BODY OF REPORT

SEATO Medic Study No.81	Serological Classification and Detection of Leptospirosis in Thailand
Project No. 3A 025601 A 811	Military Medical Research Program S. E. Asia
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Subtask 01:	Military Medical Research Program SEASIA (Thailand)
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	Division of Medical Research Laboratories
	Department of Veterinary Medicine
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Objective: The objective of this study is to determine serological prevalence of leptospiral agglutinins in wild or domestic animals in Thailand.

Description: The field teams of the Department of Livestock Development collect

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and supply livestock sera to the laboratory. This sera is collected in the routine survey programs under field conditions, separated in the field by clot extraction and shipped to Bangkok with wet ice as refrigerant. The serological test conducted is the microscopic agglutination-lysis test utilizing eighteen (18) live diagnostic antigens, serially grown in liquid Stuart's media containing rabbit serum. All sera is examined at 4-fold serial dilutions positive results are based up 75% or more agglutination. Of the eighteen (18) antigens employed, eleven (11) are obtained from local isolates and seven (7) WHO/FAO supplied by the Walter Reed Army Institute of Research.

Progress: During the report period sera was obtained from domestic animals in twenty-eight (28) provinces in Thailand. Sera was also obtained from domestic livestock and rodents in the area of Thunyaburi to correlate serological response with isolates obtained (SEATO Medic Study No. 80F). Serological titers continue to be of low order seldom exceeding 1:25 for cattle and buffalo (Annual Report 1963-64 SEATO Medic Study No. 81 pages 459-472). Local isolates have been substituted as antigens for serotypes L. bataviae, L. pomona, L. hyos, L. autumnalis, L. canicola, L. icterohemorrhagiae, L. pyrogenes, L. alexi, L. grippotyphosa, L. javanica and L. australis. Leptospiral cultures having heavy concentrations of growth at 5-7 days are essential as antigens in the conduct of the agglutination-lysis test. Initial dilutions of serum vary according to species; rodents rodents 1:25, swine 1:10, cattle and buffalo 1:25, dogs 1:25. Standard criteria used in determining serological response is:

75% or more agglutinations	+
50-75% agglutination	$\pm$
25-50% agglutination	$\mp$
25% or less agglutination	–

Results obtained from a random selection of seven hundred and seventy-eight (778) buffalo sera obtained in twenty-eight provinces are shown in Table I. Consistently higher percentages of serological reactions occurred to L hyos and L. autumnalis antigens.

Sera aliquots were obtained from five hundred and thirty-three (533) cattle from twenty-two (22) provinces primarily in northern and eastern Thailand. Serological affinity for L. hyos, L. wolffi and L. borincana antigens predominated. Serological response of cattle sera to the hebdomadis group (L. borincana and L. wolffi) antigens appeared to be greater than that encountered in buffalo sera. Agglutinin response to L. hyos appear to predominate in both buffalo and cattle especially in the northern provinces. Results of the cattle sera are shown in Table II.

Sera obtained from 110 swine in the area surrounding Thunyaburi was examined

Table I

BUFFALO SERA

	andaman	butembo	celledoni	bataviae	pomona	djaziman	hyos	autumnalis	ballum	canicola	ictero	pyrogenes	alexi	grippo	borincana	wolffi	javanica	australis
Patumthani	5	1		3	6		21	13	1	4	1	3		4	4	3		
Lampoon	1		1	1			1	1	3				1	4		2		
Lampang							1				1					1		
Sukhothai							1	2								1		
Burirum	1			2		1		4							1			
Chaiyapoom					3		2	1				2				1		
Rajburi	1				1	1						1						
Nakorn Rajsrima							1	1			2	2						
Cha Cherng Sao						1			1			3			1			
Udornthani							1									1		
Chainat								1										
Roi Ed																		
Sakol Nakorn	3			2			3	4										
Khonkaen																		
Ayudhaya	2						1					1				2		
Kalasin	1			1			1								1			
Cholburi				3			1	1		1	2	2			2			
Prachuabkirikant							1											
Rayong							1	1								1		
Loey							1					1						
Surin							1					1			1			
Lopburi								3				2			1			
Dhonburi								1			1				1			
Tak								1		1				1				
Kanchanaburi											1					1		
Saraburi												1						
Ubolrajthani															1			
Srisakate															1			

Table II

CATTLE SERA

	andaman	butembo	celledoni	bataviae	pomona	djaziman	hyos	autumnalis	ballum	canicola	ictero	pyrogenes	alexi	grippo	borincana	wolffi	javanica	australis
Lampoon	3			2			3	1	3						7		2	
Lampang				2			2	2	1							10		
Tak									1	1			1		4	1		
Rajburi							4		1						3	3		
Prae							2		1			1		1		2		
Kalasinth															1			
Singhburi															1			
Pitsanulok							3	1				1			4	4		
Lopburi															2			
Chaiyapoom								1				1				1		
Kanchanaburi																1		
Sukhothai								1										
Nakorn Rajsima								1									1	
Burirum								1										
Utaradit								1										
Kampangpetch			1															
Surin	1									1					2			
Kalasin										1								
Roi Ed							1											
Nakorn Pathom									1						2			
Thonburi								1	1									
Loey											1	1						

Table III

SWINE SERA

	1:25	1:100	1:400
L. pomona	9		
L. hyos	1	1	1
L. autumnalis	1		
L. canicola	2		

and results are shown in Table III. The results seem to confirm previous observations that L. pomona constitutes the predominant leptospiral swine infective organism. This organism was recovered from the water collected at a swine farm (SEATO Medic Study No. 80F).

Sera was obtained from one hundred and ninety-two (192) rodents trapped at the study area referred to above. Serological affinity to three (3) of the eighteen (18) serotypes antigens was demonstrated in eighteen (18) of the sera tested.

No isolates were recovered from the kidneys of six (6) rodents whose sera demonstrated affinity for the L. canicola antigen. The following Table V demonstrates the correlation of isolates recovered by kidney culture techniques with serological response of individual rodents. Since this laboratory does not have a stock of specific L. bataviae antisera and the L. djatzi and L. hyos antisera agglutinate to the L. bataviae antigen, isolates TR 170 and TR 231 may be L. bataviae.

Sera was obtained from forty-six (46) dogs at Udorn and serological affinity to the L. canicola antigen was demonstrated in twenty-nine (29), partial affinity in four (4) and negative results in thirteen (13) of the sera tested. Titers obtained are as follows:

Titers	1:25	1:100	1:400
Sera	11	13	5

Table IV

RODENTS

	1:25	1:100	1:400	1:1600
L. canicola	5		1	
L. javanica	6	1	1	
L. bataviae/hyos	2	1	1	

Table V

RODENT TITER/ISOLATE CORRELATION

Rodent	Titer		Isolate
TR 22	1:25	L. javanica	L. javanica
TR 52	1:100	L. javanica	L. javanica
TR 73	1:25	L. javanica	L. javanica
TR 74	1:400	L. javanica	L. javanica
TR 105	1:25	L. javanica	L. javanica
TR 109	1:1600	L. hyos/L. bataviae	L. djatzi
TR 119			L. javanica
TR 120	1:100	L. javanica	
TR 123	1:100	L. javanica	L. javanica
TR 124	1:25	L. javanica	L. javanica
TR 144	1:25	L. hyos/L. bataviae	L. djatzi
TR 170	1:100	L. hyos/L. bataviae	L. djatzi or L. hyos
TR 231	1:25	L. hyos	L. djatzi or L. hyos

Discussion: Area and species differences in the prevalence of leptospiral agglutinins offer information pertinent to the epidemiology of the disease in wild and domestic animals in Thailand. Serological titers were obtained in 23.9% of buffalo and 19.7% of cattle sera tested. Serological response of cattle sera to hebdomadis group antigens appears to be greater than that encountered in the buffalo sera where consistently higher numbers of L. autumnalis reactions were demonstrated. Agglutinins response to L. hyos antigen is prevalent in the sera obtained from both species. Agglutinin response results of swine sera, although predominately L. pomona did indicate that both L. hyos and L. canicola must also be considered in the epidemiology of the disease. Agglutinin response of rodent sera correlates with isolation results in infections caused by L. javanica and L. djatzi/L. hyos. No isolates were recovered from rodents whose sera elicited agglutinin response to L. canicola. Since only kidneys were cultured in each instance, it is quite concievable that L. canicola may have localized in another organ of the body and therefore was not recovered.

A high incidence of L. canicola was demonstrated in the dog sera collected at Udorn as well as in recovery of isolates from the kidneys collected from the same animals. It is apparent that this organism is the primary causative agent of leptospirosis in the canine species in Udorn and must be considered in the diagnoses of "fevers of unknown origin" in the human population of the same area.

Summary: Sera collected from five hundred and thirty-three (533) cattle from twenty-two (22) provinces, seven hundred and seventy-eight (778) buffalo from twenty-eight(28) provinces, one hundred and ten (110) swine and two hundred and eleven (211) rodents from Thunyaburi province, were examined. Agglutinin response of cattle and buffalo sera to serotypes L. hyos and L. autumnalis predominated with much lower percentages reacting to one of the sixteen (16) remaining serotype antigens. Swine sera reactions were predominately L. pomona and rodent sera L. canicola or L. javanica. Agglutinin response of canine sera collected at Udorn was predominately L. canicola.