Antibodies to Leptospirosis in Rodents from Thailand Using a Modified Human Diagnostic Assay

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Abstract

The number of reported cases of Leptospirosis in Thailand has grown since 1996. Identification of major reservoirs and endemic areas is essential in surveillance of *Leptospira* species in Thailand. To assist in the effort of surveillance, a dipstick assay for detecting *Leptospira* antibodies in mammals was adapted from a human diagnostic assay and tested in a field trial in Thailand. Antibodies to *Leptospira* were detected in 18 of 60 wild rodents. Four of 9 culture positive rodents were positive by the dipstick assay. The proportion of sera positive for antibodies by dipstick was correlated with positive culture outcome using McNemar test for correlated proportions (0.83, P> 0.05). The dipstick assay was effective in detecting antibodies to *Leptospira* in mammals and may be useful in resource poor areas or under circumstances where the microagglutination test (MAT) is not practical.

Key word : Leptospirosis, Rodent, Rattus, Antibody, Mammal

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Leptospirosis is endemic to Thailand with most cases occurring during the rainy season(1). A number of serogroups are present in Thailand with *Leptospira bataviae* being the most frequently encountered in Bangkok(2) and *L. autumnalis* and *L. hebdomadis* the two most frequently encountered

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Table 1. Results of *Leptospira* spp. antibody detection by dipstick assay and by culture.

Species	Nong Bunnak				Chalermprakiat				Chiang Rai		
	Dipstick		Culture		Dipstick		Culture		Dipstick		Not
	+	-	+	-	+	-	+	-	+	-	Cultured
Bandicota indica	3	11	7	7	0	2	1	1			
Rattus exulans	8	25	0	25	1	3	0	4			
Rattus rattus	1	1			1	3	1	3			
Rattus losea									4	6	

in rural areas in north and northeast Thailand[2,3]. The *Leptospira* species isolated from rodents in Thailand are *L. australis*, *L. autumnalis*, *L. bataviae*, *L. canicola*, *L. cynopteri*, *L. grippotyphosa*, *L. javanica*, *L. pomona*, *L. pyrogenes*[1,4].

Annual case reports of Leptospirosis in Thailand have increased from 55 in 1982[5,6] to 2,331 cases in 1997[7]. Most affected persons have been farmers. In 1998, a network in Thailand was established to identify risk factors and reservoirs of *Leptospira*. In this paper, we describe the adaptation of a human diagnostic kit for detecting antibodies (Ab) to *Leptospira* in rodent species.

MATERIAL AND METHOD

Antibody detection assays

We tested whether Protein A (Kirkegaard and Perry Laboratories KPL) could detect immunoglobulins (Ig) in a variety of mammal species using dot blot analysis. One serum sample from the following mammals were tested for non-specific Ig; Asian elephant (*Elaphus maximus*), cat (*Felis catus*), cow (*Bos taurus*), dog (*Canis familiaris*), gibbon (*Hylobates pileatus*), horse (*Equus caballus*), langur monkey (*Trachypithecus phayrei*), pig (*Sus scrofa*), roof rat (*Rattus rattus*), bandicoot rat (*Bandicota indica*), house mouse (*Mus musculus*), Ryukyu mouse (*Mus caroli*), lesser rice field rat (*Rattus losea*), Polynesian rat (*Rattus exulans*), and water buffalo (*Bubalus bubalis*). Protein A detected immunoglobulins in all mammal species tested using the dot blot method. This information was used to adapt a commercially available human diagnostic dipstick assay for detecting antibodies to *Leptospira* (Integrated Diagnostics, Inc., Baltimore, MD, USA). Culture of *Leptospira* spp. was used to compare and validate the dipstick assay.

Only the dipstick from the dipstick assay was used and no kit reagents were used. The methods for the dipstick assay were: 1) dipsticks blocked 5 min (5% skim milk/PBS), 2) strips washed 3x (PBS 0.1% Tween 20) (5 min each), 3) 2 ul serum (non-dilute) added/incubated at room temp 1 h, 4) HRP labeled Protein A (KPL) 1:500 in 1 per cent skim milk (PBS), 5) dipsticks washed as step #2, 6) TMB 1 component substrate (KPL) added/incubated 30 min, 7) reaction stopped 3x in dH_2O.

Study areas

Sixty rodents were collected in three areas of north and northeastern Thailand using live traps baited with banana (Table 1). Serum and tissue samples collected from Nongbunnak and Chalermprakiat districts of Nakhon Ratchasima provinces were tested by culture and antibody detection using the dipstick assay. Rodents from Chiang Rai province were only tested by the dipstick assay and not by culture. *Rattus rattus* inoculated with *L. biflexa* and uninfected *R. rattus* were used as positive and negative controls for the dipstick assay. The presence or absence of *Leptospira* antibodies were tested using McNemar test of correlated proportions (Statsoft, Statistica).

RESULTS

Eighteen samples were positive by the dipstick assay. In Nong Bunnak district, 3 of 14 (21%) *B. indica*, 1 of 1 *R. rattus*, and 8 of 25 (32%) *R. exulans* were positive for *Leptospira* antibodies. Seven *B. indica* collected in Nong Bunnak were culture positive of which 2 were dipstick positive. In Chalermprakiat district, 0 of 2 *B. indica*, 1 of 4 (25%) *R. rattus* and 1 of 4 (25%) *R. exulans* were positive for *Leptospira* antibodies. The one *R. rattus* positive by the dipstick assay was also culture positive and one *B. indica* was culture positive. In Chiang Rai, 4 of 10 (25%) *R. losea* collected in rice fields were positive for antibodies to *Leptospira*. The proportion between dipstick assay and

culture positive was positively correlated, not significantly different (0.83, P>0.05), using McNemar test for correlated proportions.

DISCUSSION

The identification and control of reservoirs is vital in prevention of Leptospirosis. In a previous study in Bangkok, Thailand, 31 per cent of rodents were positive for leptospiral antibodies by the microagglutination technique MAT[4]. The only rodent species found in the previous study to be positive were *R. norvegicus* a commensal rodent. Our finding of 30 per cent antibody positive rodents is similar, however, we did not capture *R. norvegicus* because of the difference in habitats between the two studies. The rodent species in the current study are commonly found in agricultural settings, particularly rice fields, where farmers may readily come into contact and become infected with *Leptospira* species. Rats infected with *Leptospira* may or may not have leptospiral antibodies. Four of 9 rodents found to be culture positive had antibodies detected by the dipstick assay. Antibodies were detected in 10 rodents that were negative by culture (Table 1). As indicated by the data, the dipstick method may be effective for detecting Ab in a variety of mammal species in resource poor areas or under field settings where the use of MAT may be impractical.

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การใช้ชุดตรวจวินิจฉัยแบบ dipstick สำหรับตรวจหาภูมิคุ้มกันต่อเลปโตสไปโรซิสในหนูในประเทศไทย

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มีรายงานผู้ป่วยโรคเลปโตสไปโรซิสในประเทศไทยเพิ่มมากขึ้นตั้งแต่ พ.ศ. 2539 การตรวจค้นหาสัตว์รังโรคและพื้นที่เสี่ยงต่อการติดเชื้อเลปโตสไปราจึงมีความสำคัญที่ควรต้องเฝ้าระวัง ดังนั้นเพื่อช่วยให้การเฝ้าระวังโรคมีความสะดวกมากขึ้น จึงได้ทดสอบการใช้ชุดตรวจวินิจฉัยโรคในผู้ป่วยแบบแท่งจุ่ม (dipstick) ตรวจหาภูมิคุ้มกันต่อเลปโตสไปราในหนูและได้ทดสอบการใช้ในสนามในประเทศไทย พบว่าสามารถตรวจพบภูมิคุ้มกันต่อเลปโตสไปราในหนูจำนวน 18 ตัวอย่างจากจำนวนทั้งหมด 60 ตัวอย่าง และตรวจพบภูมิคุ้มกัน 4 ตัวอย่างจากหนู 9 ตัวที่การเพาะเชื้อเลปโตสไปราให้ผลบวก การทดสอบความสัมพันธ์ของสัดส่วนระหว่างตัวอย่างน้ำเหลืองที่ให้ผลบวกโดยชุดตรวจวินิจฉัยกับการเพาะเชื้อด้วยสถิติ McNemar พบว่าวิธีการตรวจทั้งสองวิธีมีความสัมพันธ์กัน (0.83, P > 0.05) ชุดตรวจวินิจฉัยโรคในผู้ป่วยแบบแท่งจุ่ม (dipstick) สามารถตรวจหาภูมิคุ้มกันต่อเลปโตสไปราในหนูได้อย่างมีประสิทธิภาพ และอาจเป็นประโยชน์ต่อการนำไปใช้ในพื้นที่ที่มีข้อจำกัดในการตรวจวินิจฉัยด้วยวิธีอื่น ๆ หรือ Microagglutination test (MAT)

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