

ANNUAL PROGRESS REPORT

SEATO Medic Study No. 80-C: Isolation of Leptospire from Thailand -
Domestic Livestock Abattoir Study Utilizing
Direct Kidney Culture

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

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 by direct culture the kidneys from cattle, buffalo and swine presented to the Bangkok Abattoir from many provinces in Thailand; to recover those serotypes causing the spectrum of agglutinin responses obtained. In an attempt to isolate some of the organisms illiciting the diversified and significant agglutinin response in domestic livestock, a kidney sampling study was started at the Bangkok Abattoir. Swine, cattle and buffalo are presented to this abattoir from

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many of the provinces in Thailand, particularly the northeast. Kidneys are removed from these animals in as aseptic a manner as possible on the killing floor and placed in sterile specimen boxes. Serum is collected from each animal from which a kidney specimen is collected. The kidneys are returned to the laboratory and placed in the refrigerator until cultured, usually within 2 hours thereafter. Cultures are put up in 6 replicate tubes semi-solid Fletcher's media containing varying percentage of rabbit serum, and six replicate tubes of semi-solid Fletcher's media containing homologous species serum (cattle, buffalo and swine). 228 cattle kidney specimens were cultured as previously described. Bacterial contamination of cultures was less than 5%. Darkfield culture examinations were conducted at 15 and 30 days respectively. The cattle averaged 8 years of age at slaughter. No leptospire were isolated from any of the cattle cultures. 102 water buffalo kidney specimens were cultured as previously described. Bacterial culture contamination was less than 5%. Darkfield culture examinations were conducted at 15 and 30 days respectively. The buffalo averaged 9 years of age at slaughter. No leptospire were isolated from any of the buffalo cultures. 312 swine kidney specimens were cultured as previously described. Bacterial culture contamination was less than 5%. Darkfield culture examinations were conducted at 15 and 30 respectively. The swine averaged one year of age at slaughter. 24 isolates were recovered from 312 swine giving a 7.7 recovery percentage. Using the agglutination criterion of 75%+ agglutination at the 1:100 serum dilution as determining a positive reactor, none of the homologous isolate sera manifested sufficient serum agglutinins to be called a reactor. Isolates were recovered from 10 of the 15 provinces from which swine were sampled. 21 of the 24 isolates have been grouped as L. pomona; 1 L. canicola; 1 L. javanica (?) and 1 not examined. The relative ease with which leptospire can be isolated from swine with an overall 12% agglutinin response as opposed to the difficulty in recovering isolates from cattle and buffalo with a 25% overall agglutinin response is thought to be a reflection of the differences in age at which the animals were examined.

BODY OF REPORT

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

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Objective: Inasmuch as direct kidney culturing methods offer the best chances for recovering leptospires, the object of this study was to examine by direct cultures, the kidneys from cattle, buffalo and swine presented to the Bangkok abattoir from many provinces in Thailand, to recover those serotypes causing the spectrum of agglutinin responses obtained by serology.

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Description and Progress: In an attempt to isolate some of the organisms illiciting the diversified and significant agglutinin responses in domestic livestock, a kidney sampling study was started at the Bangkok abattoir. Swine, cattle and buffalo are presented to this abattoir from many of the provinces of Thailand, particularly the northeast. The market age for swine is one year; thus supplying the laboratory with an optimum animal for leptospiral recovery. A national law prohibits the slaughter of young cattle and buffalo because of their value to the economy as work animals. Because of this law, no cattle or buffalo less than 6 years of age were available for specimens, average age respectively was 8 and 9 years.

Groups of animals for study were selected prior to slaughter. An attempt is made to diversify the areas from which the animals originated. Area identification is accurate to the province, but not more specifically. Blood is collected at the point of bleeding out, and an attempt is made to quickly estimate the age. A tag is placed on the animal at this point and its point of origin recorded. When the animal reaches the point where the body wall is opened, it is identified by its tag. At this point it is aged as accurately as possible by means of dentition wear, and one kidney is removed as aseptically as possible and placed in a sterile specimen box. An effort is made not to remove the peri-renal fat, as this is thought to serve as a deterrent to surface bacterial contamination of the kidney. About 15 kidneys can be worked up in the laboratory daily if no other laboratory work is scheduled. The specimens are transported to the laboratory (about 5 km distance) and immediately placed in the refrigerator. No longer than $1\frac{1}{2}$ hours elapses before the first kidney is cultured, and $3\frac{1}{2}$ hours before the last kidney is cultured. The blood is centrifuged, serum collected, and frozen at - 20 F. Cultural methods are as follows:

In an aseptic manner, the peri-renal fat is removed and discarded. The kidney is halved mid-sagittally and placed with both cortical surfaces up. The cortical surfaces are flamed with a bunsen burner until the entire surface is discolored tan. The kidney capsule is then removed. If the capsule had been previously torn or partially removed, the kidney is flamed again, after removal of the remainder of the capsule. Using a new scalpel, superficial cortical surfaces are cut away, leaving natural colored cortical tissue underneath. Kidney scrapers, special constructed, but looking like enlarged jar lids with nail holes punched in one end and covered with Al foil at the other, are run across the cortical surface. Collected within the scraper is a mushy homogenate of the entire cortical surface. The Al foil is opened, and an aliquote of homogenate is picked up with a cotton-tipped applicator stick and broken off into a screw-cap tube containing 1.5 cc of liquid Stuart's media without rabbit serum. Previously, cotton tipped applicator sticks had been weighed with and without kidney homogenate to determine what amount would approximate .15 grams of homogenate. In actual culturing, what appears to be .15 grams is approximated by eye only. .50 cc of this approximated 1:10 suspension is serially diluted in 2 replicate tubes of 4.5 cc of liquid Stuart's media producing approximated 1:100

and 1:1,000 dilutions. One drop from the 1:100 dilution is cultured in each of 3 tubes of semi-solid Fletcher's media containing 8, 12, and 16% rabbit serum, and 3 tubes of Fletcher's media containing 10% homologous species serum. One drop of 1:1000 dilution is cultured in each of 3 tubes of semi-solid Fletcher's media containing 8, 12 and 20% rabbit serum, and 3 tubes of Fletcher's media containing 10% homologous species serum. The cultures are darkfield examined at 15 and 30 days for presence of leptospire. Bacterial culture contamination has been less than 5%. Homologous species serum must be agglutinin negative by microscopic agglutination lysis test before it is used for culturing purposes.

Buffalo

102 water buffalo kidneys were examined from water buffalo originating in 15 different provinces of Thailand. All were negative for leptospire. Serological examinations have not been done on these animals.

Cattle:

228 cattle kidney specimens were examined from cattle originating in 22 different provinces of Thailand. All were negative for leptospire. Serological examinations have not been done on these animals.

Swine:

312 swine kidney specimens were cultured as previously described from swine originating in 15 different provinces of Thailand. Data is as follows:

Province:	No. of swine	No. positive	Swine Isolate	Identification
1. Ang Thong	15	2		42, 43
2. Bangsue, Bangkok	14	6	248, 250, 252, 253, 254, 256	
3. Buri-Rum	5	-		
4. Cha Cherng Sao	8	3	259, 260, 261	
5. Chiangmai	25	-		
6. Khon Kaen	15	1		24
7. Lopburi	11	1		286
8. Nakorn Naiyoke	15	-		
9. Nakorn Pathom	39	3	151, 158, 200	
10. Pichit	16	1		121
11. Pitsanuloke	7	1		306
12. Rajaburi	48	4	105, 117, 119, 283	
13. Roiet	56	-		
14. Sakol Nakorn	15	-		
15. Singhburi	23	2		236, 239

173 swine were examined from 8 of these provinces in the Central Plain area yielding 22 isolates (recovery rate 12.6%). 139 swine were examined from 7 provinces beyond the Central Plain yielding 2 isolates (recovery rate 1.4%).

The number of cultures positive in rabbit serum at the 1:100 dilution was 20 out of 24, at the 1:1,000 dilution, 21 out of 24.

The number of cultures positive in swine serum at the 1:100 dilution was 2 out of 24, but the culture was better in rabbit serum. No swine serum cultures were positive at the 1:1,000 dilution. Usually, all percentages of rabbit serum would support leptospiral growth equally well. However, with some recovered isolates, only one or two tubes would contain leptospire. In these cases, no specific percentage of rabbit serum was found consistently any better than other percentages:

Serological data on isolates is as follows:

Swine identification:	Isolate grouped	1:10	Serology	1:100
	pomona	+ autumnalis + pomona + others		
	pomona			
	pomona			
	pomona	+ pomona - autumnalis +		
	pomona			
	pomona	- andaman + butembo djasiman		
	pomona	+ pomona autumnalis ictero borincana		
	pomona	- andaman +		
	pomona	2+ pomona		- pomona
	pomona	+ pyrogenes grippe 439 javanica		

	javanica (?)	- alexi	
	pomona	+ autumnalis + pomona - bataviae + ictero	
200	canicola	+ canicola - pyrogenes	+ canicola - pyrogenes
	pomona	- autumnalis + ictero borincana	
	pomona	+ borincana autumnalis + others	+ grippe
	pomona	+ autumnalis + ictero	
119	pomona	2 + pomona + autumnalis - javanica	- pomona
	pomona	- pomona + autumnalis + ictero	+ pomona
	pomona	- pomona + autumnalis + ictero	
43	pomona	- pomona	
42	pomona	+ ballum ictero	
248	not done	+ bataviae + javanica - boricana - ictero + andaman	+ bataviae

At the 1:100 serum dilution none of the 24 animals from which an isolate was recovered met the criterion designating a positive reactor, that is to say 75% + agglutination. 6 of the 24 animals demonstrated between 25-75% agglutination at the 1:100 dilution, but one of these developed agglutinins (grippotyphosa) contrary to the organism eventually grouped (pomona). The 1:10 serum dilution was also difficult to interpret. Usually a combination of agglutinins occurred; but when autumnalis or pomona occurred within this combination, the isolate was always grouped as pomona.

Summary: Attempts to isolate leptospire by direct culture of freshly collected buffalo (102 specimens) and cattle (228 specimens) kidneys were unsuccessful. Cultures were put up in 6 tubes of Fletcher's media containing 8, 12, 16, and 20% rabbit serum, and 6 tubes of Fletcher's media containing 10% homologous species serum. Bacterial culture contamination was less than 5%. The animals averaged between 8-9 years of age.

Attempts to isolate leptospire by direct culture of freshly collected swine kidneys (312 specimens) were successful. 24 isolates were recovered yielding a 7.7% of recovery. Twenty-one of these have been grouped as L. pomona, one as L. canicola, one as L. javanica (questionable) and one remains to be classified. Culturing was conducted as previously described, and bacterial culture contamination was less than 5%. All animals averaged 1 year of age. According to agglutination criteria used in this laboratory, none of the animal from which an isolate was recovered could be designated a serological reactor. Kidney samples were collected from swine originating in 24 different provinces of Thailand.

Conclusion: Inasmuch as the method of direct culturing cattle, buffalo and swine kidneys was identical for the three species, and in as much as this method has proven very successful with swine kidneys, a species or a sampling difference must explain the difference in isolations when comparing swine with buffalo and cattle. A mass of serological data accumulated from different provinces indicate that swine show about 12% serum agglutinins as opposed to 29% and 22% respectively for cattle and buffalo. Of course, these agglutinin percentages would vary from area to area. If agglutinin percentages are accepted as a measure of the species being infected with leptospire, then one would expect twice as many isolates from the cattle and buffalo than from the swine. Species and sampling differences must then be considered. About 88% of isolates recovered from swine have been L. pomona. This organism is known to be well adapted to the swine kidney and can be shed for long periods of time. It is conceivable but not probable that the leptospire infecting cattle and buffalo in Thailand are less successfully adapted to the kidneys of these species, resulting in kidney infections of very short duration. Again, serological data infers against this. The great majority of agglutinins demonstrated in cattle and buffalo have been of a very low order (1:25 and 1:100), indicative of either residual titers or relatively non-pathogenic organisms. Further, a preponderance of hyos and hebdomadis group agglutinins in cattle and buffalo infers that these are perhaps "cattle"leptospire.

Sampling differences must then be considered. As previously stated, cattle and buffalo kidneys were obtained from animals averaging 8-9 years of age, as opposed to the one year of swine. Eight to nine years in the flooded rice-field-type land of Thailand would be a sufficient length of time for these animals to develop an immunity to the most prevalent leptospire. Until yearling and two year old cattle and buffalo can be sampled, one must resort to speculation regarding infection percentages in this animals.

Inasmuch as none of the swine from which isolates were recovered manifested significant agglutinin levels to be called a reactor, either the serological test as used lacks sensitivity, or the pathogenic nature of these infections in swine is minimal. These results support results obtained and the hypothesis conjectured in the individual swine urine section of study No. 82.