

SEROLOGICAL SURVEY OF PIGS FROM A SLAUGHTERHOUSE IN JAKARTA, INDONESIA

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Abstract

Maksud dan tujuan dari survey ini ialah untuk mempelajari akan kemungkinannya hewan-hewan babi memegang peranan sebagai reservoir ataupun amplifier dari penyakit-penyakit zoonotic di Pulau Jawa. Survey ini dijalankan ber-sama-sama dengan Namru-2 di Jakarta.

Sebagian besar specimens yang berupa darah, berasal dari babi-babi yang akan dipotong dirumah pemotongan hewan babi di Jakarta Barat. babi-babi tersebut ada yang berasal dari Jawa Tengah, Jawa Barat, serta sebagian lagi berasal dari babi-babi milik rakyat di daerah Kapok.

Pengambilan specimens dilakukan sehari sebelum babi-babi tadi dipotong. Setiap pagi lebih kurang 150 ekor babi dipotong, dimana umurnya berkisar antara enam hingga 24 bulan.

Pada survey ini telah dikumpulkan 399 specimens, sebanyak 227 specimens berasal dari babi-babi betina, 159 specimens berasal dari babi-babi jantan, serta 13 specimens berasal dari babi-babi jantan kebiri.

Hasil Pemeriksaan Laboratorium.

1. Pemeriksaan terhadap *Toxoplasma*.

Dari 166 sera yang berasal dari babi-babi Jawa Barat sebanyak 46 (28 percent) menunjukkan hasil positif (titer 1 : 8 atau lebih) Sedangkan 235 sera berasal dari babi-babi Jawa Tengah, hanya sebanyak 17 (7 percent) yang menunjukkan hasil positif (titer 1 : 8).

Titer yang lebih tinggi (1 : 1024) juga ditemukan pada babi-babi yang berasal dari Jawa Barat.

2. Pemeriksaan terhadap *Brucella suis*.

Titer 1 : 320 masih diketemukan pada semua group, akan tetapi sebagian besar serum menunjukkan hasil negatif.

Sebagian kecil sera yang berasal dari babi-babi tua dari Jawa-Barat menunjukkan adanya antibody yang lebih tinggi dari pada babi-babi muda.

3. Pemeriksaan terhadap penyakit Japanese Encephalitis. Pada semua golongan umur dari geographical-group dari babi-babi menunjukkan adanya anti-body terhadap J.E. Sera yang negatif lebih banyak berasal dari babi-babi Jawa Tengah.

4. Pemeriksaan terhadap penyakit Influenza. Titer yang menyolok terhadap penyakit Influenza A2 Hongkong terdapat pada babi-babi golongan muda maupun tua baik yang berasal dari Jawa Tengah maupun yang berasal dari Jawa-Barat.

Meskipun demikian, babi-babi dari Jawa Barat lebih banyak menunjukkan hasil yang positif dari pada berasal dari Jawa Tengah.

5. Pemeriksaan terhadap *Leptospira*.

Kebanyakan serum menunjukkan adanya anti-body terhadap *L. sentot* *L. pomona* dan *L. bangkinang*.

Sebagian besar serum yang positif berasal dari babi-babi Jawa Tengah.

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INTRODUCTION

This study was designed to learn whether domestic pigs are involved as reservoirs or as amplifying hosts of zoonotic diseases in Java. Scientific literature on the subject is meager (Ressang et al., 1959), so it was desirable to obtain information on as many diseases and from as many areas as possible. Therefore, a slaughterhouse for pigs in Jakarta which receives animals from many parts of Java was selected as the source for specimens, and specimens were limited to blood sera inasmuch as serological analyses yield maximum data with minimum laboratory manipulation.

Regrettably, technical shortcomings prevented the complete serological examinations desired for each specimen. Nevertheless, examinations were completed for antibodies against the agents of leptospirosis, Japanese encephalitis (JE), toxoplasmosis, influenza, and brucellosis, and are reported here in.

MATERIALS AND METHODS

Most specimens were from the Kapok slaughterhouse situated in the midst of a pig-raising area on the western border of Jakarta City, but a few were obtained from nearby farms. Early every morning approximately 50 pigs aged 6-24 months are slaughtered. Pigs are from other parts of West Java, or from Central Java; and are held at Kapok for one day before slaughter. Pigs from W. Java are "Tangerang pure breed", those from C. Java "Mixed breed"

Pigs were aged by checking tooth eruption patterns at the time of bleeding and by checking slaughterhouse records. The latter were also.

Antibodies to influenza were determined by the haemagglutination inhibition (HI) test (HSIUNG, 1964). Antigen was from the A2/Hong Kong/68 strain in embryonated eggs. All sera were treated by the kaolin method of Spence (1960) and the trypsin and periodate method of Sampai and Isaacs (1953). Titers of 1:40 were considered as positive.

Leptospirosis studies were according to Galton et al (1962). Sera were inactivated in a water bath at 56°C for 30 minutes and screened undiluted using the macro slide agglutination test with the following antigen pools:

- Pool 1 : *L. ballum*, *L. canicola*, *L. copenhageni*
- Pool 2 : *L. bataviae*, *L. Grippotyphosa*, *L. pyrogenes*
- Pool 3 : *L. autumnalis*, *L. pomona*, *L. wolffi*
- Pool 4 : *L. australis*, *L. tarassovi*, *L. georgia*
- Pool 5 : *L. javanica*, *L. cynopteri*, *L. celledoni*.

Pools 1-4 were from the National Communicable Disease Center, Atlanta, Georgia, and Pool 5 from Difco laboratories, Detroit, Michigan.

Undiluted sera showing positive reaction against any antigen pool were re-tested using the microscopic agglutination test. Two-fold serial dilutions of sera (starting at 1:50) were tested for agglutination against the following 29 live leptospire:

L. ballum, *canicola*, *copenhageni*, *bataviae*, *benjamin*, *schueffneri*, *grippotyphosa*, *pyrogenes*, *manilae*, *autumnalis*, *pomona*, *hebdomadis*, *australis*, *tarassovi*, *wolfii*, *javanica*, *cynopteri*, *djasimana*, *andamana*, *butembo*, *patoc I*, *sejroe*, *medanensis*, *poi*, *sentot*, *bangkinang*, *celledoni*, *semaranga* and *sao paulo*.

Studied to learn the exact origin of pigs. Sex was recorded. With the possible, occasional, exception of brucellosis, pigs were not vaccinated against any of the diseases tested here.

Daily, from 5. to 30 July 1971, a team consisting of a veterinarian (CK) and three assistants drew blood from about 40 pigs awaiting slaughter. Syringes or vacutainers were used for venipuncture of an external auricular vein. Blood was refrigerated in wet ice, returned to the NAMRU-2 Jakarta Detachment laboratories, and serum separated and stored at -40°C without preservative until analysis either in Jakarta or at the NAMRU-2 Serology laboratory in Taipei, Taiwan.

Toxoplasma antibodies were measured after sera were inactivated at 56°C for 30 minutes using a microtiter* adaption of the indirect hemagglutination (IHA) test of Lewis and Kessel (1961). The antigen was prepared at the NAMRU-2 laboratories in Taipei from the RH strain of *Toxoplasma* after the method of Jacobs and Lundi (1957). Sera were also checked by the macroscopic tube agglutination test using commercial *Brucella suis* antigen.

Japanese encephalitis (JE) antibody titers were determined by the micro-method Haemagglutination - inhibition (HI) test described by Sever (1962). The Nakayama strain of JE virus was the source of antigen and were prepared by the sucrose-acetone method of Clarke and Casals (1958) and Kaolin extraction was used to remove non-specific inhibitors.

* Microtiter system, Cooke Engineering Co., Alexandria, Virginia, U.S.A.

RESULTS

Sera from 399 pigs were obtained. Two hundred twenty seven of the pigs were females, and 13 castrated males. Sex was disregarded in the figures and tables since there were no obvious differences in results by sex.

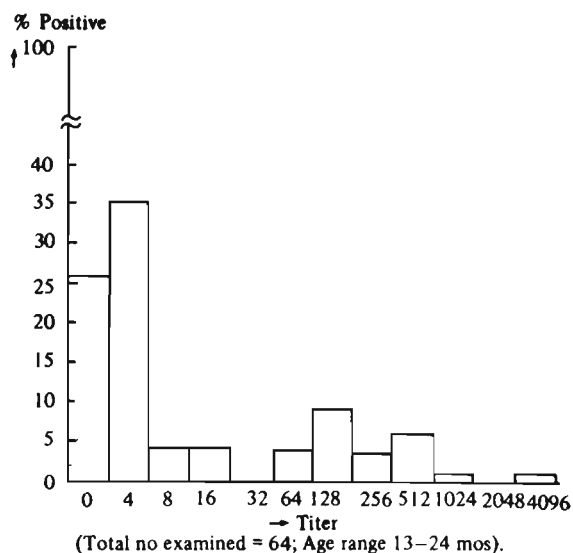
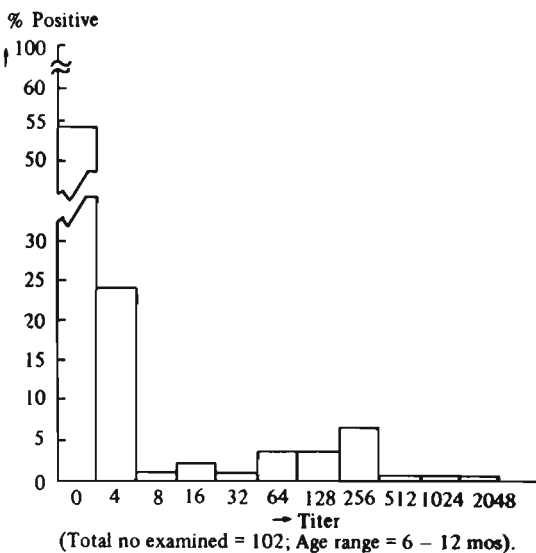
Central Java only 17 (7%) were considered positive (= 1:8). A higher prevalence of high titers (= 1:1024) was also noted in the West Java pigs and are probably associated with higher overall prevalence of infection.

Results of serological analyses are shown in Figures 1-4. Note that specimens have been separated into two age groupings - from pigs 6-12 months of age, and from pigs 13-24 months old. Although sera were also obtained from some pigs raised in East Java, these were too few to include in the study, so that only West and Central Java are here considered. For each figure, horizontal axes refer to reciprocal titers (thus 40 refers to a positive reaction at a serum dilution of 1:40). The vertical axes list positives as percentage of total number examined rather than as absolute number of positives, permitting comparison of data from both areas and age groups.

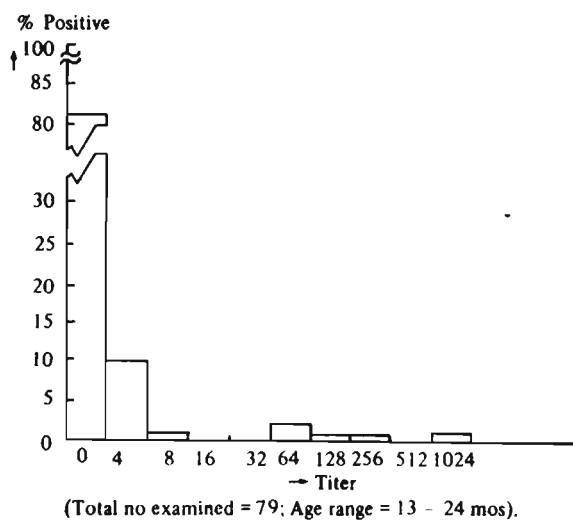
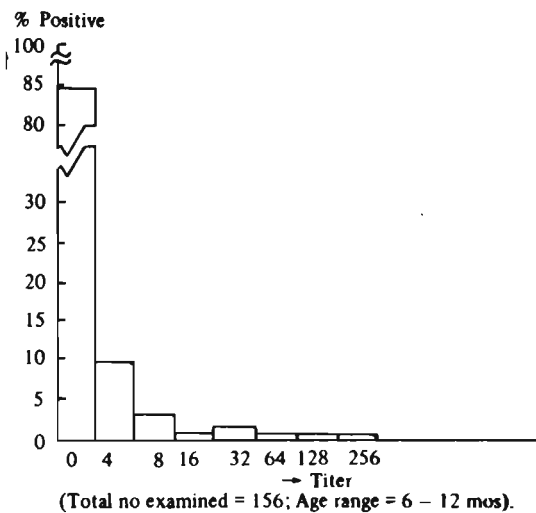
Toxoplasmosis (Fig. 1). Of a total of 166 pig sera tested from West Java 46 (28%) showed IHA titers of 1:8 or higher while of 235 sera from Central Java only 17 (7%) were considered positive (= 1:8). A higher prevalence of high titers (= 1:1024) was also noted in the West Java pigs and are probably associated with higher overall prevalence of infection.

Fig 1 HI ANTIBODIES AGAINST TOXOPLASMOSIS
IN PIG SERA FROM W and C JAVA

WEST JAVA



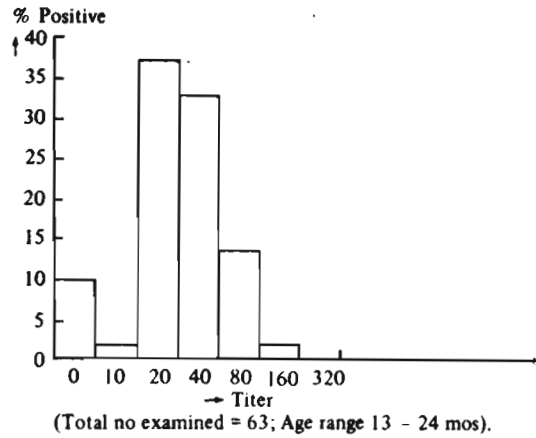
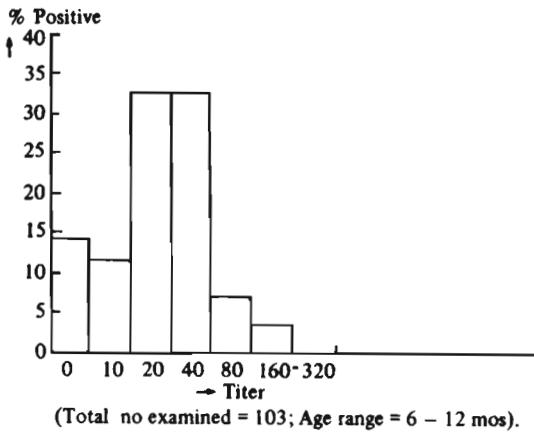
CENTRAL JAVA



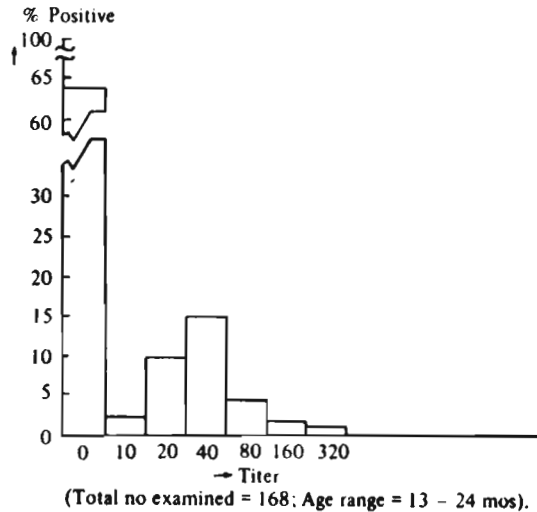
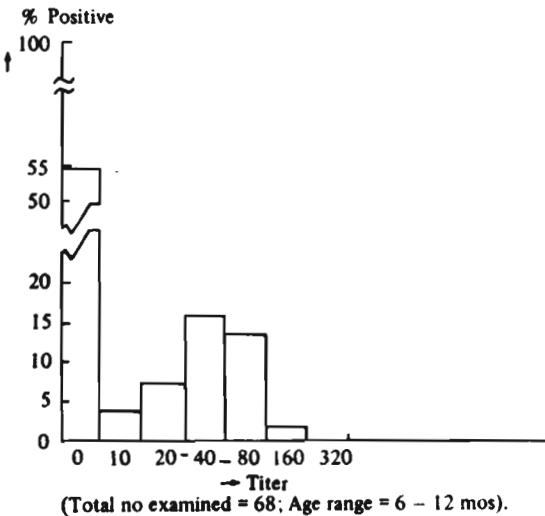
Brucellosis (fig. 2). Antibodies to *Brucella suis*, to a serum dilution of 1 : 320 in a few cases, were present in pigs of all groupings. However, most titers were negative and bar graphs showed a markedly diminishing number of animals with antibodies at higher serum dilutions (Fig. 2). Surprisingly, fewer older W. Java pigs had serum antibodies than did younger ones.

Fig 2 BRUCELLA ANTI BODIES IN PIG SERA FROM W and C JAVA

WEST JAVA



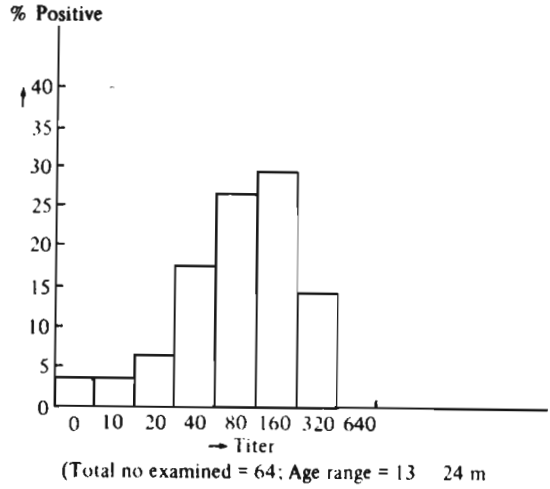
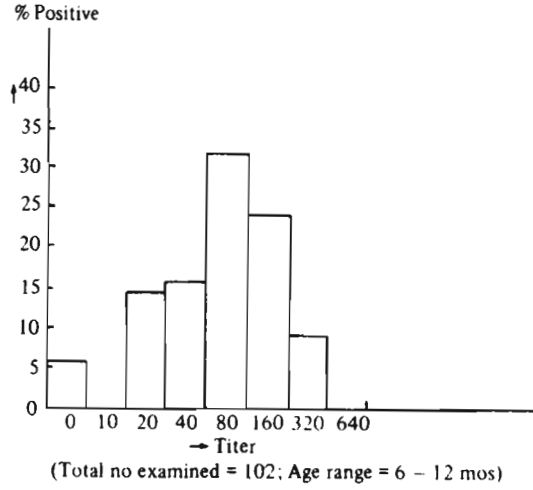
CENTRAL JAVA



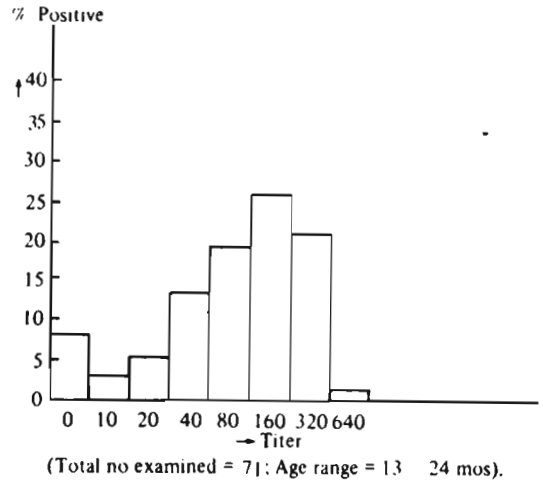
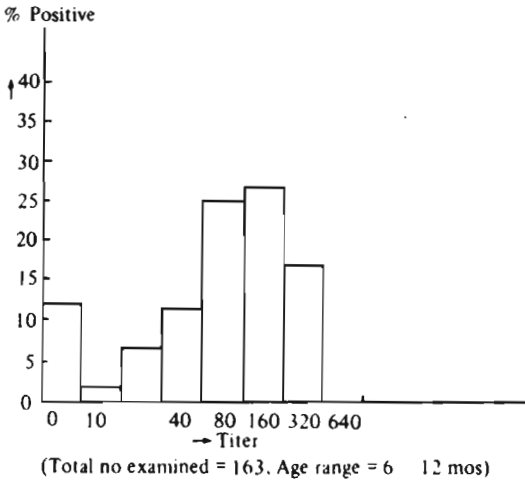
Japanese encephalitis (fig. 3). Eage age and geographical group of pigs had antibodies against JE. More negatives were obtained from Central Java, but in these pigs, as in those from West Java, an almost normal distribution around mean titers of 1 : 80 or 1 : 160 occurred.

Fig. 1. Japanese Encephalitis in pig sera from W and C Java.

WEST JAVA



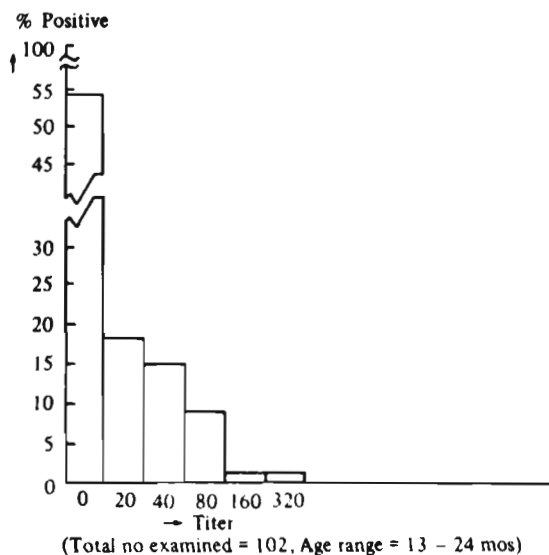
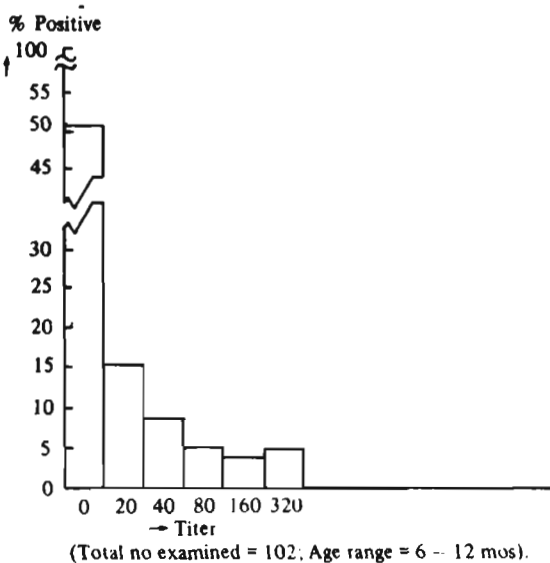
CENTRAL JAVA



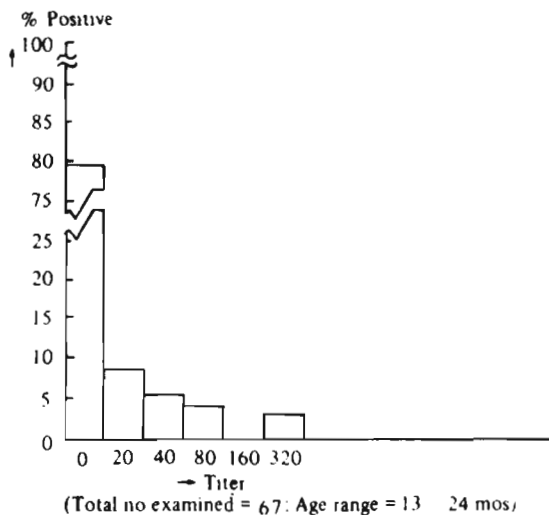
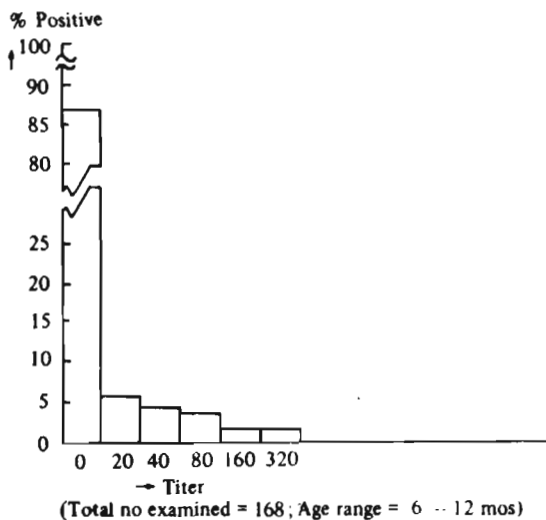
INFLUENZA (FIG. 4). Significantly elevated antibody titers against Hong Kong A2 Influenza occurred in both age groups and in pigs from both Central and West Java. Nevertheless, more W. Java pigs had positive titers; and more older than younger pigs from W. Java had antibodies at serum dilutions of 1 : 20 and above. For all groupings, there was a bellcurve distribution of antibody titers.

Fig 4 HI ANTIBODIES AGAINST A - 2/HONG KONG/1968
INFLUENZA IN PIG SERA FROM W and C JAVA

WEST JAVA



CENTRAL JAVA



Leptospirosis (Tables I and II). Most pig sera either had no Antibodies at all, or had titers against more than one **Leptospira** Serotype. Therefore, the positives by Serotype in Tables I and II total more than the actual numbers of pigs with one or more positives. As shown in Table I, Antibodies were mostly against **Leptospira Sentot**, **L. Pomona**, and **L. Bangkinang**. More positive sera were from central Java; and there were slightly more positives in younger than in older pigs from W. Java (Table II).

TABLE I. POSITIVE MICROSCOPIC AGGLUTINATION TESTS FOR LEPTOSPIROSIS
BY SERUM DILUTION AND SEROTYPE

Leptospira species	1 : 50 (No.)	1 : 100 (No.)	1 : 200 (No.)	1 : 400 (No.)	1 : 800 (No.)	1 : 1600 (No.)	1 : 3200 (No.)	TOTAL
<i>L. sentot</i>	10	31	11	7	0	2	1	62
<i>L. bangkinang</i>	10	23	11	22	2	0	0	48
<i>L. pomona</i>	2	13	11	10	6	3	0	45
<i>L. copenhageni</i>	0	19	4	5	0	0	0	28
<i>L. bataviae</i>	2	8	9	4	0	0	0	23
<i>L. tarassovi</i>	0	5	3	3	0	0	1	12
<i>L. australis</i>	0	4	1	2	0	0	0	7
<i>L. cynopteri</i>	0	3	2	1	0	0	0	6
<i>L. butembo</i>	0	2	1	1	0	0	0	4
<i>L. Djasiman</i>	0	1	3	0	0	0	0	4
<i>L. manilae</i>	0	0	1	0	0	0	0	1
TOTAL	24	109	57	35	8	5	2	240

TABLE II. MICROSCOPIC AGGLUTINATION TEST FOR LEPTOSPIROSIS
BY SEROTYPE AND AREA
JULY 1971

Area and age	Number examined	No. Neg.	No. Pos.	Number with positive reaction by serotype										TOTAL	
				<i>L. sentot</i>	<i>L. bangkinang</i>	<i>L. pomona</i>	<i>L. copenhageni</i>	<i>L. bataviae</i>	<i>L. tarassovi</i>	<i>L. australis</i>	<i>L. cynopteri</i>	<i>L. butembo</i>	<i>L. djasiman</i>		<i>L. manilae</i>
WEST JAVA															
6-12	104	61 (59%)	43 (41%)	24	18	14	5	11	7	1	0	1	1	0	81
13-24	47	32 (68%)	15 (32%)	20	14	10	11	9	0	1	1	0	0	0	66
TOTAL	151	93	58	44	32	24	16	20	7	2	1	1	1	0	147
CENTRAL JAVA															
6-12	31	16 (52%)	15 (48%)	14	13	15	9	2	2	4	4	2	3	1	69
13-24	20	10 (50%)	10 (50%)	4	3	6	3	1	3	1	1	1	0	0	23
TOTAL	51	26	25	18	16	21	12	3	5	5	5	3	3	1	92
				62	48	45	28	23	12	7	6	4	4	1	240

DISCUSSION

Pigs from W. Java were mostly of the "Tangerang Pure Breed", presumably resulting from an Importation long ago. This breed is black and small in contrast to the usual white color and large size of "Mixed breed" pigs from Central Java, thought to be the result of long interbreeding between Dutch indigenous wild pigs (*Sus scrofa*).

Results suggest that domestic pigs studied here had significant infection experience with JE, toxoplasmosis, A2 influenza and leptospirosis.

Brucella infection, if present at all, seemed to be rare. There was little difference in the pattern of antibody-titers against *Brucella* in any of the groups studied (Fig. 2). The small number of pigs with demonstrable antibodies is compatible with rare exposure of all groups to this disease, although a titer of 1:160 or higher could usually be considered evidence of disease experience (Van Peenen et al, 1963). In addition, the possibility of a vaccine response cannot be ignored since *Brucella* vaccines are available, particularly in the Jakarta area. Pigs are important reservoirs of brucellosis elsewhere (Anon., 1970) and infectious porcine abortion, etiology unknown was reported from W. Java in 1971. However, the possible role of *Leptospira*, rather than *Brucella*, infection in swine abortion should not be ignored (Hanson, 1970).

Toxoplasma can be transmitted to swine through ingestion of uncooked meat or rodents containing *Toxoplasma* cysts in their tissues, or through ingestion of infectious oocysts such as are shed in the feces of infected cats. The means by which the Java pigs became infected is unknown. The increased prevalence of antibodies in older W. Java pigs would suggest that most pigs are at an equal risk and that exposure to the agent is a function of time. The data would also suggest that the agent is more common in West Java. However, one of US (P.T.D., unpublished data) has isolated *Toxoplasma* from over 30% of 36 pigs from Solo, Central Java suggesting that the organism is more common there than indicated by the serologic tests.

Swine may be considered as potential reservoirs of *Toxoplasma* for man as well as other animals as it has been shown that if raw

pork is fed to cats they will become infected and subsequently shed oocysts in their feces -- which are highly infectious for most if not all mammals (Durfee, 1971). The prevalence of human toxoplasmosis in Java is at present unknown.

As with toxoplasmosis, the finding of a high prevalence of antibodies against JE in pigs has prompted further and more detailed research on the role of pigs in transmission of this disease. There is adequate documentation for JE in Indonesia by serological surveys (Van Peenen, 1971). The high number of pigs of all ages with antibodies points to a high, early infection rate, even in young pigs.

The role of pigs in epidemiology of A2/Hong Kong/1968 influenza has been established elsewhere (Kundin 1970) and there was serological evidence that pigs from both Central and West Java had at least been exposed at the time of this survey. Differences in percentages of positive pigs from the two areas, with a much higher percentage from both age groups in West Java, would indicate heaviest exposure there. We cannot explain this apparent geographical difference. An epidemic of human A-2 influenza did occur in Java in the spring of 1970 (Gan et al, 1971), but was not reported for 1971.

For leptospirosis, the small differences noted by area and age in table II may be because few sera were examined, particularly from Central Java. The absence of swine antibodies to *L. canicola*, *L. icterohaemorrhagiae* and other serotypes is considered as important as finding positive reactions. There is little data on importance of leptospirosis in Indonesian swine although *L. pomona* antibodies (Suratno, 1964) and isolations (Hartono and Zawawi, 1969) have been reported. It is assumed that when a single serum had reactions against more than one serotype, as often occurred, crossreaction was responsible, rather than actual exposure to more than one serotype. For example, in most cases serum dilutions against *L. pomona* were highest in pigs which also had titers against *L. sentot* and *L. Bangkinang*. Although not apparent from the tables, careful scrutiny of individual results showed *L. Pomona* to be the most common and highest. (Really high titers occurred only against *L. pomona*, *L. sentot* and *L. bangkinang*

Caution should be practiced in interpreting results of this survey. The precise role of domestic pigs in transmission of zoonotic disease to humans in Indonesia is still not clear, although results have stimulated our group to further, in-depth, research. Prior to the present study, it might have been assumed that such a role would be limited, since Indonesia is primarily a Moslem country, and the distribution of pigs in the Java country side at least is discontinuous. In fact, pig husbandry is sometimes limited by governmental regulations. However, there is no human epidemiological data indicating that any of the diseases studied here have discontinuous distribution. In addition, it is possible that other domestic animals have equally or even more important roles. Therefore, we strongly recommend that planning of any control measures take into a count the possible involvement of alternate domestic animal hosts, wild mammal hosts, and host preferences of vectors (as in the case of JE).

SUMMARY

Serological analysis for antibodies to *Toxoplasma*, *Brucella*, Japanese encephalitis, influenza and *Leptospira* were performed on 399 pig sera from W and C. Java. Significant serum antibody titers were found against all diseases with the exception of brucellosis.

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