EPIDEMIOLOGY AND CHARACTERIZATION OF LEPTOSPIROSIS AT AN URBAN AND PROVINCIAL SITE IN THAILAND

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INTRODUCTION

Leptospirosis is an endemic disease in Thailand that occurs primarily during the rainy season (Bunnag *et al.*, 1983). Both rural and urban populations may be exposed to the organism from water contaminated with urine of leptospiral reservoirs such as rodents or domestic animals. Clinically, the disease in humans may present as a fever of unknown origin with headache, muscle ache, nausea and vomiting. Leptospira can be cultured from the blood during this acute phase of the disease. A persistent renal infection often occurs in humans and animals following recovery from the clinical illness. In some patients, a severe form of the disease may occur with jaundice, hemorrhage, and renal failure; organisms may be cultured from the blood, cerebrospinal fluid (CSF) and urine. Although a variety of serogroups are present in Thailand, *Leptospira bataviae* is a frequent cause of clinical infection, and in Bangkok, it is the most common isolate from confirmed cases of human leptospirosis (Charoonruangrit and Boonpucknavig, 1964; Sitpriya *et al.*, 1980; Bunnag *et al.*, 1983). In Thai provincial hospitals, *Leptospira autumnalis* (Bunnag *et al.*, 1983) and *L. hebdomadis* (Arimitsu *et al.*, 1987) were the serogroups found most frequently.

The purpose of this study was to determine the importance of leptospirosis as a cause of fever of unknown origin (FUO) in patients at an urban and at a rural hospital. From patients diagnosed as having leptospirosis, the organism was isolated and the serogroup identified. Wild rodents and domestic animals were surveyed in the area of these two hospitals to determine the incidence and serogroup in these animals in order to identify the potential source of the human infections.

MATERIALS AND METHODS

Patients from a rural and an urban hospital with FUO and other clinical signs and symptoms of leptospirosis, including head-

ache, muscle ache, nausea and vomiting, were screened for leptospirosis. The patients included 37 adults from the Chao Phya Abhai Bhu Bejhr Hospital in Prachinburi province, and 73 children from the Children's Hospital in Bangkok. Blood or urine specimens were collected aseptically; the decision to collect blood or urine specimens was based on the duration and type of illness (icteric or anicteric). In anicteric leptospirosis, blood cultures are positive for the first week of illness after which leptospira can be isolated from the urine for one month or longer.

In both study areas, domestic animals were bled and urine was collected whenever possible. Wild rodents were live trapped, anesthetized, and bled. They were then euthanasized and a section of kidney tissue was removed. This was minced for culture using a Tenbrook tissue grinder and saline added to make a suspension. A total of 155 animals (31 dogs, 8 cats, 17 pigs, and 99 *Rattus* spp.) at 3 amphors in Prachinburi province were surveyed in October and November of 1984. During January 1984, 262 blood samples were collected from stray dogs at the Bangkok Dog Pound Center. During June to August 1984, 204 blood and 150 urine specimens were collected from dairy cattle in Bangkok. Blood and kidney specimens were collected from 75 rats trapped in various Bangkok houses in November 1984. Sera from all these animals were tested for antibody against 22 leptospira serovars (Table 1) by microagglutination titer (MAT) (Turner, 1968); blood, urine or kidney cells were cultured for serovar identification. Antisera for MAT testing were obtained from the Leptospirosis Reference Laboratory in Brisbane, Australia. Culture confirmation was accomplished at the USA Center for Disease Control in Atlanta, Georgia.

One or two drops of patient/animal blood, urine, or rodent kidney suspension were added to dual tubes containing 5.0 ml of protein supplemented semisolid Ellinghousen-McCullough-Johnson-Harris (EMJH) media and incubated at 30 °C in the dark (Turner, 1970). Cultures were examined weekly by dark field microscopy and after six weeks, samples without organisms were considered negative. Sera from FUO or suspect leptospirosis patients were tested for antibody against the 22 serovars as described previously.

Table 1
Serovars used in the leptospirosis MAT.

1. *icterohaemorrhagiae*	12. *lora****
2. *javanica*	13. *pomona*
3. *celledoni*	14. *grippotyphosa*
4. *schuffneri*	15. *hebdomadis*
5. *ballum*	16. *tabaquite*
6. *zanoni**	17. *hardjo*
7. *robinsoni**	18. *bataviae*
8. *cynopteri*	19. *tarassovi*
9. *bangkinang***	20. *panama*
10. *louisiana***	21. *shermani*
11. *djasiman***	22. *patoc*

* – Serogroup *progenes*
** – Serogroup *autumnalis*
*** – Serogroup *australis*

RESULTS

During the years 1983 and 1984, leptospirosis was confirmed in 26 of the 73 FUO patients (36%) screened at the Bangkok Children's Hospital. Fever and vomiting were the most common signs and seen in 96 and 77% of the leptospirosis positive patients, respectively. Aseptic meningitis was diagnosed in three patients with leptopirosis, while headache and abdominal and muscle pains were reported in nine patients (35%). Complete data was not available on all pa-

tients for whom blood or urine cultures were obtained due to early discharge or failure to return for follow-up interviews or treatment. There were 20 males and six females, average 10 years of age (range 6 to 14 years). In this two year study, 24 of the 26 cases occurred in the months of October, November and December 1983. During the fall of 1983 there was severe flooding throughout Bangkok due to heavy rains in the Thai countryside.

All but two of the 26 children with leptospirosis were positive for *L. bataviae* by either MAT or culture (Table 2). One child was positive on blood culture for *L. autumnalis* and a second child was positive for *L. javanica* on the MAT. Nearly half of the children (12/26) had a negative admittance MAT. None of the patients with positive admittance MAT had a positive culture for leptospirosis. Convalescent sera were not obtained from two children who had positive cultures, however all of the rest had a positive convalescent serum MAT. Seven blood cultures were positive for *L. bataviae* and one for *L. antumnalis* ; two urine cultures were positive for *L. bataviae*.

A survey of dairy cattle in eight amphors surrounding Bangkok was completed in 1984. A total of 204 cattle were bled and urine samples were obtained from 150 of them. There were 108 cows, 56 heifers, and 40 calves. Fifty-four (27%) of 204 tested sera were MAT positive. The percentage of MAT positive cattle decreased with decreasing age; 61% of cows were positive while only 7.5% of calves were positive. The serovars identified and percent positive were: *bataviae* 13%, *tabaquite* 8%, *hebdomadis* 4%; *hardjo*, *javanica* and *tarassovi* 3%; *shermani* 2%; *zanoni*, *robinsoni*, *lora*, *cynopteri*, and *ballum* $< 1\%$.

A total of 262 dogs were surveyed following the flooding of 1983, and 50 (19%)

Table 2
Serological and culture results of leptospirosis patients at Children's Hospital, Bangkok.

Patient No.	Onset	MAT Convalescent	Culture* Blood
1	–	512	+
2	–	512	–
3	ND	ND	+
4	–	4096	+
5	64	ND	–
6	16	4096	–
7	512	1024	–
8	1024	2048	–
9	–	2048	+
10	2048	4096	–
11	–	2048	–
12	32	4096	–
13	–	2048	+
14	128	1024	–
15	–	ND	+
16	256	4096	–
17	16	256	–
18	–	2048	+
19	2048	4096	ND
20	1024	1024	ND
21	–	512	–
22	512	4096	ND
23	–	4096	ND
24	1024	4096	–
25**	–	256	+
26**	512	ND	ND

\+ Positive
– Negative
ND = Not Done
* Urine cultures positive in patients No. 4 and No. 11.
** These cases occurred in fall of 1984.
Note: Serogroup *bataviae* identified in all patients except No. 3 *(autumnalis)* and No. 17 *(javanica)*.

were positive for *bataviae*. A higher percentage of adult dogs had positive MAT's for one or more serovars (54%) than did juveniles (23%) or pups (6%).

A total of 75 rodents were trapped in various houses and apartments in Bangkok. All 23 positive cultures and MATs were from *Rattus norvegicus*. The serovars identified and percent positive of total animals surveyed were: *bataviae* 15%, *javanica* 13%, *pyrogenes* and *hebdomadis* 1% each. Isolates from kidney cultures included *bataviae*, *javanica*, and *pyrogenes*.

Of 37 FUO patients screened in 1983 for leptospirosis at the Prachinburi hospital, seven were positive by blood culture or MAT. The serogroup incidence was *bataviae* (4), *pyrogenes* (1), *autumnalis* (1), and *hebdomadis* (1). The most common signs and symptoms of these patients, whose ages ranged from 17 to 33 (mean age of 25) were fever, headache, anorexia, muscle pain and constipation. No suspect patients were presented in 1984, when there was much less rainfall than in 1983.

Five of nine animal species surveyed at three amphors in Prachinburi province were positive for leptospirosis. The serogroup identified by species and percent positive of those surveyed were: *R. rattus*, *javanica* 21% and *pyrogenes* 5%; *R. norvegicus*, *bataviae* 15%, *javanica* 11% and *cynopteri* 2%; *B. indica*, *javanica* and *autumnalis* 8% each; *S. suis*, *bataviae* 18%; and *C. familaris*, *hebdomadis* 19% and *australis* 3%.

DISCUSSION

Human leptospirosis was first reported in Thailand in 1943 (Sundharagiati and Harinasuta, 1966). The typical clinical signs seen in patients with anicteric leptospirosis include fever, headache, severe muscle ache, malaise, prostration, and in rare cases, circulatory collapse. In our study we surveyed two different age groups of FUO patients. The children admitted to the Children's Hospital in Bangkok most often had clinical signs of fever, vomiting, headache, abdominal and generalized muscle pain and diarrhea. The adults admitted to the Prachinburi Provincial Hospital generally complained of fever, headache, anorexia, muscle pain and constipation. Thus, the children appeared to complain more about intestinal discomfort, particularly vomiting, abdominal pain and diarrhea, while the adults primarily complained more about generalized body discomforts such as headache, anorexia and muscle pain. The difference in clinical signs in our two groups of people could be due to the fact that for most of the children, this was their first exposure to the leptospira organism resulting in a more severe manifestation of hepatitis as evidenced by abdominal pain and vomiting. Many of the adults could have had prior exposure to the disease causing an anamnestic immune response, thereby resulting in a milder clinical manifestation of the disease. Serological determination of patients IgG and IgM would have been useful to establish whether any patients had prior exposure to leptospirosis.

The incidence of leptospirosis in FUO patients was greater in Bangkok than the rural area of Prachinburi (36% versus 19%). The relatively low incidence of leptospirosis in FUO patients in Prachinburi may be related to the higher incidence of malaria and other rural diseases that contribute to the pool of causes of FUO. Another possible explanation for the lepto incidence difference is the concentration of known leptospirosis carriers. The rat population is probably denser in Bangkok than in Prachinburi, thereby contributing to the higher incidence of leptospirosis.

During the fall of 1983 excessive rains occurred in the countryside, followed by flooding in many streets of Bangkok. In 1983 there were 7 cases of leptospirosis at the Prachinburi hospital; no cases occurred in 1984, a much drier year. Likewise at the Children's Hospital in Bangkok there were 24 cases in 1983 and only 2 in 1984. Therefore, increased exposure to rain and floodwater resulted in a marked increase of leptospirosis in both the rural and urban locations.

The predominant serogroup in both subject groups was *bataviae*, although it was far more prevalent in the children (24 of 26, or 92%) than the adults (4 of 7, or 57%). The difference may have been due to previous infection with *bataviae* at a younger age or increased exposure to various sources and reservoirs. A total of three serogroups were found in the children (*bataviae*, *autumnalis* and *javanica*). In addition to *bataviae*, the adults were positive for serogroups *hebdomadis*, *pyrogenes*, and *autumnalis*.

It is well known that a particular host species (dog, rat, cattle, etc.) may serve as a reservoir for one or more serogroups of leptospires, and conversely, that a given serogroup may be hosted by multiple animal species. Two or more reservoir hosts for the same serogroup may be present in the same geographic area and individual animals may carry and excrete leptospires belonging to multiple serogroups (Babudieri, 1958). In Thailand, rats have long been incriminated as the source of human infection (Sundharagiati, *et al.*, 1964), but the results of our study indicate that other animals could also be considered as potential reserviors.

In Bangkok, the primary serogroup in all animals surveyed (dogs, cattle, and rodents) was *bataviae*. *Javanica* was also found in all groups of animals studied, although this serogroup was more prevalent in Bangkok rats than the other species. Thus, the source of human infection with serogroups *bataviae* and *javanica* could have been any of the species studied. However, the *autumnalis* serogroup identified in one child at Children's Hospital was found in only dogs in our survey. Thus, the source of this human infection could have been a dog. In cattle and dogs, as would be expected, older animals had a higher serological incidence of leptospirosis.

The results of animal surveillance in Prachinburi province also indicate the potential for multiple species as the source of human infections. *Bataviae* was identified in both rats and pigs, *pyrogenes* only in the rat, *autumnalis* only in the bandicoot, and *hebdomadis* in only the dog. Since the animals surveyed did not necessarily come from the same location as the infected person, it is possible that the animals implicated as the infection reservoir may not necessarily be the source. The results of this study do indicate, however, that many species of animals in Thailand should be considered as potential reservoir hosts.

SUMMARY

Patients with FUOs at the Children's Hospital in Bangkok and the Chao Phya Abhai Bhu Bejhr Hospital in Prachinburi were screened for leptospirosis by blood and urine culture in addition to microagglutination testing of their serum. Animal populations in urban and periurban areas of Bangkok were surveyed for evidence of leptospira infection. Three rural sites near the Prachinburi Provincial Hospital were also surveyed. The rodents' and domestic animals' blood, urine, and/or kidney cell samples were cultured for leptospira. Sera from

these animals were also tested for leptospira antibody. The *bataviae* serovar was the most commonly detected leptospiral agent in both man and animals. Presenting symptoms varied with age with children showing primarily fever, vomiting, headache, abdominal and generalized muscle pain and diarrhea whereas adults had fever, headache, anorexia, muscle pain and constipation.

Blood samples from patients suspected of having leptospirosis were tested for antibody by the MAT and cultured in EMJH media. The following serogroups were identified: *bataviae*, *autumanalis*, *javanica*, *hebdomadis*, and *pyrogenes*. Leptospirosis incidence in humans was much higher in the rainy/flooding year of 1983 compared to the relatively dry year of 1984. Results of our animal surveillance studies indicate that in addition to rats, which have previously been mentioned, dogs, bandicoots, cattle and pigs could be the source of human leptospirosis infection in both urban and provincial locations in Thailand.

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