Kasetsart J. (Nat. Sci.) 46 : 736 - 741 (2012)

Serological Survey of Leptospirosis in Thai Swamp Buffalo (*Bubalus bubalis*) in Sakon Nakhon Province, Thailand

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ABSTRACT

The study aimed to investigate the seroprevalence of *Leptospira* infection in swamp buffalo in Sakon Nakhon province, Thailand. Blood samples were collected from 206 swamp buffalo. The microscopic agglutination test was used to test serum samples for specific antibodies against 24 serovars of *Leptospira* antigen (Bratislava, Autumnalis, Ballum, Bataviae, Canicola, Celledoni, Cynopteri, Djasiman, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Javanica, Louisiana, Manhao, Mini, Panama, Pomona, Pyrogenese, Ranarum, Sarmin, Sejroe, Shermani, Tarassovi and Patoc). The percentages of buffalo seropositivity against one or more *Leptospira* serovars were studied. Overall, the prevalence rate was 63.6% (131 out of 206). The prevalent serovars were Shermani, Tarassovi, Pomona, Sejroe, Ranarum, Bratislava, Bataviae, Hebdomadis and Mini. Shermani was the most prevalent serovar (63, 30.6%) followed by Tarassovi (24, 11.7%). Positive samples were found with titers between 1:50 and 1:800, with the highest titers being in the serovar Sejroe.

Keywords: *Leptospira*, swamp buffalo

INTRODUCTION

Pathogenic *Leptospira* is a genus of spirochetal bacteria and it is the causative agent of leptospirosis. Two hundred and fifty serovars, which affect many mammalian species including humans, have been currently identified (Faine *et al.*, 1999). Animal urine, as the shedding process of leptospires (Heath and Johnson, 1994), is a source of infection to other animals and humans (Palaniappan *et al.*, 2005). Leptospirosis is the major cause of economic loss through the abortion, stillbirth, infertility, poor milk production and death of cattle (Ellis, 1994). In buffalo, some clinical syndromes have been reported such as fever, abortion, drop in milk production, icterus and repeated bleeding (Ahmed, 1990; Silvio *et al.*, 2001).

The seroprevalence of leptospirosis in buffalo has been reported in many countries including India, Bulgaria, Brazil, Malaysia, Indonesia, Egypt, Dagestan, Sri Lanka, Italy and West Iran (Hajikolaei *et al.*, 2006).The impact of leptospiral infection in domestic animals and in humans is slowly becoming recognized and some surveys regarding infection in different kinds of animals in Thailand have been conducted (Sundharagiati *et al*, 1965; Doungchawee *et al.*, 2005). However, there have been few studies concerning the seroprevalence of *Leptospira* infection in buffalo (Kitcharonenpunya *et al.*, 2001).

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Received date : 04/04/12 Accepted date : 15/08/12

A report from the Ministry of Public Health, Thailand showed that leptospirosis serovars were found in rural and urban areas where there were swamp buffalo and cows; those serovars caused death and serious sickness in many Thai people especially those who worked in agricultural fields or their professions were associated with such animals like farmers (Tangkanakul *et al.*, 2000). A majority of the victims lacked knowledge of the infection and had insufficient protection. Many reports showed that the epidemiology of leptospirosis in Thailand reaches its peak period in the rainy season and 90% of the cases have been reported in Northeast Thailand since 2000 (Tangkanakul *et al.*, 2000; Tangkanakul *et al.*, 2005; Meeyam *et al.*, 2006).

In 2010, about 78,105 swamp buffalo were raised and provided to farmers by the Department of Livestock, Sakon Nakhon. Many districts of Sakon Nakhon province have been reported to have clinical diseases associated with leptospiral infection in humans(Patart *et al.*, 2000). The current research was conducted as an initial study to find out the types of leptospirosis serovars in Sakon Nakhon province.

MATERIALS AND METHODS

Swamp buffalo aged from 1 to 10 yr were sampled in Sakon Nakhon province. Blood samples were collected from 206 swamp buffalo during March and April 2010 in four districts of Sakon Nakhon province—namely, Pannanikom district (51 samples), Arkat-Amnauy district (71 samples), Phuphan district (55 samples) and Kudbak district (29 samples). All blood samples (10 mL) were taken from the jugular vein, put into sterile tubes and centrifuged at 4000×*g* for 10 min. The sera were separated and stored at -20 °C until used for the microscopic agglutination test (MAT). The blood sample collection procedure was approved by the Animal Care and Use Committee of Kasetsart University ChalermPhrakiat Sakon Nakhon Province Campus.

The microscopic agglutination test was carried out in microtiter plates according to Faine (1982), using reference strains of 24 *Leptospira* serovars and performed using the standard (Faine *et al.*, 1999). *Leptospira* serovars Bratislava, Autumnalis, Ballum, Bataviae, Canicola, Cellidoni, Cynopteri, Djasiman, Grippotyphosa, Hebdomadis, Icterohaemorragiae, Javanica, Louisiana, Manhao, Mini, Panama, Pomona, Pyrogenes, Ranarom, Sarmin, Sejroe, Shermani, Tarassovi and Patoc were cultured to 1×10^8 cell.mL^{-1} in Ellinghausen-McCullough-Johnson-Harris medium as the antigen for MAT. Twofold serial dilutions of serum samples starting from 1:25 were prepared in phosphate buffered saline in microtiter plates before the bacteria were added. Each plate was incubated for 2 hr at room temperature in the dark and examined for agglutination by dark-field microscopy. The serum titer was defined as the final dilution that showed 50% agglutination. Reciprocal agglutination titers of greater than or equal to 50% were considered positive reactions.

RESULTS

MAT is the reference standard test for serological diagnosis for leptospirosis. The distribution of *Leptospira* in four districts of Sakon Nakhon province is shown in Table 1. Serovar-specific antibodies to leptospires were detected by MAT at titers of 50 or more in 66.7% (34/51) of the serum samples from Pannanikom, with 61.9% (44/71) in Arkat-Amnauy, 65.5% (36/55) in Phuphan and 58.6% (17/29) in Kudbak. Of the 206 animals, 100 had antibodies against one or more of the serovars studied (48.5%). The prevalent serovars were Shermani (30.6%), Tarassovi (11.7%), Sejroe (5.8%), Ranarum (3.9%), Mini (3.9%), Hebdomadis (3.4%), Pomona (2.4%), Bratislava (1%) and Bataviae (0.5%) as shown in

Table 2. Serovar Shermani was the most prevalent serovar followed by Tarassovi. Positive samples were shown in titers between 1:50 and1:800 with the higher titers found in serovar Sejroe.

Table 1 Distribution of antibodies to leptospiral serovars determined by microscopic agglutination testing from 206 swamp buffalo sampled in Sakon Nakhon province, Thailand.

District (test buffalo)	Positive (%)	Serovar	Titer					Positive buffalo
			1:50	1:100	1:200	1:400	1:800	
Pannanikom (51)	66.7	Shermani	11	2	-	-	-	13
		Tarassovi	4	4	6	1	-	15
		Pomona	3	-	-	-	-	3
		Sejroe	1	-	-	-	-	1
		Ranarum	2	-	-	-	-	2
Arkat-Amnauy (71)	61.9	Shermani	22	7	-	-	-	29
		Tarassovi	4	1	1	-	-	6
		Ranarum	6	-	-	-	-	6
		Bratislava	2	-	-	-	-	2
		Bataviae	1	-	-	-	-	1
Phuphan (55)	65.5	Shermani	12	1	-	-	-	13
		Tarassovi	3	-	-	-	-	3
		Pomona	2	-	-	-	-	2
		Sejroe	2	1	3	1	-	7
		Hebdomadis	5	-	-	-	-	5
		Mini	4	2	-	-	-	6
Kudbak (29)	58.6	Shermani	7	2	-	-	-	9
		Sejroe	1	1	1	-	1	4
		Hebdomadis	1	1	-	-	-	2
		Mini	-	1	1	-	-	2

Table 2 Percentage of positive results for serovars Shermani, Tarassovi, Pomona, Sejroe, Ranarum, Bratislava, Bataviae, Hebdomadis and Mini from 206 swamp buffalo sampled in Sakon Nakhon province, Thailand.

Titer	Shermani (n=63)	Tarassovi (n=24)	Pomona (n=5)	Sejroe (n=12)	Ranarum (n=8)	Bratislava (n=2)	Bataviae (n=1)	Hebdomadis (n=7)	Mini (n=8)
1: 50	51	11	5	4	8	2	1	6	4
1:100	12	5	-	2	-	-	-	1	3
1:200	-	7	-	4	-	-	-	-	1
1:400	-	1	-	1	-	-	-	-	-
1:800	-	-	-	1	-	-	-	-	-
%	30.6	11.7	2.4	5.8	3.9	1	0.5	3.4	3.9

DISCUSSION

There are various standard tests in the laboratory to test for the leptospiral antibody, with the most practical being MAT. A titer at 1:100 means that the animal has been infected with the leptospira (Carter, 1986). There were no clinical signs found during the sample collection undertaken for the current study which agreed with the report of Rocha (1998) and low titers based on endemicity in the original Faine criteria were excluded as they complicate diagnosis (Faine, 1982). Serosurveying in the asymptomatic high risk group should be done with MAT and a titer of 1:50 was taken as positive in the current study.

In previous studies, the seroprevalence of leptospirosis in buffalo and other animals has been reported in many countries including Malaysia (Bahaman *et al.*, 1987) and Italy (Ciceroni *et al.*, 1995). The affects caused by serovars varied depending on the area where the pandemic occurred. The current results indicated that there were many differences found in leptospiral infection with swamp buffalo in Sakon Nakhon province. Shermani, which was the serovar reported the most by Petkanchanapong (2009), was found in every district, especially Arkat-Amnauy (40.84%, 29/71). This kind of serovar was probably a local serovar in buffalo of the Sakon Nakhon area. The most important factor that increases the spread of the leptospira in this area came from the cattle and buffalo markets where farmers who also planted rice and raised the cattle as their profession gathered to trade buffalo.

Shermani is the most common serovar in Thailand and also the major cause of sickness in infected patients, with the National Institute of Health of Thailand reporting that the frequency of Shermani in humans varied depending on the different regions of the country (Petkanchanapong, 2009). While this serovar was found in dairy cattle in the central part of Thailand, Shermani with others kinds of serovars such as Pomona, Ranarum, Sarmin, Sejroe and Tarassovi has been found in buffalo and cattle in Nakhon Ratchasima, Northeastern Thailand (Tangkanakul *et al.*, 2002). However, the main serovars detected in dairy cattle in Mexico were Hardjo and Canicola (Leon *et al.*, 2008). Thus, in summary, the infection varied in different parts of the country or in different regions.

The study in four different districts indicated that serovar Shermani was found in every district. However, the highest titer occurred with serovar Sejroe which was found in Pannanikom, Phuphan and Kudbak but not in Arkat-Amnauy. Furthermore, serovar Hebdomadis and serovar Mini were found only in the mountain areas of Phuphan and Kudbak. In addition, serovar Ranarum was found in two plain areas in Pannanikom and Arkat-Amnauy. It is possible that Serovar Ranarum is a zoonotic disease infecting the plain area of Sakon Nakhon province. This geographic difference may be a reason affecting the occurrence of these serovars.

CONCLUSION

The seroprevalence of leptospirosis was studied in four districts of Sakon Nakhon province during March and April 2010. The percentage of positive results was 63.6% from 206 swamp buffalo samples. Nine *Leptospira* serovars were detected in buffalo serum samples—namely, Shermani, Tarassovi, Pomona, Sejroe, Ranarum, Bratislava, Bataviae, Hebdomadis and Mini. The results of this study show the presence of leptospiral antibodies for different serovars in the Sakon Nakhon region. The research results may lead to effective disease monitoring and disease prevention in the future.

ACKNOWLEDGEMENTS

The authors thank the staff of the Ministry of Agriculture, Office of Livestock, Sakon Nakhon province for collecting the blood samples and also acknowledge the assistance of the Leptospirosis

Center, NIAH, Department of Livestock Development, Bangkok with the microscopic agglutination testing. This work was funded by the Faculty of Natural Resources and Agro-Industry, Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon, Thailand.

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