

ANNUAL PROGRESS REPORT

SEATO Medic Study No. 80-E: Isolation of Leptospires from Thailand -
Rodent Survey in Up-Country Grazing Land

Project No. 3A 025601 A 811: Military Medical Research Program S.E.Asia

Task 01: Military Medical Research Program S.E.Asia

Subtask 01: Military Medical Research Program SEASIA
(Thailand)

Reporting Installation: US Army-SEATO Medical Research Laboratory
APO 146, San Francisco, California

Division of Medical Research Laboratories
Department of Veterinary Medicine
Microbiology Section

Period Covered by Report: 1 April 1963 to 31 March 1964

Principal Investigator: Thomas J. Keefe, Captain, VC

Associate Investigator: Prem Brahmacharya, D.V.M. *

Assistant Investigator: Suttichai Uttasard, D.V.M. **

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

* Bacteriology Branch (Research and Education Division)
** Immunology and Serology Branch (Department of Livestock Development)

ABSTRACT

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The object of this study was to examine the rodents of cultivated fields in up-country Thailand (about 414 miles north of Bangkok) utilizing the weanling hamster as an indicator animal rather than direct kidney cultures, when preliminary results obtained in rodent examinations around Bangkok revealed low (6.9%) and exclusive L. javanica infections.

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Because of the inadequacy of the optimum sterility measures in upcountry Thailand required for direct kidney cultures, the weanling hamster was selected as an indicator animal for leptospiral presence. Rodent traps were set in and around the cultivated fields of the three northern provinces of Prae, Lampang and Lamphoon. 114 rodents were trapped; sera from 73 of these were air shipped to the laboratory in Bangkok for serological examination. Pieces of kidney cortex were obtained from 114 rodents and minced by Tenbroek grinder using liquid Stuart's media diluent. One cc of a 10% suspension of this grinding was inoculated intra-peritoneally into each of two hamsters. These hamsters were air shipped to the laboratory in Bangkok for observation. Six of the 228 hamsters, representing 5 rodent specimens died within the desired time interval of 6-21 days. Hamster kidney cultures were conducted on a total of 29 rodent specimens from Prae only, according to techniques previously described. An isolate was recovered from one rodent trapped at Prae; this was grouped as L. javanica. Sera from 73 of these rodents revealed an average 10% agglutinin rate, exclusively L. javanica. Although insufficient rodent numbers were examined to determine accurate reactor rates, the data indicates that the same degree of infection or more exists in the Prae and Lamphoon areas as in the swine study area. Much lower isolation rates (.8% upcountry vs. 6% swine study farm) indicates that hamster passage is much inferior to direct culture for recovery of L. javanica from rodent kidneys. The exclusive presence of serotype L. javanica in these rodents duplicates the swine study farm experience; and infers that for these particular species of rodents common to cultivated fields and grazing land, L. javanica infections are the most common type of infection if not the predominant infection.

BODY OF REPORT

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Objective: Inasmuch as preliminary results obtained in rodent examination around Bangkok revealed low (6.9%) and exclusive L. javanica infections, the object of this field study was to examine the rodents of cultivated

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fields in another area of Thailand 414 miles north of Bangkok as a complementary study to the first, utilizing the weanling hamster as an indicator animal rather than direct kidney culture. This study was to serve as a qualitative and quantitative check on results obtained from the first study area.

Description and Progress: Because of the inadequacy of the optimum sterility measures available in upcountry Thailand required for direct kidney culture, the weanling hamster was selected as an indicator animal for leptospiral presence.

Two geographically different areas in each of the three provinces of Prae, Lampang and Lamphoon were selected as survey sites. These were at least 5 km. or more distant from each other. Rodent traps were set in and around grasslands and cultivated areas of rice and sugar production. 114 rodents were trapped, but unfortunately none of these were identified. Sera from 73 of these animals were shipped to Bangkok on wet ice for serological examination. As aseptically as possible, pieces of left and right kidney cortex were removed from each animal and minced by Tenbroek grinder with sufficient liquid Stuart's media to produce a 10% suspension; .75 - 1.0cc of this suspension was inoculated intra-peritoneally into each of two weanling hamsters. The hamsters were air-shipped to Bangkok within 2 days for laboratory observation.

Kidneys from all hamsters dying between 5-21 days were darkfield examined and cultured as previously described for leptospiral recovery. Six hamsters representing 5 rodent specimens died within this time interval. Only RP-10 yielded an isolated, subsequently grouped as L. javanica. Hamsters representing 28 rodent specimens from Prae, surviving 21 days post inoculation were all cultured according to previously described techniques. All were negative. Hamsters representing rodents from Lampang and Lamphoon surviving 21 days were not cultured.

Sera collected from 73 of these rodents revealed the following serum agglutinins according to areas:

	Sera Examined	No Positive	% Positive	Agglutinins
Prae	17	2	11.7	<u>L. javanica</u>
Lampang	31	1	3.2	<u>L. javanica</u>
Lamphoon	25	4	16	<u>L. javanica</u>

The microscopic agglutination-lysis test was used as described under SEATO Medic Study No. 81. The criterion designating a positive reactor was 75% + agglutination at the 1:10 or higher serum dilution. No cross-agglutination patterns were evident.

Summary: 114 rodents trapped in and around the grasslands and the cultivated rice and sugar cane fields of three northern provinces of Thailand were examined for leptospiral detection and qualification. None of these rodents were identified as to species. Utilizing weanling hamster inoculation techniques, an isolate grouped as L. javanica was recovered from one of these rodents. Sera from 73 of these rodents were examined for leptospiral serum agglutinins. Seven animals demonstrated significant serum agglutinins to the L. javanica antigen exclusively, with no evident cross-agglutination patterns.

Conclusion: 17, 31 and 25 sera were serologically examined from Prae, Lampang and Lamphoon respectively, yielding 11.7, 3.2 and 16% reactor rates in that order. These are insufficient numbers to determine accurate reactor rates, but does indicate that in Prae and Lamphoon the same degree of infection or more exists as in the swine study area (6.9%). Isolation % in this latter area was 6.1, as opposed to 1 positive from 114 in the upcountry study area. In each, the exclusive "serotype" present was L. javanica. This data indicates that hamster inoculation techniques are much inferior to direct kidney culture techniques in recovering L. javanica from rodent kidneys.

Further, sufficient rodent samples were examined (148) in the mango, coconut and betel nut groves around the swine farm study area to accurately disclose whatever "serotypes" were present (L. javanica exclusively). Sufficient samples were collected from the 3 upcountry study areas to determine that, in the main, the same pattern was evolving - a predominant, if not exclusive L. javanica infection. The same species of rodents, with a predilection for L. javanica infection and relative refractoriness for other serotype infection probably existed in these four areas. This is not to infer that rodents trapped in other types of terrain will demonstrate the same exclusive L. javanica infection. Serotypes grippotyphosa, autumnalis, and sentot have been reported isolated from rodents in other areas of Thailand. The relative refractoriness of swine to L. javanica infection has already been described. Serological examination of 3,000 cattle and buffalo from many provinces of Thailand, including the same rodent study areas in Prae, indicate that L. javanica is of negligible importance (less than 1%) in livestock infection. The type of terrain from which these rodents were trapped could be accepted as typical livestock grazing land. Insufficient epidemiological data is in hand to make conclusive statements regarding rodent-livestock infection for all of Thailand, however, in the areas examined, the serotype exclusively infecting rodents was negligibly infecting livestock.