

ANNUAL PROGRESS REPORT

SEATO Medic Study No. 80-B Isolation of Leptospire from Thailand -  
A Comparison between Native and Imported  
Cattle

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 Brahmachupta, 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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This study offered the opportunity to examine qualitative and quantitative leptospiral comparisons between native cattle and recently imported cattle. A dairy farm project is located at Muack Lek 97 miles northeast of Bangkok. Here a herd of about 150 imported Red Danish dairy

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cattle (present in Thailand about 2 years) are quartered in close proximity to a large herd of about 500 native cattle. The grazing land was covered with improved pasture grasses situated in the valleys between rolling hills, about 720 ft above the sea level. Individual urine samples were collected from 18 native cattle and 85 Red Danes. Urine samples were pooled so that each pool represented about 5 cows. One cc of urine from each pool was inoculated intra-peritoneally into weanling hamsters. Sera was collected from 165 native cattle (including the 18 from which urine was obtained) and 149 imported cattle (including the 85 from which urine was obtained). Hamsters and sera were carried back to Bangkok within two days for Laboratory examination. L. pomona was recovered from pooled urine of the native cattle. No leptospire were recovered from the imported cattle. Agglutinins demonstrated by the two types of cattle were similar (hyos and hebdomadis). 41% of the native cattle and 27% of the imported cattle were reactors. None of the cattle demonstrated agglutinins to the pomona or autumnalis antigens. Inasmuch as the imported cattle had been in Thailand only 2 years, the 27% reactor rate manifested by them reflect the predominant agglutinins manifested by the native cattle as well as about 1,400 cattle previously examined from various regions of Thailand. Abberant infection, from whatever source, would be expected to produce a spotty agglutinin response in both herds. This data infers that the infection manifested by the imported cattle was probably acquired from the native herd.

BODY OF REPORT

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Objective: The objective of this study was to examine qualitative and quantitative leptospiral comparisons between native cattle and recently imported cattle.

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Description and Progress: A dairy farm project is located 97 miles northeast of Bangkok in a valley surrounded by rolling hills, elevation is about 720 ft above sea level. Here, a herd of imported Red Danish dairy cattle (present in Thailand about 2 years) are quartered in close proximity to a large herd of native cattle. Improved pastures (pangola grass, Guatemala grass etc.) comprise most the grazing land. The native cattle numbering about 500, average about 2-3 years of age. These are left on pasture all year around. A stream, about 10 ft wide in the dry season courses through most of the pastures. When animals are on pasture, all available water for drinking is supplied by this stream. The imported cattle averaging about 4 years of age, are kept stationed in a barn through the whole of the rainy season. In the 1963 dry season, they were sporadically put on pasture, and sustained some disease losses. Although the purpose of this project is to cross these pure breeds with the native cattle for upbreeding, every effort has been made to keep these two types of animals as separated from each other as possible. Anaplasmosis and piroplasmosis have been a real problem in the purebred animals, but some FVOs have been noticed as well. During the 1963 dry season the pure bred cattle on this farm had been kept indoors.

The native cattle were herded into a corral and pushed through stocks. While in the stocks, the animals were bled and urine samples were collected. The native cattle being unused to restraint, became very excited, and only 18 urine samples were collected from this group. This urine was collected into 3 pools representing 5,6,7 animals respectively. One cc of urine was inoculated intra-peritoneally into each of 5 weanling hamsters. Urine samples from 65 Red Dane cattle was pooled into 13 specimens. These were inoculated into weanling hamsters as described above. Serum samples were collected from 165 native cattle and 149 imported cattle. Serum samples (on wet ice) and hamsters were transported to Bangkok within 2 days for laboratory examination. Hamsters dying between 4-21 days post-inoculation were suspected of leptospirosis. Kidneys from hamsters surviving 21 days post-inoculation were darkfield examined for presence of leptospores. Only if hamster kidneys were darkfield positive were cultures attempted. Results of urine inoculations were as follows:

	<u>Number of Animals</u>	<u>Number of Pools</u>	<u>Hamsters Inoc.</u>	<u>Die 6-21 Days</u>	<u>Sacrificed 21 Days</u>	<u>Cultured</u>
Native	18	3	15	3 dkfld- passed	12 dkfld- discarded	3 L.pomona
Red Dane	65	13	65	0	65 dkfld- discarded	0

An isolate grouped as L. pomona was recovered from one of the native cattle pools. This represented urine from at least one animal out of a group of 5. Although no attempt was made to individualize animals apart from pools, serum samples were collected from all animals from which urine was collected. Comparative serological results are as follows:

	<u>Red Dane</u>	<u>Native Cattle</u>
No. Tested:	149	165
No. Reactors: 1:25	41	64
1:100	0	4
% Reactors:	27	41
Agglutinins L. javanica	1	0
Manifested: L. bataviae	0	1
L. hyos	13	22
L. borincana	24	24
L. wolffi	2	19

The hebdomadis antigens borincana and wolffi cross-agglutinated quite commonly, as did the hebdomadis antigens and hyos. No other cross-agglutination patterns were manifested. No L. pomona or L. autumnalis agglutinins were demonstrated in any of the 314 sera tested from the two groups of cattle, although from one of the native cows, L. pomona was isolated.

Summary: Urine from 16 native cattle, and 65 imported cattle was examined for leptospiral presence by weanling hamster passage. Pooled urine from about 5 animals was inoculated intra-peritoneally 1 cc into each of 5 weanling hamsters. An isolate grouped as L. pomona was recovered from one of the native cow pools. No other isolates were recovered.

Sera from 149 pure bred cattle and 169 native cattle were examined for leptospiral agglutinins. 41% of the native cattle were reactors principally to L. hyos and hebdomadis group antigens. One native cow was an L. bataviae reactor. 27% of the pure bred cattle were reactors principally to the L. hyos and hebdomadis group antigens. One pure bred cow was an L. javanica reactor. No L. pomona agglutinins were demonstrated in any of the 314 cattle tested, although L. pomona was recovered from one of the native cows in this group.

Conclusion: Inasmuch as the imported cattle had only been in Thailand 2 years prior to this study, its 27% reactor rate is significantly high. Except for one reactor in each group (L. bataviae - native, L. javanica - imported) agglutinins manifested in each group were identical (L. hyos and L. hebdomadis). The most consistent agglutinins manifested in about 1,400 cattle sera from many provinces of Thailand have been L. hyos and the hebdomadis group. This data would indicate that these latter agglutinins are "cattle" agglutinins elicited by leptospire with a predilection for cattle kidneys in Thailand. This can not be confirmed until leptospire eliciting these agglutinins responses can be isolated from cattle. However, heavy rodent infections with L. javanica exclusively have already been described. One of the imported cattle elicited an agglutinin response to L. javanica which can probably be interpreted as being a rodent (or dog or cat) infection. The sporadic L. bataviae agglutinin response in the one native cow is probably also indicative of an aberrant infection. Abberant infection from whatever course would be expected to produce a spotty agglutinin response in both herds. All serological data indicates that the

leptospiral infections serologically demonstrated in the imported animals were probably acquired from the native herd.

The recovery of L. pomona from the native herd in the face of no agglutinins for L. pomona in 314 animals of both herds is difficult to explain. Serological examination of several thousand cattle and buffalo sera from all over Thailand has demonstrated a surprisingly low percentage of L. pomona reactors, although this would comprise perhaps 80% of the swine infections. This isolation could be explained in the presence of our swine L. pomona isolation data; that is to say leptospiruria may not be consistent with detectable serum agglutinins, but that serum agglutinins may ascend to the point of detection after leptospiruria has occurred for some length of time. One would have expected to have isolated a leptospire eliciting L. hyos and hebdomadis agglutinins rather than L. pomona, but these serotypes may be relatively refractory to isolation via hamster inoculation techniques.