

## Seroprevalence of Japanese encephalitis Infection in Pigs in Tulungagung, East Java

### *Seroprevalensi Infeksi Japanese Encephalitis pada Babi di Tulungagung, Jawa Timur*

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#### **ABSTRAK**

Babi memainkan peran sebagai amplifying host untuk Japanese encephalitis (JE), sehingga surveilans infeksi JEV pada babi sangat penting dilakukan untuk mencegah transmisi virus ini ke manusia. Penelitian ini bertujuan untuk mengetahui tingkat infeksi JE pada babi sebagai penilaian risiko JE pada manusia. Daerah penelitian terletak di Kabupaten Tulungagung, salah satu kabupaten di Provinsi Jawa Timur dengan jumlah peternakan babi terbanyak. Penelitian ini menggunakan rancangan cross sectional, dilakukan di 5 peternakan babi di Kabupaten Tulungagung pada bulan April-November 2016, dengan total 63 ekor babi yang diperiksa. Darah vena diambil dari masing-masing babi dan diperiksa menggunakan IgG-Enzyme Link Immuno-Sorbent Assay (IgG-ELISA) spesifik JEV. Hasil penelitian menunjukkan bahwa tingkat infeksi JE pada sampel babi secara keseluruhan adalah 19,05% (12/63). Prevalensi Ab JE pada babi usia 4-6 bulan lebih tinggi (22,7%) dibandingkan dengan babi usia 2-3 bulan. Infeksi JE pada babi cenderung lebih tinggi (23,1%) pada peternakan yang ditemukan positif jentik Culex. Jenis kelamin, umur babi dan keberadaan jentik Culex di sekitar peternakan babi tidak berhubungan secara signifikan dengan besarnya angka infeksi JE pada babi. Keberadaan antibodi virus JE pada populasi babi di Tulungagung menunjukkan ada potensi transmisi virus JE pada manusia.

**Kata kunci:** Japanese encephalitis, babi, seroprevalensi

#### **ABSTRACT**

Pigs play a role as amplifier hosts for Japanese encephalitis (JE). Surveillance of JEV infection on pigs, is, therefore undoubtedly important to prevent its transmission to humans. This study aims to investigate the infection rates of JE in pigs as a risk of JE in human. The study area was located in Tulungagung regency, one of the regencies in East Java with the largest number of pig farms. This was a cross sectional study, involving five pig farms in Tulungagung Regency during April-November 2016, with a total of 63 pigs examined. Venous blood was examined by JEV specific IgG-Enzyme Link Immuno-Sorbent Assay (ELISA). The results showed that the JE infection rates in overall pig samples was 19.05% (12/63). The prevalence of Ab JE in 4-6 month pigs is higher (22.7%) than in 2-3 month pigs. The infections in pigs tend to be higher (23.1%) in farms found to be positive for *Culex* larvae. Sex and age of the pig and the presence of *Culex* larvae surround pig farms were not significantly associated with JE infection rates in pigs. In conclusion, the presence of JE virus antibodies in pigs population in Tulungagung indicates that there is a potency of JE virus transmission to humans.

**Keywords:** Japanese encephalitis, pig, seroprevalence

#### **INTRODUCTION**

Japanese encephalitis (JE) virus is one of the arbovirus that causes health problems in the world, including Indonesia. This virus is considered as a major cause of encephalitis in Asia, mainly in rural and suburban areas where rice culture and pig farming coexist.<sup>1</sup>

Twenty-four countries in the WHO South-East Asia and Western Pacific regions experienced endemic JEV transmission, with more than 3 billion people at risks of infection. Globally, it estimated around 68 000 clinical cases of JE each year, with approximately 13 600 to 20 400 deaths.<sup>2</sup>

This disease is mostly asymptomatic, except in pregnant cows may result in a miscarriage or a piglet will be stillborn.<sup>2</sup> Japanese encephalitis virus is included in the JEV serogroup of the genus *Flavivirus*, *Flaviviridae* family. The virus has single-stranded RNA with positive-sense in round direction, with 11 kb genome. Its virion contains seven non-structural proteins NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 as well as three structural proteins consisting of nucleocapsid or core protein (C), non-glycosylated membrane protein (M), and glycosylated envelope protein (E).<sup>3</sup> Japanese Encephalitis Virus is transmitted principally by the vector mosquito *Culex tritaeniorhynchus*, and is maintained through zoonotic cycles between pigs and swamps, with humans as incidental dead-end hosts. This mosquito species has been reported to prove positive for JE virus in Surabaya.<sup>4</sup> The mosquito breeding place is commonly in irrigated rice fields, and therefore this disease mainly occurs in rural areas.<sup>1,5</sup>

Pigs play a role as amplifying hosts, and a high density of pigs can be associated with increased number of cases of JE in certain area. It is believed that there are two prerequisites for JEV transmission, the presence of competent vectors and pigs as the main amplifying host. The close proximity of pigs to human settlements could increase the risks for JEV transmission to human.<sup>6</sup> The JE infection in Indonesia was confirmed from female *Cx. tritaeniorhynchus* mosquitoes collected at the area where suspected JE cases found in Sidotopo Wetan, Kenjeran Subdistrict and Kedurus Village, Karang Pilang District, Surabaya.<sup>5</sup> In 2014, sub-sequent JE surveillance activities were conducted in 11 sentinel sites recognized as JE endemic areas. Routine surveillance was conducted by collecting sera from suspected human JE cases. A total of 8 and 40 JE cases were confirmed in 2014 and 2015, respectively. In 2016, surveillance activities were continued in 11 provinces and a total 43 out of 326 human serum samples from individuals with JE

symptoms were confirmed positive by IgM ELISA.<sup>7</sup> Clinical manifestations of JEV infection in human are mostly asymptomatic, only small proportion develop into brain inflammation. The major clinical symptoms present in JE patients are fever, decrease in consciousness, mental status changes, paralysis, and convulsions.<sup>8</sup>

The potential of transmission from pigs to humans makes JE surveillance in pigs undoubtedly important. Positive pigs containing JE specific Ab were reported in several previous research in several areas in Indonesia, namely 53.6% in Jakarta, West Java, and Central Java 88-97%, Bali 64-80%, East Java 6%,<sup>9</sup> Riau 94% and North Sumatra 70%. In Surabaya, the JE specific antibody is found in several animal species including orangutans, cattle, pigs and goats, with the highest rates of infection in pigs.<sup>8</sup> Confirmation of JE cases was conducted in East Java Province, especially in Jombang District where there were 8 cases of JE in humans in 2005-2006. Tulungagung regency is one of the districts in East Java province with the largest number of pig farms. Pigs in Tulungagung increased from 2,972 in 2010 (19.03% of pig population in East Java Province) to 10,058 in 2013 (21.82%) and increased to 10,567 (25.23%) in 2014.<sup>9</sup> Yamanaka *et al.*<sup>10</sup> reported that JE-specific antibodies in pigs were found in Tulungagung District by 6% (96 pigs) based on hemagglutination-inhibition (HAI) test. Antibodies against JE virus were also found in 53% (n=28/52) blood samples from the pigs in East Sumba and 49% (n=60/123) in Bali.<sup>10,11</sup> In addition to pigs, there is growing evidence that other vertebrates may have a similar role in JE transmission in certain areas in Indonesia. This is also important to consider for understanding JEV transmission ecology. Ducks and chickens were shown likely to produce a JEV viremia sufficient to infect mosquitoes.<sup>6,12,13</sup>

Previous studies showed that pigs can act as the main reservoir of JE virus in Indonesia, therefore investigation of JEV

infection on pigs is indeed important to be carried out. This study aims to investigate the infection rates of JE in pigs as the main reservoir in order to assess the risk of JE in human. Pigs are the main amplifying host and due to this fact, pig husbandry may increase the risk of human JEV transmission as pigs are often kept in close proximity to households. The understanding of JE infection in pigs can serve as an indicator of the possibility of the threat of JE transmission to humans, especially if the location of pig farms close to the settlement.

## METHODS

This study was a cross sectional survey conducted in two sub-districts in Tulungagung regency, namely Ngunut and Kalidawir, during April-November 2016. Those areas have pig farms in Tulungagung Regency. Pigs were grouped into two categories, one from 2 to 3 month and another from 4 to 6 month of age. A total of 67 pigs was examined from the five farms in the area of study. The sample selection carried out purposively in accordance with the permit obtained from the owner of the farm. In addition to surveying pigs, a survey of mosquitoes and breeding sites was carried out in the research activities, both in pig farms and around pig farms (houses of residents, around cages of cattle and chickens).

At least 5 ml of venous blood was taken from auricularis externa vein in large pigs or saphenous vein in small pigs. Pigs are not sedated at the time of bleeding. Each sample of pigs was recorded on the characteristics of pigs (age and sex). Blood specimens were stored in an upright position in the refrigerator at 2-8°C for one night in order for the serum to separate from the blood cells. The following day, the serum was taken with pipette and then inserted into a separate microcentrifuge tube. Then serum samples were transferred to Laboratory of Microbiology, Molecular Biology and Immunology of R & D Center, P2B2 Banjarnegara. During transport to the

laboratory, serum samples were stored in ice box at temperature of 2-8 °C. Once arrived in laboratory, serum samples were transferred to a frozen cabinet at -20 °C in the laboratory until examined. Serum tests were subjected to ELISA, a standard examination technique for JE according to WHO guidance.<sup>14</sup> Pigs were categorized as infective with JE if the results showed positive JE antibody.

Data were analyzed descriptively to determine the magnitude of JE infection rates and categorized by region and pig characteristics (age and sex) and the presence of *Culex* larvae surround pig farms (less than 50 m from the cage). *Larvae* that were found in study area were kept in the laboratory to identify the species. Bivariate analysis with chi square test (CI 95%) was carried out to assess the relationship between sex of pig and the presence of *Culex* larvae surround pig farms with infection of JE. Spearman correlation test was used to determine whether there was relationship between age of pig with JE infection.

This research has received approval from the Ethics Committee of the Agency for Research and Development of Health, Ministry of Health of Indonesia (No approval: LB.02.01/5.2/KE.159 /2016, dated 4 April 2016). Blood collection on pig was conducted after permission from the owners of pig farms.

## RESULTS

In this study, 63 of pigs from five pig farms were examined, most of which came from Ngunut subdistrict. Based on the result, the prevalence of pigs containing specific JE antibody in the area of study is 17.9 % (Table 1).

Table 1. Number of Pigs Examined and Positive JE Antibodies at Each Farm in Tulungagung District

Sub district	Farms	Number of pigs examined	Number of pigs with positive Ab JE	Percentage (%)
Ngunut	Farm A	14	3	21.4
	Farm B	12	3	25.0
	Farm C	13	3	23.1
	Farm D	11	2	18.2
Kalidawir	Farm E	13	1	7.7
Total		63	12	19.05

The prevalence of positive JE antibody on female pigs is slightly more than on male group. Based on the age of pigs, the prevalence of positive JE antibody in 4-6 months pigs are higher (22.7%) than in 2-3 month pigs.

Nevertheless, statistical test results showed no association between sex, age of pigs and the existence of *Culex* larvae surrounding pig farms with JE infection in pigs (Table 2).

Table 2. Number of Pigs Examined and Positive JE Antibodies in Tulungagung District by Age and Sex

Pigs characteristic	Number of pigs examined	Positive Ab JE		Negatif Ab JE		<i>p-value</i>
		total	%	total	%	
Age (months)						0.341
- 2-3	41	7	17.1	34	82.9	
- 4-6	22	5	22.7	17	41.5	
Sex						1.000
- Male	26	5	19.2	21	80.7	
- Female	37	7	18.9	30	81.1	
Existance of <i>Culex</i> larvae arround the pig farms						0.700
- Presence	13	3	23.1	10	76.9	
- Absence	50	9	18	41	82	

Tabel 3. The Presence of *Culex* Breeding Site Inside and Arround the Pig Farms

Pig Farms	Presence of <i>Culex</i> larvae	Type of breeding sites	
		Inside pig farms	Arround pig farms
Pig Farm A	Negative	-	-
Pig Farm B	Negative	-	-
Pig Farm C	Positive ( <i>Culex</i> sp.)	Water reservoir	Puddle of household waste water, water reservoir in chicken farm, puddle of waste water from cattle pens
Pig Farm D	Negative	-	-
Pig Farm E	Negative	-	-

Table 3 showed that *Culex* larva only found around one pig farm (pig farm C), both inside and around pig farm. The distance of the *Culex* larva breeding found around the pig farm is 50-250 meters from the Ab JE positive pig farm. JE infections in pigs tend to be

higher (23.1%) in farms found to be positive for *Culex* larvae. However, the results of the fisher exact test showed that the presence of *Culex* larvae around pig farms was not significantly associated with JE infection in pigs.

## DISCUSSION

Pigs as amplifying hosts for JE viruses play important role for disease transmission, despite this virus will replicate without causing clinical symptoms in pigs. During the course of viremia, pigs can be bitten by *Cx. tritaeniorhynchus* mosquitoes, which in turn, passes the virus to other pigs or to humans as dead end host.<sup>5</sup> The results of this study indicated that pigs with positive JE antibody were found in all five farms in the area of study. Positive female pigs is slightly more than the male. and pigs of 4-6 months are higher (22.7%) than of 2-3 month. The prevalence of positive pigs in this study had slightly higher than that conducted by Ompusungu *et al.*<sup>8</sup> who examined pigs with JE antibody in 12 province in Indonesia (15 regencies) in 2012, namely 14,2 % of pigs with positive JE. However, several previous studies showed higher prevalence than the current study. High rates of infection reported in previous studies were 88-97% in West Java and Central Java, 94% in Riau, and 84% in West Kalimantan.<sup>15</sup>

The prevalence reported in other countries mostly also showed higher rates, for example in Cambodia at 63.5%,<sup>16</sup> in Vietnam between 31-79%,<sup>5</sup> and in Bangladesh between 16-42%.<sup>17</sup> Utilizing the Haemagglutination Inhibition (HI) test, in Thailand the prevalence was found between 0-66.8%,<sup>18</sup> in Japan between 0-31.7%,<sup>19</sup> in Laos between 87.4-90.2%,<sup>20</sup> and even in Vietnam reported all 100% tested pigs contained JE virus antibodies.<sup>5</sup> Ompusungu explained that the difference in prevalence of the infection could be due to many factors including pig population, sample size, age, breeding, examination methods, and time of specimen collection.<sup>8</sup> Although the prevalence in this study is lower than several previous studies, it still needs to be aware of the potential for JE transmission.

The results of this study indicated that the sex and age of pigs did not correlate with the prevalence of JE in pigs. The prevalence of JE-specific Ab in pigs aged 4-6 months

(22.7%) was higher than the prevalence of JE Ab in 2-3 months old pigs (17.1%). This result is in line with the results of research in Kapuk, Jakarta, which showed that higher rates of infection is following with increase of age. Correlations between pig age and infection rates are also reported in Cambodia,<sup>16</sup> Laos,<sup>20</sup> Vietnam,<sup>4</sup> and Bangladesh.<sup>17</sup> This difference results could be due to the difference of age limit when pigs were examined. In this study, the age limit of pigs is between 2 months to >6 months, whereas in other studies, they used age limit such as 1-7 months, or even 1.5 years to 3.5 years. The age of pigs could be correlated to the rate of JE infection because by increasing age of pigs, the chances of pigs being bitten by the vector are also higher. In the body of the pigs, immunoglobulin M (IgM Ab) is formed within 2-3 days after infection with JE virus and reaches peak after 1 week and subsequently replaced by immunoglobulin G antibody (Ab IgG). Then, maternal IgG ab to be passed down to the offspring through the milk columns and is able to protect the piglet from JE infection for 3-6 months before the Ab is lost. Immunoglobulin G specific to JEV detected in this study, which implied that antibody released because of new infection in pigs >6 months. Pigs with new infection could be a potential source of transmission through vectors to other pigs and humans during viremia. Viremia in pigs occurs within 2-5 days after infection and lasts for 2-3 days, or even longer.<sup>21</sup>

The infections in pigs tend to be higher in farms positive for *Culex* larvae but statistical analysis showed no significant association. Environmental conditions inside and around pig farms in one of the farms in Ngunut District support the existence of larvae of *Culex* sp. with the presence of several breeding sites. The distance of the breeding area is still within the flight range of *Culex* mosquitoes. Ciota *et al.*<sup>22</sup> notice that *Culex* mosquitoes flight range is a minimum of 0.16 km and a maximum of 1.98 km. The *Culex* sp. has a large role in transmitting the JE virus to pigs because this mosquito is known to prefer

animals (zoophilic). According to research in Tara-Tara Village and Talikuran Village, North Sulawesi, the availability of habitat and a large area of breeding place affect the number of mosquitoes caught. The abundance of JE infected vector will increase as well as its potential to infect human because of the high pig population.<sup>23</sup>

Our data confirmed that pigs in the area of study were found to be infected with the JE virus. This fact indicated the risk of JE transmission to human, particularly when there is contact between infected pigs and *Culex* sp. vector. Therefore, vaccination of future breeder pigs in epidemic areas could avoid the occurrence of JE.<sup>24</sup> In addition, pig farms need to be relocated farther from residential areas that include sparse *Culex* mosquitoes that become vectors in those locations. The infected pigs may also be the hosts for further spread of JEV by mosquito bites. Therefore, a short distance between dwelling places and piggeries or the habitats of feral pigs will increase the probability of exposure to JEV. Populations living in environments with high mosquito density and surrounded by piggeries are prone to JEV infection. Studies in India show that courtyards of the farmers' households without piggeries, their pigs were all raised in pig farms far away from villages (over 2–5 km). Although large numbers of *Cx. tritaeniorhynchus* and *Cx. pipiens pallens* were present in the above five courtyards, JEV was not detected from these mosquitoes.<sup>25</sup> This distance refers to maximum *Culex* flying ability, so certain distance need to be set to avoid contact with its transmitting mosquitoes *Culex*. The area of study, pig farms is somewhat separated from the settlements, but the distance is still within the range of the JE vector's flying distance. Since the JE transmission cycle is very complex because it involves several aspects such as land use pattern, pig husbandry, climate change, and human-animal relationship further studies are required for designing more efficient prevention and control strategies.

## CONCLUSION

The presence of JE virus antibodies in pig population in Ngunut and Kalidawir Subdistricts, Tulungagung Regency indicated that there is a potential of transmission of JE virus to humans in case of contact between mosquitoes as vector with the host of the amplifier.

## RECOMMENDATION

Mass vaccination in pigs needs to be done to reduce the number of pigs infected with the JE virus. If the location of the farm is not possible to be moved, it is necessary to eradicate vector around the location of the farm.

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