Survey of Leptospirosis among Rodents in Epidemic Areas of Thailand

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Abstract

To study the prevalence of infection by rodent-borne diseases in reservoir rodent species in the Northeast, Northern, Central and Southern parts of Thailand, a survey of leptospiral antibodies in rodent was conducted during the period October 1998 to April 2000. In total, sera from 1,164 rodents were collected from 10 provinces. Fifty-six sera were determined positive with the titer ≥ 1:50 by the MAT method against any of the 17 leptospira serovars used. Rodent sera collected from the Northeast, Northern, Central, and Southern Thailand, were positive at rates of 7.1%, 4.9%, 4.3% and 3.0%, respectively. The positive result of *Rattus exulans, R. rattus, Bandicota indica, R. norvegicus* and *B. savilei* were positive 6.9 (22/317), 5.0 (23/464), 3.5 (6/170), 2.6 (1/38), and 2.3 (4/175), respectively. The predominant serovars of *Leptospira* in *R. exulans* and *R. norvegicus* were *pyrogenes*, while those of *R. rattus, B.indica* and *B.savilei* were *sejroe* and *pyrogenes*; *akiyami a*; and others, respectively.

Keywords: leptospirosis, microscopic agglutination test (MAT)

Introduction

Leptospirosis is a contagious disease of animals and humans caused by infection with the spirochete *Leptospira* [1-3]. The common reservoir hosts are rodents and domestic animals, which cause leptospirosis in humans [4]. The key feature in the transmission of leptosirosis between animals and man is infection of the renal tubule and excretion of infectious leptospires in the urine of carrier animals. Urine shed from carrier animals can result indirect transmission of the infection via contamination of the mucous membranes of other animals or indirect transmission via contamination of the environment. In 2001, 8,611 cases of leptospirosis and 135 deaths were reported in Thailand. Therefore, in this study we survey the prevalence of the antibodies of leptospirosis in rodents in epidemic areas of Thailand and the incidence of infection is relatively high with humans.

Materials and methods

Rodent sera

The samples were collected during the period October 1998 to April 2000. One thousand, one hundred and sixty-four rodent sera were collected from Buriram (64), Udon Thani (60), Nakhon Ratchasima (102), Phetchabun (81), Phitsanulok (246), Chanthaburi (134), Chon Buri (150), Phra Nakhon Si Ayutthaya (160), Trang (111) and Surat Thani (56) provinces. All sera were stored in a freezer at –20˚C until testing.

Microscopic agglutination test (MAT)

The MAT was performed with a panel of 17 live leptospires prevalent in Thailand. The panel of 17 *L. interrogans* serovars were a*kiyami a, ballico, bataviae, canicola, grippotyphosa, hebdomadis, hyos, icterohemorrhagia, javanica, pomona, pyrogenes, wolffi, bratislava, copenhageni, bangkoki, sejroe,* and *hardjo*.

Table 1 The number of positive antibodies in rodent sera determined by MAT.

Province	Region (% positive)	Positive/ Total (%)	Serovars					
			Sejroe (%)	*Pyrogenes* (%)	*Copenhageni* (%)	*Pomona* (%)	*Akiyami A* (%)	Others (%)
Northeast	7.1 (16/226)		4/16 (25)	8/16 (50)	1/16 (6.3)	2/16 (12.5)	1/16 (6.3)	-
Buriram		8/64 (12.5)	1/8 (12.5)	4/8 (50)	1/8 (12.5)	2/8 (25)	-	-
Udon Thani		4/60 (6.7)	1/4 (25)	3/4 (75)	-	-	-	-
Nakhon								
Ratchasima		4/102 (3.9)	2/4 (50)	1/4 (25)	-	-	1/4 (25)	-
Northern	4.9 (16/326)		4/16 (25)	8/16 (50)	2/16 (12.5)	-	1/16 (6.3)	1/16 (6.3)
Phetchabun		10/81 (12.4)	2/10 (20)	6/10 (60)	1/10 (10)	-	-	1/10 (10)
Phitsanulok		6/246 (2.4)	2/6 (33)	2/6 (33)	1/6 (16.7)	-	1/6 (16.7)	-
Central	4.3 (19/444)		3/19 (15.8)	11/19 (57.9)	-	-	1/19 (5.3)	3/19 (15.8)
Chanthaburi		9/134 (6.8)	2/9 (22.2)	5/9 (55.6)	-	-	1/9 (11.1)	-
Chon Buri		5/150 (3.3)	-	3/5 (60)	-	-	-	2/5 (40)
Phra Nakhon								
Si Ayutthaya		5/160 (3.1)	1/5 (20)	3/5 (60)	-	-	-	1/5 (20)
Southern	3.0 (5/167)		1/5 (20)	4/5 (80)	-	-	-	-
Trang		4/111(3.6)	1/4 (25)	3/4 (75)	-	-	-	-
Surat Thani		1/56(1.8)	-	1/1 (100)	-	-	-	-
Total	4.8 (56/1164)		12/56 (21.4)	31/56 (55.4)	3/56 (5.4)	2/56 (3.6)	3/56 (5.4)	5/56 (8.9)

Table 2 Serovars of *Leptospira* in rodent sera.

Type	Positive/Total (%)	Serovars					
		Sejroe (%)	*Pyrogenes* (%)	*Copenhageni* (%)	*Pomona* (%)	*Akiyami A* (%)	Others (%)
Rattus exulans	22/317 (6.9)	1/22 (4.5)	20/22 (90.9)	-	-	-	1/22 (4.5)
Rattus rattus	23/464 (5.0)	8/23 (34.8)	8/23 (34.8)	3/23 (13.6)	2/23 (8.7)	-	2/23 (8.4)
Bandicota indica	6/170 (3.5)	2/6 (33.3)	1/6 (16.7)	-	-	3/6 (50)	-
Rattus norvegicus	1/38 (2.6)	-	1/1 (100)	-	-	-	2/4 (50)
Bandicota savilei	4/175 (2.3)	1/4 (25)	1/4 (25)		-	-	-
Total	56/1164 (4.8)	12/56 (21.4)	31/56 (55.3)	3/56 (5.4)	2/56 (3.6)	3/56 (5.4)	8/56 (14.3)

Leptospira cultures for antigen preparation were grown in neopeptone medium containing 10% rabbit serum and incubated at room temperature for 6-7 days. The cultures were examined for autoagglutination, contamination and density by darkfield microscope.

The supernatant was adjusted to a cell density of 1.5 × 10^8 cells/ml (0.5 McFarland standard) with PBS (pH 7.4). Twenty-five microliter of diluted serum (1:25 screening dilution) was added into a microtiter plate with 25 µl of properly diluted antigen (0.5 McFarland

standard). The material was mixed gently and left at room temperature for three hours. The suspension was then dropped onto a slide and the agglutination read with a darkfield microscope. When a positive reaction was seen in the screening test, serial two-fold dilutions were made for confirmation. The end-point titer was defined as the highest serum dilution giving 50% agglutination in comparison with the negative control. Sera were judged to be positive if the titer was ≥ 1:50 by the MAT method.

Result

In this study, 1,164 rodent sera were collected from 10 provinces during the period October 1998 to April 2000. The number of positive antibodies in rodent sera determined by MAT, is shown in Table 1. It was found that sera from Buriram, Udon Thani, Nakhon Ratchasima, Phetchabun, Phitsanulok, Chanthaburi, Chon Buri, Phra Nakhon Si Ayutthaya, Trang and Surat Thani provinces were positive at rates of 12.5%, 6.8%, 3.9%, 12.4%, 2.4%, 6.7%, 3.3%, 3.1%, 3.6% and 1.8%, respectively. The predominant serovars in 10 provinces were *pyrogenes*. The infection of *Leptospira* in *Rattus exulans, R. rattus , Bandicota indica, B. savilei* and *R. norvegicus* were positive 6.9 (22/317), 5.0 (23/464), 3.5 (6/170), 2.6 (1/38), and 2.3 (4/175), respectively (Table 2). The predominant serovars of *Leptospira* in *R. exulans* and *R. norvegicus* were *pyrogenes*, while those of *R. rattus, B.indica* and *B.savilei* were *sejroe* and *pyrogenes*; *akiyami a*; and others, respectively.

Discussion

The standard reference test of leptospirosis in animals is the MAT, which was used in this study, and determined positive if the titer was ≥ 1:50. Determinations of the positive titer varied in different countries, for example, the positive titers used in Australia and Malaysia were ≥ 1:25, and ≥ 1:40, respectively [5-7]. The titer of leptospiral antibodies may remain at a low level titer in humans or animals and it may persist throughout the life of the animal [3]. In this study, the results revealed that Buriram, Udon Thani, Nakhon Ratchasima, Phetchabun, Phitsanulok, Chanthaburi, Chon Buri, Phra Nakhon Si Ayutthaya, Trang and Surat Thani provinces were positive at rates of 12.5%, 6.7%, 3.9%, 12.4%, 2.4%, 6.8%, 3.3%, 3.1%, 3.6% and 1.8%, respectively. The predominant serovars in humans correlate with serovars in rodents such as *sejroe, pyrogenes* and *akiyami a*. This is in accordance with previous reports of Nakhon Ratchasima and Phitsanulok Regional Laboratory of Medical Science Department and Phulsuksombati *et al* [9-11]. This study revealed the infections of *Leptospira* in *R. exulans, R. rattus, B. indica, B. savilei,* and *R. norvegicus*. These results were different from Phulsuksombati *et al* which reported the isolation of leptospires from rodents in Northeastern region in 1999-2000. It was found that the endemic areas contained infected vectors such as *R. norvegicus, B. indica, R. losea, R. rattus, R. argentiventer, B. savilei* and *R. exulans* [9]. Since most villagers in rural areas in Thailand cultivate rice and crops, these people are at risk of contact water contaminated with urine from infected animals. An effective rat control campaign to reduce the number of field rats may contribute to an effective control and prevention of leptospirosis.

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