

ANNUAL PROGRESS REPORT

SEATO Medic Study No. 80-A: Isolation of Leptospires from Thailand
Detection and Isolation of Leptospires
from Thailand Jungle Streams

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 objective of this study is to detect and isolate leptospire from jungle streams. Recent work in Malaya has shown that clear jungle stream water of neutral pH can sustain viable leptospire for long periods of time; such streams are an important source of human infection in Malaya.

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** Immunology and Serology Branch (Department of Livestock Development)

A system of jungle streams was located on the Doi Sutep mountain range near Chiangmai, Thailand. These streams closely resembled those in Malaya from which leptospire have been consistently isolated, particularly immediately after the streams had flooded. Hamster inoculation techniques were used consistently in Malaya with excellent success. The streams at Chiangmai could be described as fast flowing, crystal-clear jungle streams, pH averaging 7.4 and located deep in primary forest. Flood water was collected by means of self-sealing water bottles positioned in the course of the streams. Surface water samples were also collected by hand, as soon after flooding as possible. All water samples were immediately inoculated into weanling hamsters, and the hamsters were air shipped to Bangkok the following day. Sixty-four water samples were inoculated into 280 hamsters. Death in hamsters between 4-21 days was considered suspicious for leptospirosis. Because of the infrequency of rain during the study period (6-15 August) only 32% of the hamsters were inoculated with flooded water. No inoculated hamsters died within the desired time interval of 4-21 days. The kidneys from all inoculated hamsters surviving 21 days were darkfield examined for presence of leptospire. Sample #38 was designated darkfield suspect, and was cultured and passed (negative results). No other hamster kidneys were cultured. Two hamsters died prior to 4 days post-inoculation; these were discarded as being bacterially contaminated. Negative results were obtained from all water samples. The negative results for leptospiral recovery from two jungle streams at flood stage in Thailand may not accurately reflect the presence of leptospire in all such streams in Thailand. Only a small number of the water samples collected (32%) were from water considered optimal for leptospiral presence. In every way, the streams and water seemed similar to those from which leptospire are consistently isolated in Malaya. Only two streams were sampled from one mountain range, insufficient sampling to predict that other streams would also prove negative for presence of leptospire. It is also possible that the small mammals living close by and frequenting the streams are scanty shedders of leptospire. One can only speculate on the negative nature of these results.

BODY OF REPORT

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Objective: To attempt to isolate and detect leptospires and to duplicate results obtained in Malaya which have shown that clear jungle stream water of neutral pH can sustain viable leptospires for long periods of time. Such streams are thought to represent one of the most important sources of leptospiral infection for troops in jungle areas.

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Description and Progress: A system of jungle streams was located on the Doi Sutep mountain range (elevation about 5,000 ft) near Chiangmai, Thailand. These streams closely resemble streams in Malaya from which leptospire have been consistently isolated in Malaya with excellent success.

The streams at Chiangmai could be described as crystal clear, fast flowing jungle streams, pH averaging 7.4, and located deep in primary forest. The stream bed was rock or sand, and non-flood stream depth ranged from 6" to 2' (pool areas in the course of the streams). Water samples were collected at the end of the rainy season, between 6-15 August. Water collected at the flood stage was considered optimal for leptospiral recovery, and self-sealing water bottles were fashioned to collect these samples. These water bottles were placed in many different locations in the course of 2 streams. The water bottles were secured in the course of the streams at staggered heights to sample different aliquotes of the stream rise. Difficulty in securing these bottles against flash floods, and infrequent rains caused only 32% of the samples to be collected from flood water. The balance of the water samples were surface-water, collected by hand, as soon after flood as possible. Water samples from any one collection were inoculated into weanling hamsters, depending upon the number of sites sampled, and the number of hamsters available. Each hamster was inoculated with 1cc of flood water intraperitoneally within one hour after the sample was removed from the stream. All hamsters were air-shipped to Bangkok within 2 days after inoculation.

Hamsters dying prior to 4 days post-inoculation were discarded as being bacterially contaminated. Hamsters dying between 4-21 days were to be considered optimal for leptospiral recovery and were to be cultured. Hamsters remaining at 21 days post-inoculation were to be sacrificed and their kidneys darkfield examined. Darkfield positive kidneys were to be cultured. Water samples collected were as follows:

(See chart attached)

Type of Sample Collected

Aug	Amt of rain	After rain	Receded surface water	Flooded surface water	Flooded water cork bottle	Streams	Locations	Hamsters	
6	heavy/light	24hrs/12hrs	clear			1	8	39	
9	moderate	1 hr	slightly cloudy	swollen muddy			8	40	
10	heavy	4 hrs	slightly cloudy 5 ham.		raise 6" 3 areas 15 ham.		7	20	
11	moderate	24 hrs	clear stream low 10 ham.		raise 3" 2 streams 10 ham.	2	7	20	
12	heavy	24 hrs	slightly cloudy stream had risen 1½' during night - 90% water bottles blew out. 25 ham.		1½' rise 3 levels water collected 15 ham.	1	8	40	424
13	heavy	24 hrs	clear - stream low 25 ham.		6" rise 3 levels 15 ham.		8	40	
14	light	24 hrs	clear pool 5 ham.		3" rise 2 levels 3 locations 35 ham.	2	4	40	
15		48 hrs	clear - low 40 ham.				8	40	

Summary: Using techniques and samples as described, 64 water samples were collected and inoculated into 280 weanling hamsters. Only 32% of the hamsters were inoculated from flood water. No hamsters died during the optimum time interval of 4-21 days. Two hamsters died prior to 4 days post inoculation and were discarded. All hamsters surviving 21 days were sacrificed and their kidneys darkfield examined. Kidneys from hamsters inoculated with water sample #38 were designated darkfield suspect, and were cultured and passed with negative results. All other kidneys were designated darkfield negative, and no cultures were attempted.

Conclusion: The negative results for leptospiral recovery from two jungle streams at flood stage in Thailand may not accurately reflect the presence of leptospire in all such streams in Thailand. Only a small number of water samples collected (32%) were from water considered optimal for leptospiral presence. In every way, the streams and water seemed similar to those from which leptospire were consistently isolated in Malaya. Only two streams were sampled from one mountain range - insufficient sampling to predict that other streams would also prove negative for presence of leptospire. It is also possible that the small mammals living close by and frequenting the streams are scanty shedders of leptospire. One can only speculate on the negative nature of these results.