

ANNUAL PROGRESS REPORT

SEATO Medic Study No. 80-D: Isolation of Leptospire from Thailand -
Cattle and Swine Urine Samples from Rajaburi

Project No. 30A 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 Brahmactpta, D.V.M. *

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

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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 recover leptospire from the urine of cattle and swine in an area of Thailand disclosing an inordinately high percentage of reactors when previous attempts to recover leptospire

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from cattle and buffalo by direct kidney culturing of abattoir specimens was unsuccessful. Efforts to recover leptospire from kidney cultures of 8-9 year old cattle and buffalo were unsuccessful on 228 and 102 cultures respectively. Individual and pooled urine sampling techniques utilizing the weanling hamster had already proven effective for recovering leptospire from swine. A limited experience with cattle urine sampling techniques at Muack Lek on 2 year old native cattle resulted in one isolate from 18 cattle urines. Past serological data from cattle at Rajaburi disclosed a 36% reactor rate, with agglutinins present for most of the 18 diagnostic antigens. Past serological data had indicated a heavy L. bataviae swine infection at Rajaburi. A field station was established at Rajaburi from which early morning trips were made to the surrounding villages to pick up samples of urine from the farmers' cattle and swine. Cattle urine samples were collected in sterile bottles and inoculated into weanling hamsters within 30 minutes after having been voided. .5cc of urine was inoculated intra-peritoneally into each of 2 weanling hamsters. Urine samples were collected from 21 swine and pooled into 6 pools. .5cc of the urine was inoculated into each of 5 hamsters as described for the cattle urine. Serum was collected from all cattle from which urine was obtained. Surface water samples were collected from large standing bodies of fresh water which were frequented by great numbers of cattle as drinking holes. 6 water samples were collected from water holes in 2 different areas and inoculated into 30 hamsters as described for urine inoculation. Cattle averaged about 2 years of age. Hamsters were shipped back to Bangkok within 2 days after inoculation for laboratory examination. 88 cattle urine samples were inoculated into 136 hamsters. No hamsters died within the desired time interval of 5-21 days. All hamsters were sacrificed at 21 days, and their kidneys cultured for leptospiral recovery. Hamster cultures representing 53 of these cattle urine samples have matured at 30 days with no isolates recovered. Urine from 33 swine was pooled into 8 samples and inoculated into 40 hamsters. Two hamsters from one urine pool died in the desired time interval. One of these hamsters was cultured as previously described, and was negative for leptospiral recovery. The 38 remaining hamsters were sacrificed at 21 days and cultured for leptospiral recovery. Hamsters representing 5 urine pools have matured 30 days and have been negative for leptospiral recovery. From the 6 water samples inoculated into 30 hamsters, no hamsters died during the desired time interval. All hamsters were sacrificed at 21 days, and their kidneys cultured for leptospiral recovery. None of these cultures have matured 30 days. One can only speculate as to the negative results obtained in these cattle and swine urine isolation attempts beyond the statement that urine sampling techniques in cattle utilizing the weanling hamster are a poor method for recovering leptospire. Other workers have demonstrated the variable nature of urine shedding in the bovine even though the kidney may be infected. The second factor of hamster pathogenicity must also be considered when using this animal as an indicator of leptospirosis. The fact that the cattle may not have been infected must be considered. But in view of the age of the animals sampled, the number of animals sampled, and rate of agglutinins prevailing in this area, several infected animals should have been encountered on random sampling.

From the 5 water samples inoculated into 30 hamsters, no hamsters died during the desired time interval of 5-21 days. All hamsters were sacrificed at 21 days, and their kidneys cultured for leptospiral recovery. None of these cultured have matured 30 days.

Conclusion: One can only speculate as to the negative results obtained in these cattle and swine urine isolation attempts beyond the statement that urine sampling techniques in cattle utilizing the weanling hamster is a poor method for recovering leptospores. Other workers have demonstrated the variable nature of urine shedding in the bovine even though the kidney may be infected. The second factor of hamster pathogenicity must also be considered when using this animal as an indicator of leptospirosis. The fact that the cattle may not have been infected must be considered. But in view of the age of the animals sampled, the number of animals sampled, and rate of agglutinins prevailing in this area, several infected animals should have been encountered on random sampling.