BODY OF REPORT

SEATO Medic Study No. 80F	Isolation of Leptospires from Thailand - Modes of Transmission.
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	Division of Medical Research Laboratories
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Objective: To evaluate by isolation of the leptospira organism, the significance of wild and domestic reservoir hosts and vehicles of transmission.

Description: Studies at Kuala Lumpur US Army Medic Rsch Unit, Institute for Med Rsch, Kuala Lumpur, Malaysia - Annual Report 1962 - 63 indicate that leptospirosis is a potential zoonotic hazard in S. E. Asia. However, preliminary reports from this laboratory were not as successful in isolating leptospiral organisms under the same conditions. A recent report from North Viet nam indicated leptospirosis may be a serious problem in areas heavily travelled by man and animals. This study consists of isolation of the leptospire organism either by inoculation

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into weanling hamsters or by direct culture in Fletcher's media enriched with rabbit serum. Kidneys of animals, as aseptically exposed plugs obtained with a Pasteur pipette, are discharged directly into Fletcher's media. Water samples collected from streams, ponds, klongs, rice paddies, buffalo wallows and mud from suspected contaminated areas are inoculated into weanling hamsters. These hamsters are observed for 30 days for signs of illness or death. Hamsters dying prior to 4 days are discarded as bacterially contaminated. Kidneys from hamsters dying from the 4th to 21st day or sick on the 21st day are cultured in Fletcher's media. Isolates so obtained are typed by standard leptospiral serological methods.

Progress: Seven (7) sites in Amphur Thunyaburi were selected to be surveyed during the rainy season. Sites 1, 2, 7 are small farms surrounded on 1 to 3 sides with rice paddies and on the other sides by ponds or flooded grass. Four to five buffalo roam the areas. Site 3 is a local village grazing grounds. Approximately forty (40) buffalo feed on the often flooded grass field which is surrounded by klongs. Site 4 is a Thai government rice experiment station. This is a well managed farm which is divided into multiple rice fields connected by canals and paths. About sixty (60) buffalo of all ages are kept in and near a covered canal which drains into a nearby pond. Site 5 is the Samran Farm. Banana lined roads interdigitate large fish ponds. There are a few storage and service buildings and a few dwellings, but most of the buildings are wood pig pens built out over the fish ponds. Some smaller concrete pens drain into smaller refuse-fish ponds. The farm is surrounded by adjacent rice fields. Site 6 includes only the swine pens and nearby ponds of the government narcotics hospital. About 100 pigs are crowded into a small area and is quite heavily rodent infested.

Three hundred and twenty (320) water samples were collected from the seven (7) sites previously described. Each site was divided into at least two stations where daily recordings of water pH were made. The water in the station having the nearest to optimum pH (6.8 - 7.4) for leptospiral survival was sampled and inoculated intraperitoneally into five (5) weanling hamsters. Hamsters were observed for 21 days and if normal discarded. One (1) hamster in the normal cages was cultured prior to discarding. Those dying between 4 and 21 days, in which no decomposition was apparent, were cultured. Hamsters in cages in which one or more deaths occurred were held for 30 days. At least one hamster in each of these cages was cultured prior to discarding at 30 days. A total of 1575 hamsters were inoculated and the following deaths occurred during this study:

Total deaths 0-4 days	116
Total deaths 4-21 days	111
Total deaths	227

It is of interest to note that clear water found in rice paddies and ponds appeared to have an acid pH when compared to cloudy or muddy water having a nearly neutral pH. All isolates were obtained from Site 5 (pH range 5.0 - 7.6). The most frequently observed pH valves at this site were 6.6 - 7.5, a satisfact-

Table I

pH AND CONDITION OF WATER

Site	pH Minimum	Maximum	Water condition
1	3.4	7.1	Clear to muddy
2	3.0	7.7	Clear to muddy
3	3.0	7.7	Clear to muddy
4	5.3	8.6	Cloudy to muddy
5	5.0	7.6	Cloudy to muddy
6	3.6	7.2	Clear to muddy
7	6.3	6.2	Cloudy to muddy

ory range for leptospiral growth. The above minimum and maximums were recorded at the various sites (Table I).

Five (5) isolates obtained from hamsters inoculated with water from the same station at Site 5 (Swine Farm) were typed L. pomona. With one exception all of these hamsters died between the 13th and 17th day post inoculation. One isolate was recovered from one hamster following the routine 30 day culture. Since the swine pens were suspended over or drained into the sites, the swine appear to be the source of infection.

Rodents were trapped nightly during the period of this study. Two hundred and forty-one (241) rodents at the various sites were trapped with twelve (12) isolates obtained. Rodents were identified by species and site from which they were trapped (Table II). Kidneys were cultured as previously described and blood collected for presence of serological agglutinins. Serological titers obtained are reported in SEATO Medic Study No. 81.

None of the isolates obtained from the rodents collected at Site 5 were the same organism isolated from the water at that site. (Table III).

Forty-seven (47) dogs were necropsied at Udorn, Thailand and the kidneys cultured. Techniques used were similar to those previously described for swine, cattle and buffalo (Annual Report pages 437-442, 1963-1964). Eleven (11) isolates were obtained and all were typed L. canicola. Kidneys were cultured from one hundred and eighty (180) stray dogs in Bangkok. Cultures checked at fifteen

(15) days have revealed twenty (20) isolates from one hundred and forty (140) kidneys. These isolates have not been typed.

Technicians investigating the disease Scrub Typhus have cultured the kidneys of the rodents trapped in the respective survey areas. The addition of 5 fluoro-racil, 150 mcgms/ml, to the Fletcher's media has decreased contamination of cultures obtained. This product has been reported as a selective agent for growth of leptospirae (Journal of Bacteriology, Vol. 87, No. 2, pages 422-426). Three hundred and twenty seven (327) uncontaminated cultures have been obtained from areas surrounding, Chiengmai, Nongkai, Udorn, Ubol, Nakorn Panom and Chiengrai. Eleven (11) isolates have been obtained, seven (7) have been serotyped L. javanica and one (1) from a rodent collected at Chiengmai typed L. alexi. Twenty-seven (27) rodent kidney cultures were collected from Cholburi with the two isolates obtained serotyped L. icterohemorrhagiae. Six (6) isolates have been obtained from rodents trapped in Bangkok, these with one exception have been typed L. djatzi-hyos, the exception being L. javanica.

Discussion: In evaluating the significance of wild and domestic reservoirs and vehicles of transmission of the leptospire organism all possibilities must be investigated. An area was chosen in Thunyaburi province and klongs, rice paddies, buffalo wallows and soil were inoculated intraperitoneally in weanling hamsters. Kidney specimens from rodents trapped in the area were inoculated in appropriate media. The results indicate that water and rodents may not be a significant reservoir of infection in that area. The acid pH of the clear water collected was such that optimum survival of the leptospire organism could not been expected. All water isolates were obtained from one site and pH recordings at this site more closely approximated that required for optimum leptospiral survival.

Isolates obtained from rodents suggest that the rodent is a significant reservoir of L. javanica and may be a reservoir for the organism L. hyos. Serological response to L. hyos has been demonstrated in a large number of cattle and buffalo sera collected.

Isolates obtained from the dogs at Udorn indicate that they are a primary reservoir of L. canicola and may play a major role in the zoonoses. Isolates obtained during the Bangkok dog survey, although untyped, appear to bear this out.

Summary: Water samples were collected daily from stations in Thunyaburi having the nearest optimum pH (6.8 - 7.4) for leptospiral survival and inoculated into weanling hamsters. Kidneys were cultured from the rodents trapped in the area and from the inoculated hamsters dying between 4-21 days post inoculation. Kidneys were cultured from stray dogs sacrificed in both Udorn and Bangkok, and from rodents trapped in these areas: Chiengmai, Nongkai and Nakorn Panom. Isolates obtained and typed were five (5) L. pomona from water; sixteen (16) L. javanica, eight (8) L. djatzi, L. hyos, two (2) L. icterohemorrhagiae and one

(1) L. alexi from rodents; and eleven (11) L. canicola from dogs.

Table II

RODENTS TRAPPED BY SPECIES

R. exulans	47
R. rattus	18
R. norwegicus	87
B. indica	89
TOTAL	241

Table III

LEPTOSPIRAL ISOLATIONS

Number	Isolates	Species	Site
5	L. javanica	B. indica	4
1	L. javanica	R. norvegicus	6
2	L. javanica	R. exulans	5
4	L. djatzi & L. hyos	R. norvegicus	5