans* (1.0%), *S. murinus* (0.8%) and *M. musculus* (1.0%). In rural areas, a total of 1,164 rodents were trapped, including *R. rattus* (39.9%), *R. exulans* (27.2%), *B. savilei* (15.0%), *B. indica* (14.6%) and *R. norvegicus* (3.3%).

It was found that 94 (5.6%) of 1,664 trapped rodents and shrews had leptospiral antibodies as shown in Table 2. In Area I (Bangkok), a seroprevalence was 7.6 per cent (38/500) while in the rural Areas II to IV, the seroprevalence were 2.9 per cent, 4.6 per cent and 7.1 per cent, respectively. A statistical trend in seropositivity between low and high endemicity for leptospirosis in rural areas was observed ($p = 0.04$).

In Area I, antibodies were detected in *R. norvegicus* only, at a seroprevalence of 8.3 per cent (38/458). In rural areas, antibodies were found in *R. exulans* (6.8%), *R. rattus* (5.0%), *B. indica* (3.5%), *R. norvegicus* (2.6%) and *B. savilei* (2.3%).

When the seroprevalence in rat species (*R. exulans* and *R. rattus*) were analyzed, there was a significant trend in increasing seropositivity in *R. exulans* between Areas I to IV (0%, 1.6%, 7.2% and 10.2%, respectively) and endemicity for leptospirosis ($p = 0.04$).

Antibodies to *Leptospira* serovars were found in 94 rats as shown in Table 3. Of 94 positive samples, single and multiple antibodies were 84 (89.4%) and 10 (10.6%), respectively. The overall leptospiral seropositivity was 110 (6.6%). Antibodies to serovars pyrogenes was most commonly detected (39.1%) followed by sejroe (19.1%), bataviae (10.0%), pomona (6.4%), autumnalis (5.5%), copenhageni (3.6%), javanica (3.6%), ballum (2.7%), tarassovi (2.7%), hebdomadis (1.8%), icterohaemorrhagiae (1.8%) and 0.9 per cent of each left while 1 (0.9%) was positive for serovar patoc. Autumnalis, bratislava and copenhageni were found only in high endemic areas (Areas III and IV).

The results of the number of detected antibodies to *Leptospira* serovars by rat species are shown in Table 4. *R. exulans* carried a high number of pyrogenes, autumnalis, pomona and sejroe. The more frequent serovars pyrogenes, sejroe and bataviae were found in *R. norvegicus*. Pyrogenes and sejroe were carried by *R. rattus*. *B. indica* carried autumalis, pyrogenes and sejroe, whereas *B. savilei* had bataviae, hebdomadis, pyrogenes and sejroe.

DISCUSSION

Leptospirosis is an endemic disease in Thailand, with a case rate of 0.3 per 100,000 population throughout the country, from a report of the Thai Ministry of Public Health (MOPH) between 1982 and 1995. However, leptospirosis was found to be sporadic in north-eastern provinces in 1996 with a case rate of 1.4 per 100,000 population. Humans were infected through direct or indirect contact with infected

Table 2. Distribution of leptospiral antibodies by 1,664 rodent and shrew species.

Rodent and shrew species	No. positives/No. rodents or shrews										P-value
	Area I	%	Area II	%	Area III	%	Area IV	%	Sub-total	%	
Rattus exulans	0/5	0	1/63	1.6	12/166	7.2	9/88	10.2	22/322	6.8	0.04
Rattus norvegicus	38/458	8.3	1/25	4.0	0/13	0	-	-	39/496	7.9	
Rattus rattus	0/28	0	3/85	3.5	16/330	4.9	4/49	8.2	23/492	4.7	0.26
Bandicota indica	-	-	0/8	0	3/73	4.1	3/89	3.4	6/170	3.5	
Bandicota savilei	-	-	1/25	4.0	3/150	2.0	-	-	4/175	2.3	
Mus musculus	0/4	0	-	-	-	-	-	-	0/4	0	
Suncus murinus	0/5	0	-	-	-	-	-	-	0/5	0	
Total	38/500	7.6	6/206	2.9	34/732	4.6	16/226	7.1	94/1,664	5.6	
P-value	◄				0.04					►	

Table 3. Detection of antibodies to *Leptospira* serovars in 94 rodents trapped in Thailand.

Leptospira serovar	No. positive								Sub-total (n = 110)	%
	Area I (n = 44)	%	Area II (n = 6)	%	Area III (n = 37)	%	Area IV (n = 23)	%		
Australis	1	2.3	-	-	-	-	-	-	1	0.9
Autumnalis	-	-	-	-	2	5.4	4	17.4	6	5.5
Ballum	3	6.8	-	-	-	-	-	-	3	2.7
Bataviae	9	20.5	1	16.7	1	2.7	-	-	11	10.0
Bratislava	-	-	-	-	-	-	1	4.3	1	0.9
Copenhageni	-	-	-	-	2	5.4	2	8.7	4	3.6
Hebdomadis	-	-	-	-	2	5.4	-	-	2	1.8
Icterohaemo-rrhagiae	1	2.3	1	16.7	-	-	-	-	2	1.8
Javanica	2	4.5	-	-	2	5.4	-	-	4	3.6
Pomona	3	6.8	-	-	2	5.4	2	8.7	7	6.4
Pyrogenes	13	29.5	3	50.0	17	46.0	10	43.5	43	39.1
Sejroe	10	22.7	-	-	8	21.6	3	13.0	21	19.1
Tarassovi	2	4.5	1	16.7	-	-	-	-	3	2.7
Wolffi	-	-	-	-	-	-	1	4.3	1	0.9
Patoc	-	-	-	-	1	2.7	-	-	1	0.9

Table 4. Number of detected antibodies to *Leptospira* serovars by rat species.

Rat species	*Leptospira* serovars
Rattus exulans (n = 32)	Autumnalis (2)*,**, Bataviae (1), Bratislava (1), Copenhageni (1), Icterohaemorrhagiae (1), Pomona (2), Pyrogenes (20), Sejroe (2), Tarassovi (1), Wolffi (1)
Rattus norvegicus (n = 48)	Australis (1), Ballum (3), Bataviae (19), Copenhageni (2), Icterohaemorrhagiae (1), Javanica (2), Pomona (3), Pyrogenes (15), Sejroe (10), Tarassovi (2)
Rattus rattus (n = 20)	Autumnalis (1), Copenhageni (1), Hebdomadis (1), Javanica (1), Pomona (2), Pyrogenes (7), Sejroe (6), Tarassovi (1)
Bandicota indica (n = 6)	Autumnalis (3), Pyrogenes (1), Sejroe (2)
Bandicota savilei (n = 4)	Bataviae (1), Hebdomadis (1), Pyrogenes (1), Sejroe (1)

* Number of positive(s) shown in parenthesis.
** High number of positives shown as underlined.

animals. Infected animals may become carriers and excrete leptospires in their urine from the favoured persistent site in renal tubules[6].

The most common reservoir animals of leptospires are rodents, dogs, cattle and pigs. In the present study, 94 (5.6%) of 1,664 rodents and shrews were positive for leptospiral antibodies using MAT. In Bangkok, the leptospiral seroprevalence of 7.6 per cent was lower than that of 30.7 per cent as previously reported by the AFRIMS group[7]. The difference can be explained by the decrease of case rate of 100,000 population from 0.15 in 1988 to 0.07 in 1999 reported by the MOPH. In 1965, a serological surveillance in rats of 10 districts in Bangkok showed 59.2 per cent (151/255 rats)[8]. A similar result by using MAT in urban areas showed seroprevalence in *R. norvegicus* between of 7.6 per cent in Bangkok in the present study and of 8.4 per cent in Turkey[9]. Leptospiral antibodies of 7.1 per cent in high endemicity for leptospirosis (Area IV) was lower than the prevalence of leptospiral reservoirs (14.3%) in rodents from the epidemic provinces[10].

Reservoirs for leptospires include wild and domestic animals especially rodents, since it has been reported that 10-50 per cent of rodents excreted the leptospires in their urine[4] and rodents have close-contact with humans. In the present study, the seroprevalence in rural areas tended to increase significantly with high endemicity for leptospirosis. A similar report showed 41 per cent isolates from rodents in an epidemic area, whereas none in a non-epidemic area[11]. However, a subsequent study demonstrated there was no difference of leptospiral isolation rates in rodents from the epidemic provinces (14.3%) and the non-epidemic one (15.3%)[10]. The reason might be due to the difference of rodent species caught. The most frequent rodents in urban areas are *R. norvegicus, R. rattus* and *R. exulans*, whereas those in rural areas are *B. indica, B. savilei, R. exulans* and *R. rattus*[12]. When the seroprevalence in rodent species trapped in both urban and rural areas were analyzed, there was an association for a trend between seropositive rate in *R. exulans* and endemicity for leptospirosis.

The distribution of serovars has changed from time to time. In Bangkok, bataviae and javanica accounted for 20.5 per cent and 4.5 per cent, respectively, compared to 70 per cent and 28 per cent, respectively, in 1965[8]. Recently, pyrogenes has rapidly progressed in Bangkok and other provinces of Thailand. Sejore, a newly introduced serovar, has also spread recently in most areas. Interestingly, autumnalis using serology found only in high endemicity areas (Areas III and IV) in the present study was similar to that previously isolated from rats among epidemic provinces[10].

More serovar types detected in high endemic areas were similar to those in a previous report[10], however, a high number of serovars was also found in Bangkok. The seroprevalence in Bangkok was not different from that in high endemic areas in the present study. These results showed that there was no difference of prevalence of leptospiral reservoirs in rodents from epidemic provinces and non-epidemic ones[10]. The results of both a high number of serovars and prevalence of leptospiral infections in rodents indicate that animal reservoirs are not the only factors affecting infection in humans. Other potential risk factors include exposure to contaminated water[13], such as walking through water, applying fertilizer in wet fields for more than 6 hours a day, plowing in wet fields for more than 6 hours

a day and pulling out rice plant sprouts in wet fields for more than 6 hours a day. In most countries, farmers and fish-farmers continue to be the major occupational risk group(14,15).

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ความชุกของแอนติบอดีย์ต่อซีโรวาร์ของเชื้อ*เลปโตสไปร่า*ในหนูซึ่งถูกดักจับในพื้นที่ที่มีการระบาดในระดับต่ำและสูงในประเทศไทย

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วัตถุประสงค์ : เพื่อสืบหาความชุกของการติดเชื้อโดยการหาแอนติบอดีย์จำเพาะต่อซีโรวาร์ชนิดต่าง ๆ ของเชื้อ-*เลปโตสไปร่า*ในหนูซึ่งถูกดักจับในพื้นที่ชุมชนและชนบทซึ่งเป็นพื้นที่ที่มีการระบาดระดับต่ำและสูงสำหรับโรคเลปโตสไปโรสิส ในประเทศไทย

วัสดุและวิธีการ : ตัวอย่างตรวจซีรั่มจำนวน 1,664 ราย ได้ถูกเก็บจากหนูในพื้นที่ที่มีการระบาดระดับต่ำและสูงสำหรับโรคเลปโตสไปโรสิส พื้นที่ทั้ง 4 แห่งถูกจัดแบ่งตามอัตราผู้ป่วย (case rate, CR) ต่อประชากร 100,000 คน ของโรคเลปโตสไปโรสิส ดังนี้ พื้นที่ I กรุงเทพมหานคร ซึ่งเป็นพื้นที่ชุมชน (CR = 0.07), สำหรับพื้นที่ชนบทประกอบด้วย พื้นที่ II (CR = 0.24), พื้นที่ III (CR = 1.97), และพื้นที่ IV (CR = 48.20) ตัวอย่างซีรั่มทุกตัวอย่างตรวจถูกสืบหาแอนติ-บอดีย์ ต่อเชื้อ*เลปโตสไปร่า*โดยวิธีไมโครแอกกลูติเนชั่น (MAT) เชื้อที่ใช้เป็นแอนติเจนประกอบด้วย 22 ซีโรวาร์ซึ่งก่อโรคได้ของ *Leptospira interrogans* ดังนี้ ออสทราลิส, ออทัมนาลิส, บอลลัม, แบงค็อก, ปัตตาเวีย, บราทิสลาวา, แคนิโคลา, เซลลีโดไน, โคเปเฮเกนไน, จาสลิมัน, กริฟโปโทโฟซา, ฮาร์ดโจ, เฮบโดมาดิส, อิคเตอร์โรฮีโมราเจีย, จาวานิกา, โปโมนา, ไพโรจินิส, ราชมัทติ, ไซง่อน, เซโร, ทารัสโซวี, และวูฟฟี สำหรับอีกหนึ่งซีโรวาร์ของเชื้อไม่ก่อโรค *L. biflexa* คือ พาท็อก

ผลการศึกษา : หนู 94 ตัว (ร้อยละ 5.6) มีผลบวกต่อเชื้อ *L. interrogans* แอนติบอดีย์ต่อซีโรวาร์ที่พบบ่อย คือ ไพโรจินิส (ร้อยละ 39.1), เซโร (ร้อยละ 19.1), ปัตตาเวีย (ร้อยละ 10.0), โพโมน่า (ร้อยละ 6.4), ออทัมนาลิส (ร้อยละ 5.5), และโคเปนเฮเกนไน (ร้อยละ 3.6), และจาวานิกา (ร้อยละ 3.6) อัตราผลบวกในพื้นที่ I, II, III, และ IV คือ ร้อยละ 7.6, 2.9, 4.6, และ 7.1 ตามลำดับ ความชุกการติดเชื้อโดยการตรวจหาแอนติบอดีย์เฉพาะในพื้นที่ชนบท พบว่ามีแนวโน้มว่าเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติกับพื้นที่ที่มีการระบาดของโรคเลปโตสไปโรสิสระดับสูง (ไค-สแคว์ร ฟอร์ เทรน, p=0.04) อัตราผลบวกของแอนติบอดีย์ตามชนิดของหนู *R. norvegicus* (หนูท่อ), *R. exulans* (หนูจี๊ด) *R. rattus* (หนูท้องขาว), *B. indica* (หนูพุกใหญ่), *B. savilei* (หนูพุกเล็ก), *Mus musculus* (หนูหริ่ง) และ *Suncus murinus* (หนูผี) ดังนี้ 39/496 (ร้อยละ 7.9), 22/322 (ร้อยละ 6.8), 23/492 (ร้อยละ 4.7), 6/170 (ร้อยละ 3.5), 4/175 (ร้อยละ 2.3), 0/4 (ร้อยละ 0), และ 0/5 (ร้อยละ 0), ตามลำดับ พบว่ามีความสัมพันธ์แบบแนวโน้มระหว่างอัตราผลบวกของแอนติบอดีย์ในหนูจี๊ด และระดับการระบาดของโรคเลปโตสไปโรสิส (ไค-สแคว์ร ฟอร์ เทรน, p = 0.04)

สรุป : หนูในประเทศไทยร้อยละ 5.6 เป็นรังโรคของโรคเลปโตสไปโรสิส การเฝ้าระวังความชุกของการติดเชื้อในหนูช่วยบ่งชี้การระบาดของโรคเลปโตสไปโรสิสได้

คำสำคัญ : โรคเลปโตสไปโรสิส, ความชุกของการติดเชื้อโดยแอนติบอดีย์, หนู, พื้นที่ที่มีการระบาดต่ำและสูง

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