

ANNUAL PROGRESS REPORT

SEATO Medic Study No. 82: Swine Abortion Syndrome

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
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, Cert. Vet Med **

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

* Bacteriology Branch (Research and Education Division)

** Immunology and Serology Branch (Department of Livestock Development)

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The objective of this study is to detect the presence and significance of three infectious disease agents on one large commercial swine farm near Bangkok in which the principle overt clinical manifestation encountered was abortion. The infectious disease agents studied were Leptospira sp., Japanese B. encephalitis virus, and Brucella sp. Leptospirosis, Japanese

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B. Encephalitis and Brucellosis are major diseases of swine in which the principle clinical manifestation in the female is abortion. One large swine herd was selected for study having an abortion rate of 30%. Brucella sp. agglutinins were present in 91% of those examined and Br. suis was isolated from three aborting sows. All adult animals tested revealed antibodies to JBE or related viruses. Maternal antibodies were evident in a high percentage of the young. Significant leptospiral agglutinins were present in 17% of the animals tested and were exclusively L. pomona. This organism was repeatedly isolated from the urine collected. Serological and isolation procedures demonstrated the presence of L. javanica in rodents trapped in the area. Negative results were obtained following attempts to isolate leptospire from the canal water.

Two (2) of twenty three (23) farm workmen demonstrated serological evidence of Brucella, and all revealed high order JBE agglutinins. Brucella suis was the primary cause of the abortions occurring on this farm. Japanese B. Encephalitis virus was widely disseminated and active immunity was developed prior to maturity, establishing resistance to abortion from this organism. The swine were heavily infected with L. pomona and the rodents with L. javanica; no cross infection was evident. In the presence of an overwhelming Brucella sp. infection it is difficult to incriminate JBE virus or leptospire with specific abortions.

BODY OF REPORT

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Final Report

Objective: The object of this study was to detect the presence and significance of three infectious disease agents at one large commercial swine near Bangkok in which the principle manifestation encountered was abortion. The infectious disease agents under study was Leptospira sp., Japanese B. Encephalitis virus, and Brucella sp. These three disease agents cause

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serious diseases of swine whose principle overt clinical manifestation in the female animal is that of abortion.

Description: The Virus Department, SEATO Medical Research Laboratory, had obtained considerable data indicating the widespread occurrence of antibodies in domestic animals to the virus of JBE. Work in Japan has indicated that swine are one of the principle reservoirs of the virus. Previous work conducted by the Research and Education Division of the Department of Livestock Development in 1954 indicated the widespread occurrence of brucellosis in some large swine herds in Thailand. Leptospiral investigations in Thailand have been limited in quantity and directed wholly to human and rodent infections.

The commercial swine farm under study is located about 20km. southwest of Bangkok, in a semi-rural area surrounded on all sides by banana plantations, coconut, mango, and betel nut groves. Many canal systems, navigable by small poled boat or canoe lace through the farm property and constitute the only means of contact between different parts of the farm. Thick undergrowth approaches the canal edge on either side, and the tree canopy is impervious to most sunlight overhead. Canal water is always muddy and covered with plant growth. Small rodents and tree shrews are abundant in the area, and rodents are quite commonly seen eating out of the swine mangers at night.

The farm is about 2-3 years old. A large percentage of the original stock was of foreign origin, Duroc Jersey and Australian Yorkshire. About 30% of the present animals are cross-breds from these originals. The farm numbers about 2,000 animals, 30 breeding boars, about 150 breeding sows, the balance feeder pigs. The abortion rate at the time of study averaged about 30% , most taking place at $1\frac{1}{2}$ -2 months of pregnancy. Farm records were not sufficiently accurate to determine conception rates. Eight adult boars were noticed to have abnormally swollen testicles. Some of these animals were feverish and off-feed.

Part A: Leptospiral Epidemiology

Leptospire are known to be able to survive for long periods in relatively clear fresh water of about neutral pH. Leptospire would be contributed to the water by any indigenous animal shedding infected urine directly or indirectly (by rain washing) into the water. An attempt was made to recover leptospire from the canal water coursing through this swine farm. Surface water samples were collected from two sites of effluent drainage (part of a canal system) from the swine farm. These were concentrated to 5cc aliquotes by Seitz filtration and inoculated lcc into each of 5

weanling hamsters. Inoculated hamsters dying 6-21 days post-inoculation were considered potentially "positive" for leptospirosis and were cultured. Hamsters dying prior to 6 days post-inoculation were considered bacteriologically contaminated and were discarded. Hamsters apparently normal at 21 days were discarded as being "negative" for leptospirosis. Six tubes of semi-solid Fletcher's media containing rabbit serum were inoculated from each suspect hamster. In an aseptic manner plugs were removed from the kidney using Pasteur pipetes - 3 tubes containing 8, 12, and 16% rabbit serum respectively were inoculated. In an aseptic manner, slices were removed from the kidney cortex and ground in a Tenbroek grinder with sufficient liquid Stuart's medium to make a 10% suspension. One drop from this suspension was inoculated into each of three tubes of Fletcher's medium containing 8, 12 and 20% rabbit serum respectively.

Rodent traps were placed in the swine houses and groves surrounding the farm. Although more traps were placed in the surrounding groves, about an equal number of animals were caught from both locations. Rodents were cultured as described for hamsters.

Urine samples from approximately 15 swine were collected and pooled into a single bottle. One (1) cc of this pooled urine was inoculated intraperitoneally into each of five weanling hamsters. Hamsters were cultured as previously described. Twenty (20) pooled samples were collected from different swine houses and inoculated into 100 hamsters. This work was an attempt to recover the range of leptospiral serotypes possibly being shed by the entire herd.

In an attempt to correlate isolate recovered with specific serum, urine samples were collected from individual swine whose serum was on hand. Urine collected from each pig was inoculated 1cc into each of five (5) weanling hamsters. Samples from 86 swine were inoculated into 172 hamsters. Inoculated hamsters were cultured as previously described.

In an attempt to demonstrate the leptospiral agglutinin spectrum manifested by the entire herd, 122 swine were selected at random and serologically examined.

In an attempt to demonstrate relationship of leptospiral infection with abortion, urine samples were collected from eight (8) swine subsequent to, and during the same week they had aborted.

On 28 August, the 23 workmen at the farm were bled to detect agglutinins to leptospire (as well as to the other disease agents under study). When cleaning the swine pens, these workmen did so mostly in bare feet, exposing themselves to leptospiral infections via the cutaneous route - if this were to occur.

Part B: Brucella Study

Swine abortions were collected for a four month period and returned to the laboratory for Brucella sp. culturing. Fifteen (15) abortions occurred during this period, and seven of these were subjected to Brucella sp. culturing. The other abortions either occurred at night, or were so grossly contaminated that cultures were not attempted. Culture methods were as follows:

1. 5-10 ml of fetal heart blood, and 2 ml of fetal stomach contents were inoculated into 50 ml each of trypticase soy broth.

2. Broth cultures were incubated at 37°C under CO₂ tension for 4-6 weeks.

3. Every four (4) days, the broth cultures were Gram stained and cultured as follows:

Gram + organisms - streaked onto trypticase soy agar with .1% dextrose and 1:1,000 crystal violet

Gram - organisms only - streaked onto trypticase soy agar with .1% dextrose. These cultures were incubated under CO₂ tension at 37°C

4. Colonies on trypticase soy agar were examined for suggestive morphology and Gram stained.

5. Typical colonies were subcultured onto trypticase soy agar slants for pure culture. If suggestive of Brucella sp. they were identified as follows:

6. From each of the above suggestive cultures, two sets of Blood Agar and Trypticase Soy Agar plates were streaked. One set of plates was incubated in a candle jar under CO₂ tension for Brucella abortus while the other set was incubated ordinarily for other Brucella species. On primary incubation, the colonies required from 72 to 96 hours to develop.

7. Gram stains were made, and if the organisms looked like Brucella morphologically, they were inoculated into urease tubes to verify. For accelerating the decomposition of urea, urease medium tubes were incubated in a water bath at 37°C instead of an incubator. Brucella suis is a fast urea decomposer, normally within an hour or slightly more. Brucella abortus is much slower.

8. Slide agglutination tests were conducted, utilizing Brucella abortus, suis and melitensis specific anti-serum.

No further diagnostic or differentiating tests were conducted (such as Basic Fuchsin or Thionin tests).

In an attempt to demonstrate the herd *Brucella* agglutinin response, 106 swine were selected at random and examined serologically. The method used was the rapid plate agglutination test as described by Huddleson. The antigen used was the *Brucella abortus* antigen produced and maintained in the Research & Education Division of the Department of Livestock Development. Serial batches of this antigen were standardized against the U.S.D.A. *Brucella abortus* strain 1119-3, or the Lederle *Brucella abortus* antigen.

On 28 August, the 23 workmen at the farm were bled to detect agglutinins to *Brucella*. In caring for aborted sows, these workmen usually handled the fetal membranes with their bare hands, even though they had been cautioned against this. Certainly, prior to this investigation, animal disease husbandry practices were always conducted with no regard to personal safety. Blood cultures for the isolation of *Brucella* sp. were also conducted on these workmen at this time according to the following techniques:

1. 5 ml of fresh whole blood was added to sealed vaccine bottles containing 100 ml of Trypticase Soy Broth supplemented by 0.1% NaCl and 0.1% Na citrate. Incubation was at 37°C.

2. This broth was Gram stained and sub-cultured to blood agar plates at 3, 7, 14, 21 and 28 days after inoculation. Incubation was carried out at 37°C in an atmosphere of CO₂ or/and under aerobic conditions.

Part C: Japanese B. Encephalitis Virus

In order to detect the presence of the JBE virus (or related virus), 88 pigs were bled in June 1962 from different houses and pens on the swine farm. Twenty (20) of these pigs were adults (9-12 months old) and 68 of these were gilts and shoats ranging from 1-3 months of age. Although no attempt was made to individualize these animals, 34 of these same animals were again bled and serologically examined in September 1962 to detect differences in agglutinin response from the first bleeding. The serological procedure conducted was the hemagglutination-inhibition test as used by the Virus Department, SEATO Medical Research Laboratory. JBE, H-I values represent 2 fold serum dilutions starting at the 1:10 serum dilution.

Specimens from aborted sows #211, 219, 227 and 6920 were set aside for Japanese B. encephalitis virus isolation attempts. In as sterile a manner as possible, portions of fetal brain, liver, spleen, lung, kidney and placenta were placed each into a sterile vial. These were immediately frozen at -20°F

and sent to the Virus Department SEATO Medical Research Laboratory for virus recovery. These specimens were stored at -70°C until inoculated into suckling mice. 10% tissue suspensions were inoculated 0.01 ml intra-cranially and 0.02 ml intra-peritoneally into 2 litters of 1 day old mice. Litters were passaged at 10 days intervals to the third mouse passage, observed for 21 days and discarded.

On 28 August, 1963, the 23 workmen at the farm were bled and serologically examined to detect agglutinins to the JBE virus (or related agent). The test conducted was the hemagglutination-inhibition test as used by the Virus Department of the SEATO Medical Research Laboratory. JBE H-I values listed represent 2 fold serum dilution, starting at the 1:10 serum dilution.

Progress:

Part A: Leptospiral Epidemiology

Results of canal water sampling were as follows:

Table 1:

# Samples	1st pass hamsters	Die 1-5 days	Die 6-21 days	Cultured	Results
69	345	11	12	9	Negative

2nd pass	Die 1-5 days	Die 6-21 days	Cultured	Results
2	1	1	1	Negative

Water pH averaged 7.16

Rainy Season:

Surface water samples were collected from the same sites as Part A during and within 3 hours after heavy rain. Samples were processed as in Part A. Nineteen (19) water samples were collected from 13 June, - 19 August 1963 and inoculated into 95 hamsters.

Table 2:

Hamsters inoc.	Die 6-21 days	Dkfld	Cultured	2nd pass	Die 6-21 days	Cultured
95	10	-9, sus 1	10-	10	1	1, all neg.

The only animal to die on second passage was that designated suspect from first passage; kidneys were darkfield negative and cultures were negative.

Results of rodent trapping were as follows: Table 3:

Rodent sera tested:	115
Significant titers:	
Javanica	8
Wolffi	1
Rodent kidneys cultured	148
Isolates recovered (all <u>L. javanica</u>)	9
% of significant titers	
% of isolates recovered	

75% leptospiral agglutinations on 1:10 serum dilutions or higher were considered significant titers for rodent sera. From the 9 rodents from which isolates were recovered, only 2 developed titers to L. javanica and others were negative. Reciprocally, of the 8 animals developing significant titers to L. javanica only 2 yielded an isolate.

Results of pooled urine samples were as follows:

Nineteen leptospire had been isolated from 8 pooled urine samples as follows: Table 4:

Can #	Hamster #	Die 4-21 days	Sacrificed 21 days	Group
4	16	dkfld-		L. pomona
	19	dkfld-		L. pomona
5	24	dkfld-		L. pomona
	25	dkfld-		L. pomona
7	34	dkfld-		L. pomona
	35	dkfld-		L. pomona
9	41	dkfld-		L. pomona
	42	dkfld-		L. pomona
	44	dkfld-		L. pomona
10	46	dkfld-		L. pomona
11	51		dkfld+	L. pomona
	52		dkfld+	L. pomona
	53		dkfld+	L. pomona
	54		dkfld+	L. pomona
	55		dkfld+	L. pomona

(Continued next page)

Table 4 (Continuation)

Can #	Hamster #	Die 4-21 days	Sacrificed 21 days	Group
12	59		dkfld+	L. pomona
20	96	dkfld-		L. pomona
	98		dkfld+	L. pomona
	99		dkfld+	L. pomona

Sacrificed hamsters which were darkfield negative were not cultured.

Results of individual swine urine samples were as follows:

Isolates have been recovered from 4 of the 86 samples. All hamsters surviving 21 days were cultured as previously described.

1. Hamsters dead 4-21 days -12 (representing 6 samples)
L. pomona recovered from 2 samples (both dkfld -) LW 42
DJ 349
2. 2 samples (4 hamsters sacrificed at 21 days were darkfield+ LW 8025
L. pomona recovered from both. LW 8077

Table 5:

Sow #	Baseline serological results	Urine-hamster inoculation	Subsequent serology
LW 42	- 26 July	5 August <u>L. pomona</u>	+ 4 Sept - 3 Dec pomona pomona
LW 8025	- 1 August	6 August <u>L. pomona</u>	+ 4 Sept + 17 Sept - 3 Dec pomona pomona pomona
DJ 349	- 26 July +	20 August <u>L. pomona</u>	- Dec pomona
LW 8077		6 August <u>L. pomona</u>	+ 4 Sept + 17 Sept (sold out of herd) pomona pomona

All serum reactions are at the 1:100 dilution.

+ = 75% + agglutination

+ = 50% + agglutination

- = 25% + agglutination

- = less than 25% agglutination

Results of herd leptospiral agglutinins were as follows:

During the course of this study, 122 swine from this farm were bled, and their sera checked for significant leptospiral agglutinins. Results were as follows:

Table 6:

	Serum dilutions		Total	% reactors
	1:100	1:400		
L. pomona	8	9	17	17
L. autumnalis	1	2	3	
L. javanica	1		1	

No leptospiral agglutinins were demonstrated in any of the 23 workmen serologically examined. No leptospire were isolated from urine of 8 aborted sows when urine was examined within the week following abortion.

Part B: Brucella Study

From seven culture attempts to recover Brucella sp. from aborted sow specimens Brucella suis was isolated from three sows. These cultures decomposed urea within 20 minutes, and agglutinated specifically with the Brucella suis specific anti-serum.

The 106 swine selected at random and examined for Brucella agglutinins exhibited a 91.5% reactor rate. Ninety-seven (97) of these swine were reactors, fifty-three (53) at the 1:400+ serum dilution. Thirteen (13) out of 15 aborted sows demonstrated Brucella agglutinins subsequent to abortion, two of these with ascending titers. The following three tables outline human and swine cultural and serological results: (See 3 following tables), tables 7, 8 and 9.

Commercial Swine Farm Abortions
20 June 1963 - 10 September 1963

Table 8

Swine #	aborted	Culture attempts	Leptospirosis	Leptospirosis
			1st bleeding	2nd bleeding
Crossbred #8	9 Sept 63	not done	26 July	neg.
37	5 June 63	not done	26 July	not bled
* DJ 190	12 June 63	neg.	26 July	23 Aug pomona 1:400 autumnalis 1:100
LW 194	Between 20 June & 20 Sept 63	not done	26 July	1:100 javonica
DJ 199	6 Aug 63	not done	1 Aug	14 Aug neg. neg. neg.
* 211	17 July 63	neg.	26 July	not bled
			26 July	1:400 pomona autumnalis 1:100
* DJ 219	17 July 63	neg.	1 Aug	23 Aug pomona 1:100 autumnalis 1:100
226	29 May 63	not done	26 July	4 Sept neg.
* 227	24 June 63	neg.	26 July	neg.
* DJ 271	25 July 63	neg.	26 July	23 Aug neg.
DJ 330	between 20 June & 20 Sept 63	not done	26 July	neg. neg.
			26 July	1:100 autumnalis 1:100
* DJ 394	20 June 63	neg.	26 July	23 Aug pomona 1:100
429	end of July	not done	26 July	neg.
* 6920	22 June 63	neg.	26 July	not bled
8032	1 Sept 63	not done	7 Sept	neg.

Table 9
Human Serology and Cultures

Specimen # 23 August	Name:	Age:	Started work	Serology		JBE H-I
				Leptospirosis	Brucella	
3	PK	24	May 63	-	-	8
4	BD	23	May 63	-	-	10
5	SS	20	July 63	-	-	9
6	SW	19	July 63	-	-	8
7	CW	21	Mar 63	-	-	7
8	PD	23	Aug 63	-	-	8
9	BL	23	July 63	-	-	9
10	SB	21	May 63	-	-	10
11	SC	16	July 63	-	-	7
12	PK	22	April 63	-	-	5
13	UT	19	July 63	-	-	6
14	IS	22	July 63	-	-	9
15	UC	14	July 63	-	-	7
16	BS	17	July 63	-	-	6
17	MS	30	2 yrs	-	-	5
18	UO	17	2 yrs	- 21 Jan 4 Mar	1:100 1:400	4
19	SU	22	-	-	-	6
20	PR	46	May 63	-	-	10
21	SD	16	July 63	-	-	5
22	MP	20	July 63	-	-	7
23	LJ	21	June 63	-	-	5
24	LP	28	April 62	-	1:400	10
25	PP	18	April 63	-	-	

Blood cultures conducted on these 23 workmen on 23 August were negative for Brucella sp recovery.

Individual # 18 presented herself to the SEATO Medical Research Laboratory on 21 January as a patient suspected of brucellosis. She complained of chronic low-grade fever, chills, headache, and loss of weight. Serum titers are recorded, blood cultures attempted on 21 January were negative for Brucella sp. She was treated for brucellosis and recovered uneventfully. This individual also informed us that individual # 24 was also sick in the same manner at this time. He was not seen at the SEATO Medical Research Laboratory, but was treated with broad spectrum antibiotics and presumably recovered.

Part C: Japanese B. Encephalitis Virus

Of 33 pigs bled in June 1962, all 20 adults (9-12 months old) had H-I antibodies to JBE or related viruses. Young gilts and shoats had high percentages of JBE antibody with a tendency to lose antibody with increasing age (Table 10). When some of these piglets were bled again in September 1962 (Table 12), gilts and shoats in House 1, pens 1 and 2 had lost H-I antibody for JBE virus while virus transmission for Group B agents was occurring in House 1, pen 3 (manifested by acquisition of high H-I titers and increase in number of positives). Thus, during the summer of 1962, patchy virus transmission of Group B arthropod borne agents was occurring. Apparently, 9-12 months residence at this swine farm was sufficient for all or nearly all pigs to become infected with JBE (or related) virus. Whether any sows become pregnant without having had a JBE virus infection early in life is problematical. On the basis of evidence obtained by the Virus Department, SEATO Medical Research Laboratory that JBE virus transmission occurs maximally during the early rainy season in the Bangkok vicinity, it seems likely that some yearling sows pregnant at that time of the year might still be susceptible and acquire a JBE infection. Abortions attributable to these infections should occur between May and August. The following tables 10 and 11 illustrate these findings:

Table 10:

JBE H-I Antibody in Swine Study Farm Pigs June 1962

Age Group:	JBE <u>No. Pos./No. sampled</u>
9-12 months	
1 month	
2 months	21/34
3 months	

Table 11:

JBE H-I Antibody in Gilts and Shoats in 3 Pens in Swine Study Farm, June and September 1962

Pen Number:	JBE	
	<u>June</u>	<u>September</u>
	9/9	0/9
	4/4	0/4
	12/19	15/21

All of the 23 swine farm workmen bled on 28 August 1963 demonstrated high-order H-I agglutinins to the virus of JBE, or related virus (refer to last column of Table 9).

Attempts to isolate the virus of JBE from specimens from aborted sows numbering 211, 227, 219 and 6920 were all negative.

Summary:

Part A: Leptospiral Epidemiology

Results of canal water sampling were as follows:

During the dry season (November 62 - March 63) sixty-nine water samples were passed through 345 weanling hamsters. Twelve hamsters died during the desired time interval, nine hamsters were cultured, all were negative for leptospires. During the rainy season (June - August 1963) nineteen water samples were collected during or within 3 hours after heavy rain. Samples were passed into 95 weanling hamsters; ten hamsters died during the desired time interval, all were negative for leptospires.

148 rodents were trapped from in and around the swine houses and groves. Nine isolates (all *L. javanica*) were recovered - isolation % 6.1. One hundred fifteen of these rodents examined serologically exhibited a 6.9% agglutinin rate, almost exclusively *L. javanica*.

Freshly voided "clean" urine from groups of apparently normal swine (15/group) was inoculated into weanling hamsters. Twenty pooled samples collected from different swine houses resulted in *L. pomona* recovery from 8 samples (40% recovery rate).

Individual urine samples from 86 apparently normal swine were passed through 172 weanling hamsters. L. pomona was recovered from urine of 4 sows, isolation % 4.6. At the point of leptospiruria, none of the 4 sows demonstrated significant leptospiral agglutinins.

122 apparently normal swine, less than 1 year of age were examined for leptospiral agglutinins. 17% of these exhibited significant agglutinins, 17 L. pomona exclusively, 3 L. autumnalis exclusively and 1 L. javanica.

Urine from 8 aborted sows was passed through weanling hamsters the week of the abortion for leptospiral recovery with negative results. The twenty-three workmen examined on 23 August 1963 demonstrated no leptospiral agglutinins.

Part B: Brucella Study

Fifteen swine abortions occurred during a 4 month period. Specimens from 7 aborted sows were culturally examined for Brucella sp; Brucella suis was isolated from 3 of these specimens. Thirteen out of fifteen aborted sows demonstrated Brucella agglutinins subsequent to abortion, two of these with ascending titers.

106 Apparently normal swine were examined for Brucella agglutinins. Ninety-seven of these were reactors (91.5%) fifty-three at the 1:400+ serum dilution.

On 23 August 1963, 23 workmen were examined for evidence of Brucella agglutinins. Two individuals demonstrated significant Brucella species agglutinins, one of these to an ascending degree during the course of a disease clinically consistent with brucellosis.

Part C: Japanese B. Encephalitis Virus

In June 1963, a group of sows and young piglets (1-3 months old) were bled for evidence of JBE hemagglutinins. The 20 sows elicited hamagglutinins of a high order to JBE. The 52 young gilts and shoats elicited hemagglutinins of a low order (maternal antibody). In September 1962, 32 of this same group of pigs were examined for JBE agglutinins. Most of the pigs had lost all of their low order agglutinins. Some of the pigs converted to high order agglutinins.

All of the young adult pigs and sows examined in July 1963 elicited high order JBE agglutinins. The twenty-three workmen examined on 23 August 1963 all demonstrated high order JBE hemagglutinins. Specimens from

4 aborted sows were culturally examined (suckling mouse passage) for presence of JBE virus with negative results.

Conclusions:

Part A: Leptospiral Epidemiology

It can be stated with reasonable assurance that the indigenous rodents at the study farm moderately contributed L. javanica (perhaps exclusively) and the swine at the study farm heavily contributed L. pomona to the fresh water canals coursing through the farm. Although the water pH was favourable for leptospiral maintenance (avg. pH 7.16) none were recovered from this water. It must be assumed that the canals, always muddy and covered with plant growth, were inimicable for leptospiral survival.

Because the swine gave almost exclusively evidence of L. pomona infection, and the rodents of L. javanica infection, and knowing that many rodents frequented the swine mangers at night, it can be assumed that L. pomona is relatively non-infective for these particular rodents, and L. javanica is relatively non-pathogenic for swine. In this particular study the humans gave evidence of no leptospiral infections, indicating that these two serotypes under these particular circumstances, were relatively non-pathogenic for humans. Because of the overwhelming Brucella infection, it became impossible to incriminate leptospires in the abortion syndrome.

There was no positive correlation between rodents with agglutinin response and rodents from which isolates were recovered; only two animals demonstrated homologous agglutinins. Reciprocally of the eight rodents demonstrating agglutinins, only two yielded a homologous isolate. Speculating on the parasitic course (rather than the acute clinical course) of this infection in rodents, it is perhaps true that serum agglutinins are illicit secondary to long and heavy kidney infection. The development of high order serum agglutinins may in turn rid the kidney of the infection. There is insufficient experimental data to support this hypothesis, but the possibility is enticing.

In the framework of the agglutination criteria establishing a reactor, it is significant that random serology of this heavily infected herd disclosed no reactors higher than the 1:400 serum dilution. This may indicate that the microscopic agglutination-lysis test as used lacks sensitivity, or that swine do not typically develop high order agglutinins subsequent to L. pomona infections. Further, from the 4 animals from which isolates and serum was available, some striking features are apparent. In two animals, serum agglutinins were not present at the point of leptospiruria. Low

order serum agglutinins \pm + @ 1:100 dilution were detectable $1\frac{1}{2}$ months subsequent to detectable leptospiruria, and then disappeared 4 months subsequent to leptospiruria. This data confirms past experience that swine leptospiral serum agglutinins fall off rapidly after infection. This data implies that leptospiral serum agglutinins in swine are typically of low magnitude. This data further implies that the highest order of serum agglutinins are not present at the time of leptospiruria, but develop from that point. Knowing that L. pomona is a serotype well adapted to the swine kidney, one can speculate on the parasitic rather than clinical nature of this infection in swine. Further transmission studies, mimicing natural infection, must be done before any conclusions on L. pomona pathogenesis in swine are warranted.

Part B: Brucella Study

The data indicates that Brucella suis was the primary cause of swine abortion on this farm. In the presence of an overwhelming Brucella sp infection, it becomes extremely difficult, if not impossible, to incriminate JBE virus or leptospiruria with specific abortions.

Part C: Japanese B. Encephalitis Virus

Evidence of JBE virus conversion of pigs during the rainy season of 1962 on the study farm, plus additional data collected by the Virus Department, SEATO Medical Research Laboratory from a consecutive study of birds (maximal antibody acquisition May-July) definitely points to a marked seasonal transmission of JBE virus in and near Bangkok during the early rainy season. Thus, a situation paralleling that in Japan and Taiwan is evident. Pigs born during the rainy season and protected by maternal antibody probably remain susceptible throughout the cool and dry season. Those pigs bred in February and March and 2-3 months pregnant in May and June might well be infected with JBE virus. Unfortunately, no viral recoveries were made from fetal parts submitted during the abortion study, and the role of the JBE virus in this study farm abortion syndrome must remain speculative.