ANNUAL PROGRESS 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
Task 01:	Military Medical Research Program S. E. Asia
Subtask 01:	Military Medical Research Program SEASIA (Thailand)
Reporting Installation:	U.S. Army-SEATO Medical Research Laboratory, APO 146, San Francisco, California
	Division of Medical Research Laboratories
	Department of Veterinary Medicine
Period Covered by Report:	1 April 1963 to 31 March 1964
Principal Investigator:	Thomas J. Keefe, Captain, VC
Associate Investigators:	Achit Chotisen, D.V.M., Ph.D. *
	Preecha Klainil, D.V.M. **
Reports Control Symbol:	MEDDH-288
Security Classification:	UNCLASSIFIED

* Chief, Immunology and Serology Branch (Research and Education Div.)
** Immunology and Serology Branch (Dept.of Livestock Development)

ABSTRACT

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The objective of this work was to study the qualitative and quantitative leptospiral agglutinins prevailing in Thai domestic livestock, an effort being made to establish area and species differences. 1,370 cattle sera, 790 buffalo sera, and 567 swine sera were collected in Thailand from

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5 northern provinces, 4 eastern provinces and 5 provinces in the sub-Bangkok Central Plain. These were examined for leptospiral agglutinins and an attempt was made to note area and species differences. Results are as follows:

a. Leptospiral agglutinins manifested in random sampling of livestock are typically of a low order and seldom exceed 1:25 (buffalo and cattle) or 1:100 (swine).

b. Percent reactors in buffalo and cattle are essentially similar (+22 %) with a tendency to diminish in frequency as one moves away from the Central Plain.

c. From "positive" areas, agglutinins manifested by swine are typically less frequent than cattle and buffalo (less than 17%); particularly noteworthy is the very low evidence of swine infection (negative serology - 2% isolation) in areas outside the Central Plain.

d. Hebdomadis group and hyos agglutinins are consistently illicited by cattle and buffalo from all areas.

e. L. autumnalis agglutinins are thought to be negligible or absent in the Central Plain (no buffalo examined), but occur in buffalo second in frequency to only hebdomadis in areas of the North and Northeast. Buffalo illicit a 7-15 fold-higher autumnalis agglutinin response than do cattle examined from the same areas.

f. Although evidence of bataviae and canicola infections exist, L. pomona appears to constitute 84-92% of the swine infections. Central Plain swine infections are heavy (17% serologically - 12% isolation); infections beyond are scanty.

g. Serological and isolation data in the leptospil infections of rodents frequenting the grazing land of 4 provinces discloses a predominant if not exclusive L. javanica infection which is of negligible importance in livestock infections (.66 is maximum infective rate in cattle).

Area and species differences were disclosed in prevailing livestock leptospiral agglutinins which, when compared with the small amount of livestock isolation data on hand, offers a degree of clarification to the epidemiology of these infections. By inference, areas of additional study have been pointed out to clarify the epidemiology of human infections. The following conclusions may be warranted or inferred.

a. The consistency and prominence of the hebdomadis group and L. hyos agglutinins in cattle and buffalo from all areas, in the presence of meager rodent data indicating the lack of these infections in rodents, indicates that the serotypes causing these agglutinins are "cattle" organisms. The ability of these serotypes to cause clinical disease in buffalo and cattle is not known and the importance of these organisms in causing human infections and/or disease is not known.

b. L. pomona appears to constitute 84-92% of the swine infection, whose intensity is centered in the Central Plain region. This may explain the prevalence (although very low) of cattle and buffalo pomona agglutinins within the Central Plain, and apparent lack of them without. Intensive swine husbandry is thought to enhance pomona infections.

c. L. autumnalis agglutinins from buffalo in the north and northeast are significantly high (5-9% infectivity rate), 7-15 fold higher than cattle agglutinins in the same area. There is no concrete evidence of significant autumnalis agglutinins in regions of the Central Plain examined (also a relative lack of buffalo in these regions). This infers that the buffalo is either environmentally disposed or species disposed to autumnalis infections, and constitute a potential of human autumnalis infection in areas of great buffalo densities.

d. From 4 different provinces examined, the exclusive rodent infection (grazing land) was disclosed to be L. javanica, whose highest infectivity rate in livestock was .66%. This infers that the rodent role in livestock infections has yet to be clarified in Thailand.

BODY OF REPORT

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Objective: This work is a qualitative and quantitative serological study of the leptospiral agglutinins manifested by Thai domestic livestock, and complements livestock isolation studies conducted by the same laboratory. This study has attempted to establish area and species agglutinin differences among livestock. Once baseline data on livestock leptospirosis

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has been formulated, the degree to which these animals can be implicated in human leptospirosis can be studied. Minor rodent studies were conducted to determine their role in livestock leptospirosis.

Description: The field teams of the Department of Livestock Development have supplied this laboratory with livestock sera and accompanying background information. Aliquotes of sera collected by these teams in their routine survey programs are given to this laboratory for examination. These teams have further been made available for special collection trips in exclusive support of this program. These sera are collected under field conditions, separated in the field by clot retraction, and shipped to Bangkok on wet ice. Of the 3,000 + sera these teams have collected during the past year, this laboratory has examined 790 water buffalo sera, 1,370 cattle sera, and 567 swine sera collected from 5 provinces in Northern Thailand, 4 provinces in eastern Thailand, and 5 provinces in the sub-Bangkok Central plain area. The serological test conducted in this laboratory is the microscopic agglutination-lysis test, utilizing the 18 live diagnostic antigens originally supplied by the WHO/FAO Leptospirosis Reference Laboratory at the Walter Reed Army Institute of Research. The antigens employed by this laboratory are as follows:

L. andaman CH 11
L. butembo Butembo
L. celledoni Celledoni
L. batavia Swart
L. pomona Pomona
L. djasiman Djasiman
L. hyos Mitis Johnson
L. autumnalis (FBF)
L. ballum M-127
L. canicola H.U.
L. ictero M-20
L. pyrogenes Salinem
L. alexi HS-616
L. grippotyphosa M.V.
L. borincana HS-622
L. wolffi 3705
L. javanica V.B.
L. australis A - Balico KM

All sera are examined against this battery of live antigens serially grown in liquid Stuart's media containing 12% rabbit serum. Antigen cultures showing heavy concentrations of leptospires at 5-7 days growth are mandatory for conducting the agglutination-lysis test. All sera are examined at 4-fold serial dilutions depending upon the species of animal examined. Initial dilutions of serum are as follows for the following species:

rodents -1:10; Swine 1:100; cattle and buffalo 1:25; human 1:100; cat and dog 1:100. The standard criteria used in measuring serological reactivity is based upon the minimum species serum dilution and the following:

75% or more agglutination	+
50-75% agglutination	±
25-50% agglutination	∓
25% or less agglutination	

All serological reactions previously mentioned or to be mentioned are based on these criteria. The only "reactors" recorded then were those animals whose serum demonstrated 75% or more agglutinating capacity at the minimum species serum dilution.

Groups specific anti-sera for classifying isolates is also supplied by the Walter Reed Army Institute of Research. These anti-sera are as follows:

L. djatzi p.58
L. hyos Mitis Johnson p. 57 WHO
L. pomona Pomona p. 57 WHO
L. djasiman Copenhagen p. 61 WHO
L. canicola H.U. p. 59
L. icterohemorrhagiae M-20 p. 59
L. zanoni p. 58 WHO
L. alexi HS-616 p. 59
L. sentot Copenhagen p. 61 WHO
L. autumnalis AB Akiyama A. p. 57
L. australis A Ballico KM p. 57
L. ballum M-127 p. 58
L. grippotyphosa M.V. p. 58
L. medanensis p. 58 WHO
L. javanica Veld. Bat. p. 59
L. pyrogenes R-046

Progress: The following 11 tables summarize most of the serological data collected thus far. Pertinent details will be pointed out, if indicated, at the end of the series of tables.

Rector Percentage/Area:

Cattle (Table I):	North			East		Sub-Bangkok Central Plain		
	Nan	Muack Lek		Sisaket	Korat	Nakorn Pathom	Rajiburi	Cholburi
# Tested	302	314		36	92	297	316	23
Reactors: 1:25	41	105		1	20	75	79	7
Reactors: 1:100+	2	4		0	0	13	34	4
% Reactors: 1:25	13	33		3	22	25	25	30
% Reactors: 1:100+	0.7	1		0	0	4	11	17
Area % Reactors:	23.5			12.5		37.3		

The 3% reactors for Sisaket should perhaps be considered too low, and due to the small numbers tested. The 47% reactors for Cholburi should perhaps be considered too high due to the small number tested. Eliminating these percentages then area reactors for cattle becomes North 24%, East 22%, Central Plain 32%. The 20% difference in reactors between Nan and Muack Lek may be an accurate reflection of the more northerly location of Nan by 530 km. This table points out two things, primarily:

(1) Agglutinins manifested in random sampling of cattle are typically of a low order, and seldom exceed 1:25

(2) Percentage of reactors diminishes more or less as one moves out from the Central plain area North or East.

Buffalo (Table II):

	North		East	
	Nan	Prae	Sisaket	Korat
# Tested:	504	142	43	101
Reactors: 1:25	117	21	6	30
Reactors: 1:100+	11	6	1	0
% Reactors: 1:15	23	15	14	30
% Reactors: 1:100+	2	4	2	0
		22		23

This table illustrates two points primarily:

(1) Agglutinins manifested in random sampling of water buffalo are typically of low order and seldom exceed 1:25.

(2) Percentage of reactors, as compared with cattle, for provinces of the North and East are about the same, ± 22%.

Swine (Table III):

	North		North East	East	Sub-Bangkok Central Plain		
	Nan	Prae	Sakol Nakorn	Korat	Bangkae	Nakorn Pathom	Rajiburi
# Tested	60	88	30	83	122	171	45
Reactors:							
1:100	-	-	-	-	10	9	10
1:400+	-	-	-	-	11	3	5
% Reactors:							
1:100	-	-	-	-	8	5	22
1:400+	-	-	-	-	9	2	11
Area % Reactors:	0		0			19	

This table is strikingly different from I-II, and the following appears evident:

(1) Agglutinins manifested in random-sampling of swine are typically of low order and seldom exceed 1:100 (In the limited amount of work conducted in this laboratory, agglutinins have been an exclusive manifestation of past infection and have never been observed with swine showing leptospiruria or infected kidneys).

(2) From "positive" areas, agglutinins manifested by swine are typically lower than those of cattle and buffalo (The 33% positive reactors for Rajburi should be considered improbably high, and due to the small number of animals tested).

(3) Especially noteworthy is the lack of agglutinins in areas beyond the Central Plain.

Table IV-Cattle Agglutinin Rates/Area

	Nan	Muack Lek	Sisaket	Korat	Nakorn Pathom	Rajiburi	Cholburi
# Tested	302	314	36	92	297	316	23
% Reactors	14	34	3	22	29	36	47
L. andaman		.64			1.0	2.2	
butembo	.66				1.0	1.7	
celledoni	.33						
bataviae	2.3	.32				2.5	
pomona	1.9				1.9	2.8	
djasiman	.33					.94	
hyos	1.7	11.4	2.8	11.9	6.7	4.1	
autumnalis	.33			1.1	.33	4.6	
ballum					.66	.94	
canicola						.22	
ictero					.66	2.8	
pyrogenes				1.1	.66	.64	
alexi				1.1	.66	.94	4.3
grippo					1.4	.32	
borincana	2.3	15.8			8.1	2.8	39.3
wolffi	2.6	6.6		6.5	7.0	3.8	4.3
javanica	.66	.32			.33	1.3	
australis	.33					.32	

Sisaket and Cholburi columns should be disregarded because of the small number of animals tested. Several things appear evident from the table.

(1) The Central Plain area demonstrates a greater variety of agglutinin response than do other areas.

(2) L. hyos and the hebdomadis group(L. borincana and wolffi) show consistently high percentages of reactors in all areas tested. This tendency has been manifested throughout the study, so much so that one wonders whether these agglutinins represent leptospires with a special predilection for cattle and buffalo kidneys.

(3) The paucity of L. pomona agglutinins (particularly outside the central plain area) and L. javanica agglutinins is noteworthy.

(4) L. autumnalis and L. pomona share cross-agglutinating properties. Except for the possibility of autumnalis infections at Korat, most autumnalis agglutinins listed here should be considered possible pomona infections.

(5) All other agglutinin responses listed here show nothing particularly striking, and not knowing the inciting organism, one can only speculate as to the epidemiology of these infections.

Table V: Buffalo Agglutinin Rates/Area

	Nan	Prae	Sisaket	Korat
# Tested	504	142	43	101
% Reactors	25	19	16	30
L. andaman				
butembo	.39	.71		
celledoni	.19			
bataviae	.99	2.1		1.9
pomona	.39	1.4		
djasiman				
hyos	4.6	2.1		4.9
autumnalis	4.8	6.3	6.9	8.9
bullum	.19			
canicola	.19			
ictero	1.6	.71	2.3	.99
pyrogenes	.78			2.9
alexi	1.2	.71		2.9
grippo	.39			.99
borincana	4.8	2.8	2.3	.99
wolffi	4.4	2.1	4.7	4.9
javanica	.19			
australis	.39			

The following appears evident:

(1) Buffalo share the variety of agglutinin response already demonstrated in cattle, and dependent upon area. No buffalo were examined from the Central Plain area. It can be speculated that if the same numbers of buffalo were examined at Prae, Sisaket and Korat as had been examined at Nan, the same variety of low order agglutinins (.19 etc) would have been ilicited.

(2) As with cattle, the hebdomadis group (borincana and wolffi), shows a significantly high % of agglutinins in all areas tested. L. hyos is very consistent, but not to the same degree as was evident in cattle.

(3) L. autumnalis agglutinins are thought to be consistent and significant, and probably do not represent L. pomona cross reactions (L. autumnalis has been isolated from a water buffalo). L. autumnalis agglutinins appear second in frequency to the hebdomadis group. Because no serology was conducted on buffalo from the Central Plain, and L. autumnalis has been isolated from one buffalo, it is impossible to conclude at this time whether the frequency of L. autumnalis is a species phenomenon or an environmental phenomenon.

(4) All other agglutinin responses listed here show nothing particularly striking, and not knowing the inciting organism, one can only speculate as to the epidemiology of these infections.

Table VI - Swine Agglutinin Rates/Area

	Nan	Prae	Sakol Nakorn	Korat	Bangkae	Nakorn Pathom	Rajiburi
# Tested:	60	88	30	83	122	171	45
% Reactors	0	0	0	0	17	7	33
L. andaman							
butembo							4.4
celledoni							
bataviae							20
pomona					14	5.2	
djasiman							
hyos							
autumnalis					2.4	.59	
ballum						.59	
canicola						.59	
ictero							

(Continued next page)

Table VI - Swine Agglutinin Rates/Area (Continuation)

	Nan	Prae	Sakol Nakorn	Korat	Bangkae	Nakorn Pathom	Rajiburi
pyrogenes							
alexi							4.4
grippo							
borincana							2.2
wolffi							
javanica					.8		
australis							

The following appears evident:

(1) The lack of agglutinin response in provinces in the North and North East has already been commented upon.

(2) L. autumnalis reactors listed under Bangkae should be considered L. pomona. This area represents one large swine farm in which exclusively L. pomona was repeatedly isolated from swine urine.

(3) The L. bataviae agglutinins listed for Rajiburi are considered significant, but misleading because of low numbers tested. Four leptospires have been isolated from 48 swine kidneys originating in Rajiburi; all have been grouped as L. pomona.

(4) This table, plus isolation data, indicates that L. pomona constitutes the predominant leptospiral swine infection (84-92%) which is more prevalent within the Central Plain area than without. As stated before, this is a probable reflection of swine husbandry practices.

(5) The lack of L. hyos agglutinins is noteworthy, as is the fact L. hyos has not been recovered from the many swine leptospires isolated to date.

(6) L. hyos is supposed to be a serotype second only to pomona in its ability to infect swine. It can be strongly inferred that L. hyos cattle and buffalo agglutinins are illicited by a serotype antigenically related to L. hyos, but none-the-less a different serotype non-infective for pigs.

(7) The dimunition of cattle and buffalo pomona agglutinins in the North, East and Northeast , relative to the Central Plain may be a direct reflection of the apparent decreased pomona swine infection for these areas.

Table VII Rodent Agglutinin Rates/Area

	Prae	Lumpang	Lampoon	Bangkae
# Tested	17	31	26	122
% Reactors	12	3	15	7
L. andaman				
butembo				
celledoni				
bataviae				
pomona				
djasiman				
hyos				
autumnalis				
ballum				
canicola				
ictero				
pyrogenes				
alexi				
grippo				
borincana				
wolffi				.8
javanica	12	3	15	6.5
australis				

The following appears evident:

(1) From the four provinces tested, variable percentages of rodents demonstrated leptospiral agglutinins. This may be a function of the low number of animals tested.

(2) From the four provinces tested, the exclusive infection was L. javanica, whose infection rate for livestock has never exceeded .66%. Although data is still meager, the inference is present (at least in these four provinces) that the predominant to exclusive serotype infecting the rodents of cultivated fields is of negligible infecting potential for livestock

Table VIII - Area Agglutinins Rates/Species

Korat

	Buffalo	Cattle	Swine
# Tested	101	92	83
% Reactors	30	22	0

(Continued next page

Table VIII - Area Agglutinins Rates/Species (Continuation)
Korat

	Buffalo	Cattle	Swine
L. andaman			
butembo			
celledoni			
bataviae	1.9		
pomona			
djasiman			
hyos	4.9	11.9	
autumnalis	8.9	1.1	
ballum			
canicola			
ictero	.99		
pyrogenes	2.9	1.1	
alexi	2.9	1.1	
grippo	.99		
borincana	.99		
wolffi	4.9	6.5	
javanica			
australis			

Table IX - Area Agglutinins Rates/Species

Sisaket

	Buffalo	Cattle
# Tested	43	36
% Reactors	16	3
L. andaman		
butembo		
celledoni		
bataviae		
pomona		
djasiman		
hyos		2.8
autumnalis	6.9	
ballum		
canicola		
ictero	2.3	
pyrogenes		
alexi		
grippo		
borincana	2.3	
wolffi	4.7	
javanica		
australis		

L. autumnalis agglutinins (thought not to represent prevalent cross-agglutinins) are 8 fold more prevalent in buffalo than cattle in Table VIII, 6.9 fold more prevalent in Table IX, and 15 fold more prevalent in Table XI. As stated, insufficient data has accumulated to indicate whether this reflects an enviromental difference, or species - serotype predilection.

Table X - Area Agglutinins Rates/Species

Prae

	Buffalo	Swine	Rodents
# Tested	142	88	17
% Reactors	19	0	12
L. andaman			
butembo	.71		
celledoni			
bataviae	2.1		
pomona	1.4		
djasiman			
hyos	2.1		
autumnalis	6.3		
ballum			
canicola			
ictero	.71		
grippo			
borincana	2.8		
wolffi	2.1		
javanica			
australis			12

It appears that the 12% javanica infection listed for rodents is probably high because of the small number of rodents tested. However, this exclusive rodent infection is not reflected at all in the buffalo or swine from the immediate regions.

Table XI - Area Agglutinin Rates/Species

Nan

	Buffalo	Cattle	Swine
# Tested	504	302	60
% Reactors	25	14	0
L. andaman			
butembo	.39	.66	

(Continued next page)

Table XI - Area Agglutinin Rates/Species (Continuation)

Nan

	Buffalo	Cattle	Swine
celledoni	.19	.33	
bataviae	.99	2.3	
pomona	.39	1.9	
djasiman		.33	
hyos	4.6	1.7	
autumnalis	4.8	.33	
ballum	.19		
canicola	.19		
ictero	1.6		
pyrogenes	.78		
alexi	1.2		
grippo	.39		
borincana	4.8	2.3	
wolffi	4.4	2.6	
javanica	.19	.66	
australis	.39	.33	

Summary: A qualitative and quantitative study for leptospiral agglutinins was conducted on 790 buffalo sera, 1,370 cattle sera, and 507 swine sera collected from 5 provinces in northern Thailand, 4 provinces in eastern Thailand and 5 provinces in the sub-Bangkok Central Plain. An attempt was made to note area and species differences. The following appears evident:

a. Leptospiral agglutinins manifested in random sampling of livestock are typically of a low order and seldom exceed 1:25 (buffalo and cattle) 1:100 swine.

b. Percent reactors in buffalo and cattle are essentially similar (± 22%) with a tendency to diminish as one moves away from the Central Plain.

c. From "positive" areas, agglutinins manifested by swine are typically less frequent than cattle and buffalo (less than 17%); particularly noteworthy is the very low evidence of swine infection (negative serology, 2% isolation) in areas outside the Central Plain.

d. Hebdomadis group and hyos agglutinins are so consistently illicited by cattle and buffalo from all areas, that one might consider them cattle and buffalo infections.

e. Although evidence of L. bataviae and L. canicola infections exist, L. pomona appears to constitute 84-92% of the swine infection. L. pomona in swine is very prevalent in the Central Plain region (17% serologically, 12% isolation) but diminishes to negligible importance beyond. (Negative serologically North, Northeast, -2% isolation).

f. The prevalence of L. pomona agglutinins in cattle and buffalo is surprisingly low in all areas tested (2.8% maximum infective rate) but this diminishes to negligible in the North and Northeast.

g. L. autumnalis agglutinins are negligible or absent in the Central Plain (no buffalo examined) but occur in buffalo second in frequency to only hebdomadis in areas of the North and Northeast. Buffalo illicit a 7-15 fold higher autumnalis agglutinin reaction than to cattle

h. Serological and isolation data on the leptospiral infections of rodents frequenting the grazing land of four provinces discloses a predominant if not exclusive L. javanica infection which is of negligible importance in livestock infections (.66 maximum infective rate - rattle).

Conclusion: Area and species differences were disclosed in prevailing livestock leptospiral agglutinins which, when compared with the small amount of livestock isolation data on hand offers a degree of clarification to the epidemiology of these infections. By inference, areas worthy of additional study have been pointed out to clarify the epidemiology of human infections. The following conclusions may be warranted or inferred:

a. The consistency and prominence of the hebdomadis group and L. hyos agglutinins in cattle and buffalo from all areas, in the presence of meager rodent data indicating the lack of these infections in rodents, indicate that the serotypes causing these agglutinins are "cattle" organisms. The ability of these serotypes to cause clinical disease in buffalo and cattle is not known, and the importance of these organisms in causing human infections and/or disease is not known. Further, inasmuch as hyos agglutinins have never been detected in swine, nor has hyos ever been isolated from the many swine isolates recovered to date; together with the fact that hyos is a serotype with a special predilection for swine kidneys second only to pomona, may indicate that agglutinins manifested by cattle and buffalo are illicited by an organism antigenically similar to L. hyos but otherwise different.

b. Serological evidence of L. pomona infection in cattle and buffalo is surprisingly low, but as one leaves the Central Plain agglutinins become almost negligible. This may be a direct reflection of the heavy L. pomona swine infection within the Central Plain, and very low pomona swine infection without the Central Plain. This in turn may be a direct reflection of the intensive swine husbandry practiced within the Central Plain enhancing swine to swine transmission, as opposed to the relatively free grazing practices employed without the Central Plain, discouraging swine to swine transmission. Because of the intensive swine infection within the Central Plain, the pomona

diagnostic antigen should be included in this area with others when attempti to serologically diagnose human leptospirosis.

c. L. autumnalis agglutinins are second in prevalence in buffalo in the north and northeast only to the hebdomadis group. Cattle from the same area show a relative lack of autumnalis agglutinins. The cattle and swine of the Central Plain area (no buffalo examined) showed no clear evidence of autumnalis infection. We have insufficient knowledge presently to determine whether this indicates a buffalo predilection for autumnalis infection (buffalo to buffalo transmission) or whether this is an abberant infection from another source (rodent) occuring significantly in buffalo because of this creature's constant immersion in water. In any event, it is speculated that human autumnalis infections would be of much more importance in the North and Northeast than in the Central Plain area.

d. The rodents of cultivated fields collected from one province near Bangkok and three in the distant north showed exclusive L. javanica infection. Javanica infection appears to be negligibly important in livestock. Although data is meager, the inference is clear that one of the most prominent rodent leptospiral infections is of negligible importance in livestock infections. The agglutinins manifested to the 13 remaining antigens (pomona, autumnalis hyos, hebdomadis and javanica excluded) were always relatively infrequent, occured sporadically, and their origin was unknown. Although serotypes grippotyphosa and canicola have been isolated from a rat and swine respectively, and L. javanica from a cat, and there is serological evidence of significant bataviae infection in dogs and swine; much more extensive epidemiological work must be done to determine where these serotypes fit in the cycle of livestock and human infections.