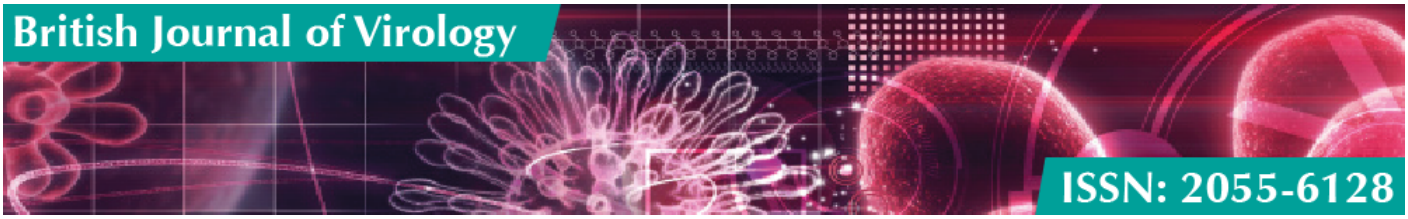

Seroprevalence of Japanese Encephalitisin East Sumba, Indonesia

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Abstract

Japanese encephalitis (JE)is a vector-borne *viral disease of several continents. The objective of this study isto determinethe JE seroprevalencein pigs in East Sumba and Slaughterhause in Kupang. For this purpose, blood samples were collected from 52 pigs frompig-populated areas of East Sumba. The antibodies against the JE were measured using competitive enzyme linked immunosorbent assay (C-ELISA). The results show the seropositivity in 28 of 52 (53%) samples in East Sumba. These findings suggest the presence of JE in East Sumba and its potential to cause infectionsin humans.*

Keyword : Japanese Encephalitis, Viral, Zoonosis.

Introduction

Japanese encephalitis (JE) is a vector-borne and emerging viral disease that occurs in South Asia, Southeast Asia, East Asia, and the Pacific (Solomon *et al.* 2003).Pigs play an important role as major amplifying host (Yamanaka *et al.* 2010) and potentially a health risk to the public (Ritchie *et al.* 2007). *Culex* mosquitoes may be responsible for JEV activity and may act as the amplifying host of JEV(Hurk *et al.* 2008). Clinically,JE is manifested by thefebrile illness, aseptic meningitis or encephalitis which terminates insensorium, seizures and focal neurological deficit and acute flaccid paralysis (Dong *et al.* 2004).

Migration of mosquitoes from one island to another is often the case that may increased incidence of JE (Samuel *et al.* 2008). JE causes significant epidemics of encephalitis, especially in children below 10 years of age, causing 10,000 deaths annually (Saxena 2008, Diagana *et al.* 2007). Thought JE is reported worldwide, its prevalence is relatively higher in Asia where it poses public health threats throughout the year (Olsen *et al.*2010; Nidaira *et al.* 2007).

According to the results of research PATH and NIHRD (2006), based on a survey of 15 hospitals in 6 provinces in Indonesia, it was found that JE is common in children less than 15 years old.JE virus (JEV) infections have been previously reported in Indonesia. In 2006,12 patients who were clinically diagnosed symptoms of encephalitis have been found infected with JEV in Bali (Sendaw *et al.* 2005). In 2009, JE antibodies were found in pigs in6 districtsof the East Nusa Tenggara Province. The highest prevalence of JE in pig serum (100%) was detected in Manggarai and West Manggarai districts(Santhia *et al.* 2009). However, JE antibodieshas yet not been detected in Sumba districtfor any existing prevalence of JE infections in animals and their potential threat to human population.

Materi and Method

Blood samples were collected from 52 pigs in East Sumba on areas where pig population are high. Samples collection with random system without clinical signs of the diseases. The age of the pig varied from 6 month to 3 years. Blood samples were taken from the jugular vein of the animals. Blood tubes were centrifuged at 3,000×g for 10 min, and the samples were transferred to sterile tubes, then examined in Laboratory of Animal Disease, Denpasar-Bali for the antibodies.

Competitive enzyme-linked immunosorbent assay (CELISA) was applied ofr the detection of JE specific antibodies according to the proceduredescribed by the Santhia*et al.*(2000). Competitive ELISA Reagents were used washing solution (PBS-Tween- 20), skin milk PBST solution, substrate solution, stopper solution (sulfuric acid). Antigens and antisera are used mainly JE virus antigens that have been terminated by gamma rays, a monoclonal antibody (Mab),conjugate (antimouse HRP conjugate), viral antigens, JE virus positive serum control, and negative control serum JE virus. Elisa plate coated with JE virus antigen (1:400) and added to 50 mL on all plates . The plates were incubated at 37 ºC for 1 hour. The plates were washed 3 times with wash solution (PBS-Tween). Tested serum samples diluted 1:10 with PBS-Tween solution added on each plate hole. Positive control serum was added to the diluted series hole A11-12 to E11-12, negative control serum, respectively added on the hole series AF11-12 and G11-12, while hole H11-12 added buffer. The plates were incubated at 37 ºC for 30 minutes. The plates are not washed, but immediately added to 50 mL monoclonal antibody (Mab) (1:6.000) on all holes except hole H11-12 subsequently incubated at 37 ºC for 30 minutes. The plates were washed 3 times with wash solution (PBS tween), after it was added 50 mL conjugate (1:4.000). Plate was washed at 37 ºC for 30 minutes. The plates were washed 3 times with wash solution (PBS-Tween). Added 50 mL of substrate solution on all subsequent holes plates were incubated at 37 ºC for 10 minutes. The reaction was stopped by adding a solution of sulfuric acid. The reaction is read on an ELISA reader using a 450 nm filter. Seropositive and seronegative then obtained.

Results

Analysis of blood serum samples were obtained. 50 blood serum samples were taken from 50 pigs were tested by Competitive ELISA (Table 1). 38 samples or 78% were found seropositive for antibodies against JE virus (Figure 1).

Discussion

Samples were collected from East Sumba areas. Prior to sampling, a survey was conducted in residential locations that maintain a pig farm through pig maintenance system both extensively and intensively. intensive system was teken the pig(intensive maintenance is done by). Extensively maintenance system is a system which tied livestock to a tree or form a pole around the yard in a house or garden. Livestock rearing systems is closely associated with the transmission of the disease. this fact caused Japanese encephalitis vectors (Culex mosquitoes) can

easily infect JE virus from pigs to humans. beside that the location of pig maintenance with home has become an important source of disease transmission.

Sampling sites in both locations are rural areas Kambaniru and abattoirs located in the village Kambaniru. From the survey result, are known about 70 % Kambaniru villagers maintain pigs with extensive maintenance system and the rest use intensive maintenance system. Livestock rearing systems will be closely associated with the transmission of the disease has been known to cause Japanese encephalitis vectors Culex mosquitoes can easily infect JE virus from pigs to humans. Besides the close distance between the location of maintenance pigs with home owners/residents becoming an important source of study. Based kambaniru village location , it is known that the lowland village and closed to the beach area. It also can be a source of important information to the distribution of Culex mosquitoes and many low-lying areas on the coast. From the characteristics of the location of the houses were also obtained pig owners/residents adjacent to rice fields. Rice fields are known as a placed of Culex mosquito breeding.

Analysis of blood serum indicates that 78% were found seropositive for antibodies against JE virus.The results of this study complement national data on the results of serological tests that indicate the presence of Japanese encephalitis in animals that have been spread in Indonesia. This study fits with previous studies conducted Santhia*et al.*(2000). The results showed that antibodies against JE virus found in pigs from several regions in Indonesia (Sendow*et al.* 2005) . Climate and nature condition in East Sumba, strongly support the Culex mosquito breeding, which also has been known as the vector responsible for this disease disseminate the views. JE disease which is the same vector borne diseases like malaria and dengue fever, which is transmitted by mosquitoes. Cases of malaria and dengue fever becomes endemic, which occurs each year in East Sumba, can be estimated to assess the comparison further to JE.

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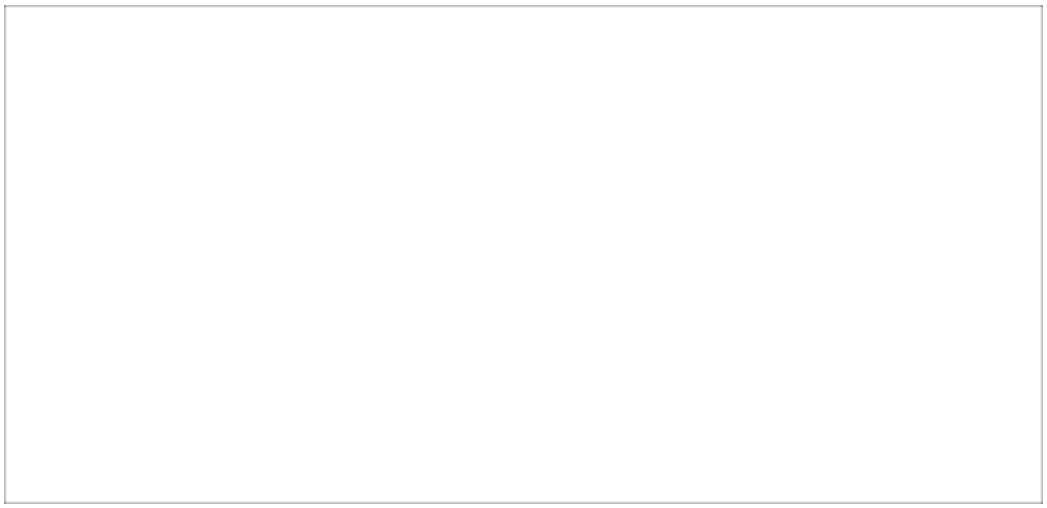

Figure 1. Positive samples in East Sumba with antibody level

Table 1.Seropositif samples in East Sumba

Sex	Number of Samples	Seropositive	Maximum antibody titer	Extensively Maintenance System
Male	29	19	69.3	21
Female	23	19	69.1	15