Seroprevalence of Japanese Encephalitis Virus in Pigs and Cattle: An Evaluation from Tangerang Regency

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Abstract. Japanese encephalitis (JE) is a zoonotic vector-borne disease in humans and animals in the Asia Pacific. This RNA virus is transmitted to humans through mosquito bites from viral amplifiers, such as pigs, poultry, bats, and other vertebrates. In the spread of JEV, herons are considered the reservoir, pigs as the main amplifying host, and the Culex mosquito as a vector. Although the number of cases of JE in humans is low, Indonesia is considered an endemic area for JE. The purpose of this study was to evaluate the seroprevalence of JE in pigs and cattle in Tangerang Regency, the area with the largest pig population in Banten Province. From June - to July 2019, a total of 101 samples of pig blood and 110 samples of cattle blood were collected from 13 pig farms and 42 cattle farms. Characteristics of livestock management were recorded for further descriptive analysis. From 101 samples of pig blood and 110 samples of cattle blood, only 94 pig serum and 94 cattle serum were obtained for testing by Japanese encephalitis virus (JEV) Specific IgG ELISA; the seroprevalence results for pigs were 6.38% and 7.44% for cattle. The follow-up PCR test for porcine and bovine serum found none of the samples with the JE virus. Based on seroprevalence results and livestock management characteristics, we suspect that the JE antibody in reactive pig serum was inherited from their sow before being imported from outside the Tangerang district. However, judging from the location of the source of the positive JEV IgG cattle, almost all of which were obtained locally, there is a possibility that JEV transmission occurred in Tangerang Regency. We suggest the need for JE virus control in breeding farms in the area of origin of pigs that enter the Tangerang Regency area, considering that the introduction of the virus can occur through the transportation of cattle or piglets between regions.

Keywords: Japanese encephalitis · seroprevalence · pigs · cattle

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1 Introduction

Japanese encephalitis (JE) is a vector-borne zoonotic disease in humans caused by the Japanese encephalitis virus (JEV), a member of the Flaviviridae family and the flavivirus genus [1]. This RNA virus is transmitted to humans through mosquito bites from viral amplifiers, such as pigs, poultry, bats, and other vertebrates [2]. Mosquitoes, as JE vectors especially from the genus Culex [3]. In the spread of JEV, herons are considered the reservoir, pigs as the main amplifying host, and the Culex mosquito as a vector [4].

Indonesia is one of the countries considered to be JE endemic. JE has been identified all through the Indonesian archipelago from West to East [5]. JE infection in humans in Indonesia is rarely reported because it has similarities with other encephalitis symptoms and barriers to disease etiological analysis. However, the lack of JE case reports does not indicate that this disease does not exist in Indonesia [6]. Studies have shown that JE cases always occur in Indonesia, with the biggest outbreak cases in Bali, [7].

Several studies have shown evidence of the spread of JEV in mosquitoes and some animals that are scattered throughout Indonesia [5, 8–10]. Swine, domesticated or wild, serves as an amplifying host in endemic areas. The herons are considered important reservoir transmitters and can spread the virus to new areas through seasonal migration. Other vertebrate animals are considered dead-end hosts because there is not enough viremia to infect mosquitoes [11].

JEV infection in pigs does not show any characteristic symptoms. As for infection in pregnant female mothers, it can cause reproductive abnormalities, miscarriage of the fetus, stillbirth, or birth with neurological disorders [4]. Generally, JEV infection in other animals does not cause symptoms. However, some symptoms of JEV infection have been reported in horses [12] and cattle [13]. Examination of dead-end host animals such as cattle can be an indication of local transmission in the area [14].

Some of the risk factors for JEV transmission include the presence of migratory birds from endemic areas, vector mosquitoes, pig farms near rice fields, climate change, standing water, or the introduction of animals from JEV-infected areas [15]. The presence of pigs as the main amplifying host has a central role in JEV transmission [4].

Tangerang Regency is the region with the highest pig population in Banten Province. Some farms are backyard farms that have existed for generations before the area was populated with residential areas. Until 2018 there were around 1.739 pigs and 31.785 cattle scattered in several sub-districts of Tangerang Regency included, 36.163 hectares of paddy fields. With the existence of several risk factors for JEV transmission, it is necessary to evaluate the spread of JEV in Tangerang Regency. This study is the first evaluation effort undertaken to determine the potential risk of JEV transmission in pig and cattle populations serologically in Tangerang Regency. Moreover, this study can serve as an example of evaluation for other regions in with similar conditions that have never previously reported cases of Japanese encephalitis.

2 Material and Methods

2.1 Study Design

This study used a cross-sectional approach using purposive sampling. This research was conducted in 4 sub-districts in Tangerang Regency, namely in Panongan, East Sepatan, Tigaraksa, and Solear with consideration of the history of cases of encephalitis reported by hospitals in Tangerang Regency in 2017–2018. The location of the farm is chosen by considering affordability and access. There were 13 pig farms and 42 cattle farms, both backyard and commercial farms. Sampling was carried out in the period June–July 2019 and obtained 101 pig blood samples and 110 cattle blood samples. All blood samples were taken from the jugular vein and carried out by officers from the Subang Veterinary Center. The owner or head of the farm is interviewed for filling out a livestock management questionnaire. The blood sample (5 ml) was put in a vacutainer and centrifuged for 15 min at 2500 rpm. The serum is separated and stored at − 20 °C for further processing.

2.2 Serological Analysis

Serological tests were carried out using Competitive ELISA Kit JE-IgG in pigs (Porcine Japanese Encephalitis Virus Antibody ELISA Kit) (BT Laboratory) (Cat. No eD0449Po) and cattle (Bovine Japanese Encephalitis Antibody IgG ELISA Kit (BT Laboratory) (Cat no EA0008Bo). The ELISA test was carried out according to the procedure recommended by each of these kits. Of 101 pig serum and 110 cattle serum, only 94 pig serum and 94 cattle serum were obtained which were continued to the ELISA test.

2.3 PCR Assay

The extraction of viral RNA was carried out using a QIAamp Viral RNA Mini Kit (Qiagen) following the manufacturer's instruction. JEV detection was carried out by one step RT-PCR on the NS3 gene [16] using the consensus primers FP (5′-AGA GCG GGG AAA AAG GTC AT-3′) and RP (5′-TTT CAC GCT CTT TCT ACA GT-3′). The primers corresponded to a 162-bp product. The JEV RT-PCR was performed with SuperScript III One-Step RT-PCR System with the Platinum Taq DNA Polymerase Kit (Invitrogen, Life Technologies, Carlsbad, USA). Thermocycling conditions were as follows: 50 °C for 30 min; 94 °C for 2 min; 40 × [94 °C for 15 s + 55 °C for 30 s + 68 °C for 1 min] followed by final extension step at 72 °C for 5 min. Products were electrophoresed in 2% agarose gel and visualized by SYBR safe DNA gel staining (Invitrogen, Life Technologies).

2.4 Livestock Management Data Collection

A total of 13 questions related to livestock management were asked to the owner of the farm or the head of the farm manager. Questions to be asked to farm owners are as follows: a) individual animal: type of animal (pig/cattle), age, sex, health status, type of breeding, b) livestock management: type of farm (open/closed), animal origin (local, imports from outside the region), population, type of feed, history of abortion, history of death, and vaccination status. The data was then analyzed descriptively.

3 Results

Based on the ELISA test results, it was found that the seroprevalence of JEV in cattle was higher than in pigs. The results of the Reverse Transcriptase Polymerase Chain Reaction

Table 1. Seroprevalence and RT-PCR JEV in Pig and Cattle

Livestock	IgG Positive	Seroprevalence	RT-PCR
Pig	6 (94)	6,38%	0 (6)
Cattle	7 (94)	7,44%	0 (7)

(RT-PCR) test on 13 samples of JEV positive porcine and bovine IgG serum did not find any serum samples with JEV antigen (Table 1).

The individual characteristics of the pig samples whose serum was taken have an almost balanced sex proportion, but all of them are crossbreed (imported), and almost all pigs sampled were pigs aged 3–4 months. The samples of cattle taken are mostly female and calves (0–11 months). The Ongole cross breed cattle (PO cattle) are the most obtained. Of all the pigs and cattle sampled, all were in good health. The percentage of positive pigs IgG JEV all occurred in young pigs (0–12 months), crossbred (imported), healthy, and more common in male pigs. The percentage of positive cattle for IgG JEV all occurred in calves (0–11 months), were in good health, and more occurred in female cattle, as well as PO cattle (Table 2).

Based on the management of the farm, of the 13 selected sample pig farms, all of them raise crossbred (imported) pigs, mixed-sex, closed breeding type (one type), concrete pigpen, and abortion has never been reported. Most of the farms are located in Panongan, a commercial farm with a population of more than 100 heads, concentrate feed types, livestock sources from other areas, most of the farms have had livestock deaths, and most of them carry out vaccination (pig cholera vaccination). Of the 13 pig farms in the study sample, there were 4 farms with positive pig samples for IgG JEV. Based on the characteristics of livestock management, the farms with positive IgG JEV pigs all raise crossbred pig (imported), mixed-sex, closed farms (one type of animal), concrete pigpen, livestock sources from other areas, and never had abortions. Most of the farms with positive IgG JEV pigs are in Sepatan Timur, feed on concentrates, had livestock death, and pig cholera vaccination, while the population was balanced between large and small farms (Table 3).

This study obtained samples from 42 cattle farms, including commercial and backyard farms, which are spread over 4 sub-districts. Based on livestock management, most farms have PO cattle, only raise female cattle, located in Panongan, small livestock population ($<$100 head,) open farm, soil stable floor type, grass/hay feed, local livestock origin (Tangerang District), never reported abortion or death of livestock. Of the 42 cattle farms, there were 6 farms with positive cattle for IgG JEV. These farms all raise PO breeds, livestock population $<$ 100 heads, never reported abortion, and livestock death on the farm. Most farms with IgG JEV positive cattle raise cattle with mixed-sex, soil type, grass/hay + concentrate feed, and local livestock origin. For livestock locations with positive cattle for IgG JEV, they come from Panongan and Tigaraksa, also with open and closed farm types (Table 3).

Table 2. Individual characteristics of pigs and cattle

Characteristic	Percentage	IgG Positive	IgG Negative
Pig			
Sex			
Male	42 (45%)	4 (10%)	38 (90%)
Female	52 (55%)	2 (4%)	50 (96%)
Age			
1–2 month	8 (9%)	0 (0%)	8 (100%)
3–4 month	56 (59%)	4 (7%)	52 (93%)
5–6 month	25 (27%)	2 (8%)	23 (92%)
> 7	5 (5%)	0 (0%)	5
Breed			
Local	0 (0%)	0 (0%)	0 (0%)
Crossbreed (import)	94 (100%)	6 (6%)	88 (94%)
Health Status			
Healthy	94 (100%)	6 (6%)	88 (94%)
Sick	0 (0%)	0 (0%)	0 (0%)
Cattle			
Sex			
Male	21 (22%)	2 (10%)	19 (90%)
Female	73 (78%)	5 (7%)	68 (93%)
Age			
Calves (0–11 month)	91 (98%)	7 (8%)	84 (92%)
Yearling (12–17 month)	1 (1%)	0 (0%)	1 (100%)
Old (>17 month)	1 (1%)	0 (0%)	1 (100%)
Breed			
Angus	7 (7%)	0 (0%)	7 (100%)
Brahman Crossbreed	18 (19%)	0 (0%)	18 (100%)
Limousin	1 (1)	0 (0%)	1 (100%)
PO Cattle	57 (61%)	5 (9%)	52 (91%)
Simmental Crossbreed	11 (12%	2 (18%)	9 (82%)
Health Status			
Healthy	94 (100%)	0 (0%)	94 (100%)
Sick	0 (0%)	0 (0%)	0 (0%)

Table 3. Characteristics and management of pig farms

Characteristic	**n (percentage)**	**IgG Positive**	**IgG Negative**
Pig			
Breed			
Local	0 (0%)	0 (0%)	0 (0%)
Crossbreed (import)	13 (100%)	4 (31%)	9 (69%)
Sex			
Single sex	0 (0%)	0 (0%)	0 (0%)
Mixed-sex	13 (100%)	4 (31%)	9 (69%)
Location			
Panongan	7 (54%)	1 (14%)	6 (86%)
Sepatan Timur	3 (23%)	3 (100%)	0 (0%)
Tigaraksa	1 (8%)	0 (0%)	1 (100)
Solear	2 (15%)	0 (0%)	2 (100%)
Population			
$<$ 100	9 (69,2%)	2 (22%)	7 (78%)
$>$ 100	4 (30,8%)	2 (50%)	2 (50%)
Type of Farm			
Open farm	0 (0%)	0 (0%)	0 (0%)
Closed farm	13 (100%)	4 (31%)	9 (69%)
Floor type			
Soil	0 (0%)	0 (0%)	0 (0%)
Wood	0 (0%)	0 (0%)	0 (0%)
Concrete	13 (100%)	4 (31%)	9 (69%)
Feed			
Concentrate	8 (61,5%)	3 (38%)	5 (62%)
Kitchen waste + concentrate	5 (38,5%)	1 (20%)	4 (80%)
Livestock Source			
Other area	7 (53,8%)	4 (57%)	3 (43%)
Same area (Tangerang)	6 (46,2%)	0 (0%)	6 (100%)
Abortion			
Yes	0 (0%)	0 (0%)	0 (0%)
No	13 (100%)	4 (31%)	9 (69%)
Death			

(*continued*)

Table 3. (*continued*)

Characteristic	**n (percentage)**	**IgG Positive**	**IgG Negative**
Yes	7 (46,2%)	3 (43%)	4 (57%)
No	6 (53,8%)	1 (17%)	5 (83%)
Vaccination Status[1]			
Yes	8 (61,5%)	3 (47%)	5 (63%)
No	5 (38,5%)	1 (20%)	4 (8%)
Cattle			
Breed			
Ongole Crossbreed (PO)	36 (86%)	6 (17%)	30 (83%)
Brahman cross	1 (2%)	0 (0%)	1 (100%)
Simmental	4 (10%)	0 (0%)	4 (100%)
Limousin	1 (2%)	0 (0%)	1 (100%)
Sex			
Female only	29 (69%)	1 (3%)	28 (97%)
Mixed-sex	13 (31%)	5 (38%)	8 (62%)
Location			
Panongan	25 (60%)	3 (12%)	22 (88%)
Sepatan Timur	1 (2%)	0 (0%)	1 (100%)
Tigaraksa	7 (17%)	3 (43%)	4 (57%)
Solear	9 (21%)	0 (0%)	9 (100%
Population			
< 100	40 (95%)	6 (15%)	34 (85%)
> 100	2 (5%)	0 (0%)	2 (100%)
Farm type			
Open farm	34 (81%)	3 (9%)	31 (91%)
Closed farm	8 (19%)	3 (38%)	5 (63%)
Floor Type			
Soil	30 (71%)	4 (13%)	26 (87%)
Concrete	12 (29%)	2 (17%)	10 (83%)
Feed			
Grass/hay	33 (79%)	2 (6%)	31 (94%)
+ Concentrate	8 (19%)	4 (50%)	4 (50%)
+ tofu waste	1 (2%)	0 (0%)	1 (100%)

(*continued*)

Table 3. (*continued*)

Characteristic	n (percentage)	IgG Positive	IgG Negative
Livestock Origin			
Local	41 (98%)	5 (12%)	36 (88%)
Other area	1 (2%)	1 (100%)	0 (0%)
Abortion			
Yes	1 (2%)	0 (0%)	1 (100%)
No	41 (98%)	6 (15%)	35 (85%)
Livestock Death			
Yes	1 (2%)	0 (0%)	1 (100%)
No	41 (98%)	6 (15%)	35 (85%)

[1] Pig chorela vaccination

4 Discussion

East Sepatan Sub-district is an urban area and is close to Tangerang City. The Sub-districts of Panongan, Tigaraksa, and Solear are close to each other and belong to rural areas. Based on the results of our previous study, it was shown that the environmental characteristics of almost all pig and cattle farms were found stagnant water and close to paddy fields [17]. This shows the existence of a risk factor for JEV transmission, i.e. the presence of potential JEV vector mosquito breeding sites around pigs and cattle pens in the four sub-districts. The potential risk is also indicated by the location of several farms adjacent to residential areas. In line with the risk of mosquito breeding, our previous study also showed that the potential JEV mosquito vector has spread around pig farms in Tangerang District, with *Culex vishnui* and *Culex quinquefasciatus* as the dominant species [17]. Some of these *Culex* species have been reported as JEV vectors in Indonesia [5]. Althought RT-PCR result found no mosquitoes with JEV antigen within mosquito samples [17], a study showed persistent circulation of JEV in a region can occur through direct transmission from infected to healthy pigs without vector assistance [18].

The results of the prevalence of IgG JEV in pigs and cattle in this study were small when compared to the results of other studies. Research by Podung in North Sulawesi Province seroprevalence in pig reached 30%. Highest seroprevalence found in Minahasa Regency (43.8%), followed by Tomohon City (29.4%) and the lowest in South Minahasa Regency (17.6%) [19].

Research by Sendow obtained the seroprevalence of JEV in pigs and cattle in several regions of Indonesia by 11% and 51%. Seroprevalence of pigs in Riau and North Sumatra Provinces reached 94% and 70%, respectively [20]. The research of Ompusungu obtained seroprevalence of all pig samples in Indonesia of 14.2%. Seroprevalence of JEV in pigs in Tulungagung, East Java was 19.2% [21].

Apart from low seroprevalence, a follow-up RT-PCR test for reactive serum did not find any JEV antigen in the examined serum. This result is in line with the health conditions of pigs and cattle that do not show symptoms of illness. The low seroprevalence and the absence of JEV antigen in the serum indicate that the risk of JEV transmission in Tangerang District is low. Especially when it is associated with the potential vector which also does not find any mosquito samples with JEV [17]. Some studies have also reported low antigen detection results of reactive pig IgG JEV serum [22, 23]. Viremia in pigs can be detected as soon as 1–5 days post-infection and persists within three to five days. Viremia is relatively fast compared to other important pig diseases such as African Swine Fever which can last from three weeks to two months. This is also why JEV is difficult to isolate in cross-sectional or longitudinal studies when the blood draw period is sparse [4]. PCR results for cattle that are negative for JEV antigen are possible because all cattle are in good health, also because the sample is taken from the serum. Several other studies reported successful isolation of bovine JEV from cattle with symptoms and samples from the cerebrum [13, 24].

Based on several criteria for the characteristics of livestock management, there are several things that become our focus regarding the risk factors for JEV transmission. Factors on the location of the farm, source of livestock, incidence of abortion, and death of livestock are our focus in this discussion. These factors are very important related to the risk of JEV transmission. We consider the other livestock management factors to be very low to non-existent in terms of the study design and the risk of JEV transmission.

Based on the characteristics of the JEV IgG positive farms, all sources of pigs were obtained from areas outside Tangerang Regency. Also, abortions were not reported on farms and almost all farms had reported pig deaths. These three things indicate that it is likely that the reactive pigs had developed JEV antibodies before entering the farm. However, considering that maternal anti-JEV antibodies from the parent disappear between 1.5–4 months with a median of 2 months [4] and the emergence of JEV viral antibodies can occur within 3–7 days after infection [25], it is possible that local transmission of JEV in pigs still exists in our study. Especially by looking at the age of IgG JEV reactive pigs in the range of 3–6 months. The absence of data on the arrival date of the pigs raised on each farm becomes our limitation in concluding this.

Although there are several risk factors for JEV transmission in cattle, this does not indicate the risk of JEV transmission to humans because cattle are considered dead-end hosts and vector distractor [15, 26, 27]. Cattle are also considered a sentinel species to provide warning of local transmission and in general, JEV infection cause no symptoms [2]. Judging from the location of the source of positive JEV IgG cattle, which are almost all obtained locally, there is a possibility that JEV transmission has occurred in Tangerang Regency. If you look at the location of IgG JEV reactive farms in pigs and cattle, there is one location in the same sub-district, namely Panongan Sub-district. In this sub-district, pig and cattle farms were detected positive for IgG JEV. Another studies have shown that the presence of JEV infection in pigs is followed by infection in cattle [15].

In Indonesia, JEV infection in livestock has been reported since 1975 and until now Bali Province has been declared the only JEV endemic area [7]. Reports on the seroprevalence of JEV in livestock in several regions in Indonesia are generally not followed up with JEV examination in humans. This is due to limited surveillance and diagnostic capabilities. Livestock management data together with seroprevalence data in livestock can be used as a guide for the prevention of zoonotic disease through vaccination [28].

Although with some limitations, our study shows the possibility of the introduction of the Japanese encephalitis virus from other regions in the Tangerang Regency through livestock trading and transport. We predict that our case may also occur in other parts of Indonesia or even globally. We suggest that good measurement and action are necessary as preventive measures in these areas. To the best of our knowledge, this research is the first Japanese encephalitis seroprevalence research conducted to livestock in Tangerang Regency. Furthermore, our study demonstrates the importance of livestock management data in seroprevalence studies as a tool for the prevention of zoonotic diseases.

5 Conclusion

Although with some limitations, our study shows the possibility of the introduction of the Japanese encephalitis virus from other regions in the Tangerang Regency through livestock trading and transport. We predict that our case may also occur in other parts of Indonesia or even globally. We suggest that good measurement and action are necessary as preventive measures in these areas. To the best of our knowledge, this research is the first Japanese encephalitis seroprevalence research conducted to livestock in Tangerang Regency. Furthermore, our study demonstrates the importance of livestock management data in seroprevalence studies as a tool for the prevention of zoonotic diseases.

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