Genotyping Survey the Leptospira in Bovine Urine Samples in Thailand by PCR-Based Method: A Non Matching with the Antibody Titer by MAT

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Introduction

Leptospirosis is a common disease in wildlife and livestock. The disease is caused by spirochete named *Leptospira* spp. Infection of this microorganism in livestock causes serious economic loss. It is a contagious disease causing abortion in 25-30% infected cow (14). Leptospiral mastitis was also reported in dairy cows (1, 9). In Thailand, incidences of Leptospiral abortion in cattle herd and dairy farm were sporadically reported in 1997 and 1999 (10, 15). The dairy cattle and feedlot cattle are also recognized as a reservoir for this microorganism (13, 14, 20). It is believed that these reservoir cows are leptospires carriers for the infection in human. However, there is no correlation between the *Leptospira* serovars in microscopic agglutination test (MAT) positive cows and the *Leptospira* serovars reported in *Leptospira* suffered patients residing in the same area (17). We, therefore, decided to survey the genospecies of *Leptospira* in cow reservoirs and their MAT titer. With the development of Polymerase Chain Reaction (PCR) - based technique, scientists can detect *Leptospira* DNA in clinical samples such as; blood, CSF, urine, etc (2, 4, 7, 11, 12, 16, 18, 19). Seven genospecies of pathogenic leptospires have been detected by two sets of primer (7). Using this PCR-based method and MAT, we can group genospecies of leptospires shed from cow urine and we did compare these genospecies with serological MAT results.

Materials and Methods

Serum and urine samples were collected from cows at area of Bangkok, Pratumdranee, Nakornprathom, and Nakornrachaseema. Serum were serological titrated by the microscopic agglutination test (MAT), a test utilized routinely at the National Institute of Animal Health (NIAH) as described in standard protocol (3). Urine samples were kept in transport media prior to be detected the *Leptospira* DNA by Polymerase Chain Reaction (PCR) -Based method followed the technique described by Gravekamp, C.H. et al (7). The 24 Leptospira references were tested with primer sets of Gravekamp, C.H. et al (7).

Results and Discussion

The 24 leptospire references were tested with PCR primers. The PCR product of 285 bp in size was detected from the reactions with L.interogans (serovars; autumnalis, canicola, djasiman, hebdomadis, icterohaemorrhagiae, pomona, and pyrogenase), L.noguchii (serovar louisiana), L.borgpetersenii (serovars; ballum, javanica, sejroe), L.santarosai (serovar shermani), L.weilii (serovar sarmin), L.meyeri (serovar semaranga), and L.inadai (serovar lyme).

TABLE 1. Relation between Genospecies of *Leptospira* in cow urine samples identified by PCR method[a] and sero-specific titer of each cow detected by MAT.

Genospecies (by PCR)[a]	Sero-specific titer detected by MAT[b] [# titer]							
	cyn	gri	heb	mi	ran	sej	tar	neg
563 bp PCR product size (8 positive) :- L. kirschneri	-	-	-	-	1[c]	2[c]	2[d] 2[c]	3
285 bp PCR product size (3 positive):- L. interrogans L. noguchii L. borgpetersenii L. santarosai L. weilii L. meyeri (only semaranga) L. inadai (only lyme)	-	-	2[c]	1[d]	-	2[c]	2[d] 1[c]	-
340 bp PCR product size (4 positive):- L. inadai (only inadai)	-	-	2[c]	1[d]	-	1[c] 1[e]	2[d] 1[c]	1
Ambiguity[f] PCR product size (3 positive):- unclassified genospecies	-	-	1[c]	1[g]	1[c]	1[g]	3[c]	-

[a] Test on primers designed by Gravekamp, C.H. et al, 1993 (7).

[b] Serovars :- cyn;cynopteri, gri; grippotyphosa,heb; hebdomadis, mi; mini, ran; ranarum, sej; sejroe, tar; tarassovi, neg;negative MAT.

[c] titer 1:40, [d] titer 1:20, [e] titer 1:160, [g] titer 1:80

[f] PCR product around 630 bp.

However, there was no PCR product shown off from the reaction of L.borgpetersenii serovar tarassovi and L.meyeri serovar ranarum. The PCR product from L.kirschneri serovars cynopteri and grippotyphosa is 563 bp in size. The PCR product was not shown off with reactions from L.biflexa serovar andamana, maintenon, and patoc. There was an anomalous 240 bp product size with the reaction of L.biflexa serovar saopaolo. The

L.inadai serovar inadai (strain 92152-1) gave a 340 bp PCR product.

Comparing groups of leptospira species identified from antigen in bovine urine by PCR method with cows' serological titer detected by MAT, we found a non-matching between these identifications from the same cows (Table 1). As shown in Table 1, carrier cows, with L.kirschneri antigen detected by PCR from urine sample, did not have any antibodies titers to either cynopteri or grippotyphosa. Positive PCR cows shown positive MAT with hebdomadis, mini, ranarum, sejroe, tarassovi, and patoc, as shown in Table 1.

TABLE 2. MAT results from cows with negative PCR results.[a]

MAT titer	Sero-specific detected by MAT[b] (per-cent)							
	heb	mi	ran	sej	tar	pat	neg	Note
> 1:40	-	-	-	4.5	3.4	-	62.5	85[c]
1:40	5.7	4.5	7.9	6.8	19.3	1.1		
1:20	-	4.5	1.1	-	9.1	-		
Total	5.7	9.0	9.0	11.3	31.8	1.1		

[a] Test by primers designed by Gravekamp, C.H. et al, 1993 (7).

[b] Serovars:- heb; hebdomadis, mi; mini, ran; ranarum, sej; sejroe, tar; tarassovi, pat;patoc, neg;negative MAT.

[c] Positive on direct exam under dark-field microscope.

TABLE 3. MAT results from all sampling cows.

MAT titer	Sero-specific detected by MAT[a] (per-cent)							
	heb	mi	ran	sej	tar	pat	neg	Note
> 1:40	-	1.0	-	5.8	2.9	-	57.3	87.4[b]
1:40	9.7	3.9	8.7	10.7	24.3	1.0		
1:20	-	5.8	1.0	-	13.6	-		
Total	9.7	10.7	9.7	16.5	40.8	1.0		

[a] Serovars :- heb; hebdomadis, mi; mini, ran; ranarum, sej; sejroe, tar; tarassovi, pat;patoc, neg;negative MAT.

[b] Positive on direct exam under dark-field microscope.

Cows with negative PCR results had MAT titer to hebdomadis, mini, ranarum, sejroe, tarassovi, patoc, and negative titer as 5.7, 9.0, 9.0, 11.3, 31.8, 1.1, and 62.5 per-cent, respectively, as shown in Table 2. In addition, all sampling cows shown MAT titer to hebdomadis, mini, ranarum, sejroe, tarassovi, patoc, and negative titer as 9.7, 10.7, 9.7, 16.5, 40.8, 1.0 and 57.3 per-cent, respectively (Table 3). Regarding to these results, cows with negative PCR results are prone with MAT positive. In addition, 26.6% of cows with PCR positive was negative MAT. These results replied that MAT could not be used solely to evaluate the disease and carrier status in cows since there is existence of seronegative carriers (8) and seropositive non-excreting animals (5, 6). There is also error prone in epidemiological prediction if we rely only on MAT. The unmatching MAT and genotyping of antigen from urine samples suggested some physiological relation between cows' carrier status and cows' immune status. As shown in Table 1, eigth cows (53.3%) shown PCR positive for L. kirschneri while all of them carried no antibodies titer for cynopteri and grippotyphosa. In contrast to L. kirschneri PCR positive cows, there is only 3 cows (20%) with 285 bp PCR positive which have correct seropositive (Table 1). We doubt that the seropositive of any antibodies may protect cows from being a carrier of that *Leptospira* spp.

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