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## Survey of Japanese encephalitis reactor on pigs in north Sulawesi

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**Abstract**

Several studies have shown that pigs are considered as the main reservoir of Japanese encephalitis virus transmission in Indonesia, in addition to having isolated Japanese encephalitis virus in pigs, pigs are also the type of livestock that is most often reported to contain Japanese encephalitis antibodies with a relatively high infection rate. This study aims to determine the spread and magnitude of Japanese encephalitis virus infection in pigs by detecting the presence of Japanese encephalitis antibodies using the Competitive Enzyme Linked Immuno Assay (C-ELISA). By knowing the spread of Japanese encephalitis virus infection in pigs in North Sulawesi Province, it can indirectly be used as an indicator of the possible threat of Japanese encephalitis virus transmission to humans, especially if the location of pig farms is close to residential areas.

The results of serological examination using the Competitive ELISA method on 50 samples of porcine serum in 3 districts/cities, 30% of Japanese encephalitis reactors (15 samples were positive). The JE reactor in pigs in Minahasa Regency is the highest (43.8%) compared to Tomohon City (29.4%) and South Minahasa Regency (17.6%). The proximity of pigs to humans can be seen from the location of the settlements adjacent to the location of pig farms. Pigs located in Minahasa Regency which are close to settlements are the highest risk factors for the transmission of the Japanese Encephalitis virus.

**Keywords:** C-ELISA, Japanese encephalitis, pigs, reactor

**Introduction**

Japanese encephalitis is a zoonotic viral disease transmitted by mosquitoes. This disease is caused by an arbovirus (arthropod borne virus) from the Flavivirus family that attacks the central nervous system (Central Nervous System). In nature, this virus can survive in wild birds (such as cranes) and other animals, especially pigs. In humans, this virus can cause serious neurological diseases. There is a group of cases, in which 300-1000 people are clinically suspected of being infected by the Japanese encephalitis virus. This disease presents with general symptoms such as headache, high fever, stiff neck (neck stiffness), abnormal movements (tremors and convulsions in children), impaired consciousness and coma. Case Fatality Rate of this disease ranges from 20% - 40% <sup>[1]</sup>.

Japanese encephalitis is caused by viruses belonging to group B arboviruses, single chains of which are often joined to proteins and are called nucleoproteins. Japanese encephalitis virus is a member of the genus Flavivirus (family Flaviviridae) <sup>[2]</sup>. Several countries indicate that the types of mosquitoes as vectors for the spread of the Japanese encephalitis virus are mosquitoes, including: *Culex tritaeniorhynchus*, *Cx. fuscocephalus*, *Cx. gelidus* and *Cx. quinquefasciatus*. This vectors are widely distributed in Asia, including Japan, Korea, China, India, Thailand, Philippines, Malaysia, Vietnam, Taiwan and Indonesia <sup>[3-5]</sup>.

The cycle of transmission of the Japanese encephalitis virus, namely: mosquitoes - animals - mosquitoes - humans. Infectious mosquitoes or vectors are mainly played by the *Culex* which have zoophilic properties, which prefer to bite animals than humans. The important vertebrate animal as a reservoir is pigs, because in pigs high levels of viremia can occur for a long time (4 days), so pigs are also referred to as Amplifying Hosts <sup>[6]</sup>.

Efforts to improve the diagnosis of Japanese encephalitis continue to be made to obtain an accurate and efficient diagnostic model. Diagnostic techniques that are a WHO standard, such as ELISA or HI are generally intended to detect Japanese encephalitis antibodies. Not many techniques have been developed to detect DNA from this virus. To avoid cross reactions that occur in ELISA and HI techniques, an accurate and specific diagnostic method is needed so that the diagnosis of Japanese encephalitis can be made correctly.

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Recently, serological examination techniques using the HI test and ELISA have given very satisfactory results, but this test was carried out on killed or dead livestock [7].

This study aims to determine the Japanese encephalitis reactor in pigs in several areas in North Sulawesi Province. The results of this study will add to the seroepidemiological data of Japanese encephalitis from North Sulawesi Province.

## Materials and Methods

### Serum

Total serum samples from 50 pigs were collected from 3 regions, namely: 17 samples from the Tomohon area; 16 samples from Minahasa; and 17 samples from South Minahasa. The sampling technique used is purposive sampling by grouping serum samples from male and female pigs. Serum samples were collected through the anterior vena cava. Serum is separated, then stored in a refrigerator after recording the location of the sample and the sex of the livestock. Serum was then tested serologically using the C-ELISA test.

### Serological Test

In this study, serological examination was performed using the Competitive ELISA (C-ELISA) test. Inactivated JE antigen and JE monoclonal antibody, obtained from the Australian Animal Health Laboratory, Geelong, Australia.

- The percentage of inhibition was calculated using the formula:

$$\% \text{ inhibition} = 100 - \frac{(X - Y)}{(Z - Y)} \times 100\%$$

### Where

X = OD of tested serum

Y = OD background

Z = negative serum OD

Determination of the reactor is based on the % inhibition. If the % inhibition was more than 50%, the serum was positive for the JE group.

## Results and Discussion

The results of the examination of serum pigs in three pen locations are presented in table 1. It was found that there were positive reactors for Japanese encephalitis antibodies in the serum samples of pigs at each location of the pens, namely five samples of serum from 17 samples of serum tested in the city. Tomohon (% reactor: 29.4%); samples obtained in Minahasa Regency were 7 positive samples from 16 samples of serum test (% reactor 43.8%); samples obtained in South Minahasa Regency as many as 3 positive samples, from 17 test serum samples (% reactor = 17.6%).

**Table 1:** Japanese encephalitis reactor in pigs in three cage locations

	Locations		
	Tomohon	Minahasa	Minahasa Selatan
Female			
Samples	9	9	8
Positive	3	6	2
Negative	6	3	6
% Reactor	33,3%	66,7%	25%
male			
Samples	8	7	9
Positive	2	1	1
Negative	6	6	8
% Reactor	25%	14,3%	11,1%
Total Samples	17	16	17
Positive	5	7	3
Negative	12	9	14
% Reactor	29,4%	43,8%	17,6%
Total Samples	50		
Total Positive	15		
Total Negative	35		
% Reactor	30%		

Thus, from all serum samples tested, which were 50 samples, it was found that the positive test results of the Japanese encephalitis reactor were 15 samples (% reactor = 30%). The highest reactor prevalence was found in Minahasa Regency with a reactor% value of 43.8%, when compared to pigs in other cage locations, while the lowest reactor prevalence was found in Tomohon City, amounting to 17.6%. There are very few data on the incidence of Japanese encephalitis in humans and livestock in North Sulawesi Province. Especially in livestock, Japanese Encephalitis virus infection does not cause typical clinical symptoms (asymptomatic). Therefore, to diagnose Japanese encephalitis virus infection, serological examination is absolutely necessary.

The cycle of transmission of the Japanese encephalitis virus is wider, namely: mosquitoes - animals - mosquitoes - humans. The most important animal as a reservoir is pigs (6). Reservoir

host is a vertebrate animal that is a source of agent carriers, so that the disease can occur sustainably or continuously without the animal showing clinical symptoms or mild disease symptoms. Because pigs can develop high levels of viraemia for a long time (4 days), so pigs are also referred to as amplifying hosts. Therefore, it is clear that the role of pigs is more important than the mosquito that transmits it. The cycle of transmission in pigs alone can cause an area to become endemic for Japanese encephalitis in animals (8). This study supports this statement, where at the location of pigs in Minahasa Regency, the results of the Japanese encephalitis infection reactor were 43.8%.

Several studies on the prevalence of Japanese encephalitis virus infection in pigs have been carried out, such as in North Sumatra it was found that the prevalence of Japanese encephalitis reactor was 28%; in South Sulawesi (50%); Irian

Jaya (11%)<sup>[9]</sup>, as well as Riau Province using the HI method, the JE reactor yields 94%, North Sumatra 70%<sup>[10, 11]</sup>, in Bali (59.4%)<sup>[12]</sup>, Solo and Pontianak respectively 90.7% and 100%<sup>[6]</sup> and the study in Sintang, West Kalimantan with the HI test from a total sample of 68, obtained a positive HI sample of 39 samples (57.4%). Another study conducted on serum from pigs in Bali and East Java using the HI method, obtained positive results for Japanese encephalitis 49% and 9%, respectively<sup>[13]</sup>.

Based on the results of research on the prevalence of Japanese encephalitis antibodies in pigs in three pig locations, it can be said that North Sulawesi Province has become an endemic area for Japanese encephalitis in pigs. By knowing the spread of Japanese encephalitis infection in pigs in North Sulawesi, it can indirectly be used as an indicator of the possible threat of Japanese encephalitis transmission to humans, especially if the location of pig farms is close to residential areas. In this study, where the prevalence of antibodies was highest (43.8%) was in the location of pigs in Minahasa Regency, very close to settlements. This is supported by a study (12) conducted at the Sanglah Hospital in Bali, where 73 of the 155 encephalitis patients (47.1%) were positive for Japanese Encephalitis and of the Japanese Encephalitis positive patients, 57.4% lived close to rice fields, and 59.4% of patients with encephalitis have or are adjacent to a pig farm. It was also found that pigs kept around the patient's house would increase the risk factor for Japanese encephalitis by 4.28 times compared to no pigs around the patient's residence<sup>[14]</sup>.

Pigs are one of the favorite animals as domestic pets. Data on the population of pigs in North Sulawesi Province in 2011 – 2015 (BPS, 2015) averaged 397,879 heads and continues to increase every year. This data on the population of pigs in North Sulawesi is the 7th number of livestock populations among 33 provinces in Indonesia that have data on the population of pigs, where the pattern of smallholder farming is still dominant. The habit of smallholder farmers who still raise pigs that are close to their homes or settlements and do not pay attention to cage sanitation, is very likely to spread the Japanese Encephalitis virus. For example, in this study it was found that the pig pens in Minahasa Regency, which had a very high prevalence of Japanese encephalitis antibodies, were located very close to settlements and Manado City compared to the other two pen locations.

With the discovery of the prevalence of Japanese encephalitis antibodies in pigs, the cycle of transmission of this Japanese encephalitis virus has also been found directly in pigs. In other studies, which have found types of mosquitoes that have the potential to be vectors of the Japanese encephalitis virus, the incidence of Japanese encephalitis virus transmission to humans is very possible through mosquito bites<sup>[15]</sup>, and it was even identified that *Culex gelidus* is a vector of transmission of the Japanese encephalitis virus in Indonesia<sup>[5]</sup>.

Furthermore, there are still many breeders who only raise their livestock just to fatten them. Usually, pigs are kept until they are 6 months old for slaughter, then they are replaced with new and younger pigs that are more susceptible to viral infections. This is supported by<sup>[6]</sup>, that the turnover of new pigs (turn over) can be high (a lot and often) or low depending on the size of the farm. Once the pig is infected with the Japanese encephalitis virus, after recovering it will be immune to the disease and last a lifetime. Thus, older pigs are usually immune and do not serve as amplifying hosts anymore. At low turnover values, there will be many old pigs

that are not important in the spread of the Japanese Encephalitis virus. Conversely, if the turnover value is high, where the pigs are often replaced with new pigs that are still susceptible to Japanese encephalitis virus infection and can continue the cycle of spreading the virus.

### Conclusion

The highest Japanese encephalitis infection reactor was in the pig group in the Minahasa area with a reactor% of 43.8%. This means that the seroepidemiology of Japanese encephalitis infection is highest in pigs in Minahasa Regency. Japanese encephalitis virus has the potential to be transmitted to humans, because its maintenance is close to settlements. The potential for transmission is supported by the presence of *Culex* sp. which acts as a vector.

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