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Seroepidemiological evidence for the presence of Japanese encephalitis virus infection in ducks, chickens, and pigs, Bali-Indonesia



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ABSTRACT

Background: The presence of various animals, such as: ducks, chickens and pigs in households increases the potential risks of zoonosis from animal to human. One of the diseases is Japanese encephalitis (JE). The seroepidemiological studies on the presence JE among animals especially those raised in household is very important for emerging and reemerging disease control program. Ducks, chickens and pigs have long been considered as carrier and even the amplifier hosts of Japanese encephalitis virus (JEV) replication. The presence of the animal hosts and mosquitoes as vector could result in transmission of the JEV to humans.

Methods: A seroepidemiological study of the presence of Japanese encephalitis virus (JEV) was conducted by collecting sera and detecting the antibody against JEV in ducks, chickens and pigs in Bali. As pig is the amplifying animal of JEV, comparison JEV antibody between

ducks reared in households with pig nearby and with no pig were also determined the presence of antibody against JEV was examined by using indirect enzyme-linked immunosorbent assay (ELISA). The serum samples with over cut off value (COV) of optical density were considered as those containing Ab against JEV.

Results: Antibody against JEV was demonstrated in ducks (20.6%), chickens (36.7%) and pigs (32.2%) evaluated in this study. Moreover, there was no significant difference ($p > 0.05$) in the prevalence of antibody against JEV in ducks kept closely with pigs compared to the antibody in the ducks reared without pigs around.

Conclusion: This study convinced that antibody against JEV is found in ducks, chickens and pigs in Bali. Indicating that these animals was infected or previously infected by the virus.

Key words: Japanese encephalitis, Zoonoses, household, ELISA

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INTRODUCTION

Most households in Balinese rural community keep small flock of local ducks, chickens, pigs or even Balinese cattle as a secondary activity to their main agricultural activities. The community mainly rears their animals in a traditional way. The ducks, chickens and pigs are generally risen in the back yard and tend to be fed with kitchen leftover. These animals play important role in poverty alleviation and food security in rural community. This situation is quite common to be found in rural areas in Asia and Africa, where the animals are roaming freely and feeds mostly by scavenging or eating household leftovers.¹

The presence of various animals, such as ducks, chickens and pigs in households increases the potential risks of humans from contracting infectious diseases. One of the diseases is Japanese encephalitis (JE). Ducks, chickens and pigs have long been considered as carrier and even the amplifier hosts of Japanese encephalitis virus (JEV) replication. The presence of the animals and mosquitoes

as vector can result in transmission of the JEV to humans

Mosquito play an important role in the transmission of JEV.² The virus cannot directly transmit between hosts without vectors. There are many species of mosquitoes, which are competent to transmit JEV and *Culex sp.* consist majority of JEV vector with species such as *Cx. tritaeniorhynchus*, *Cx. vishnui*, *Cx. fuscocephala*, *Cx. annulloirostris*, *Cx. pipiens*, *Cx. gelidus*, *Cx. vishnui*, *Cx. annulus*, and *Cx. quinquefasciatus*. The *Aedes* mosquitoes also had been reported like *Aedes vigilax* in Australia and *Aedes japonicus* in Japan.³⁻⁷

Japanese encephalitis is an inflammatory disease in the central nervous system with high fatality rate. The disease is endemic in East, Southeast and South Asia, with around 50.000 to 175.000 human cases reported annually in the past.^{5,8-9} The causative agent is Japanese encephalitis virus (JEV) belongs to family Flaviviridae. This disease is categorized a mosquito-borne zoonotic pathogen as

its spread to humans or other susceptible animals by mosquitoes. The virus is found across a vast geographic area, such as: China, India, Japan and mostly of South-East Asia's countries. The natural transmission cycle of JEV involves an enzootic (sylvatic) mosquito-bird-mosquito and/or mosquito-pig-mosquito cycle; primarily involving *Culex spp.* Mosquitoes are the primary vectors.¹⁰ Pigs are the amplifying host,⁴ and humans are the accidental "dead end" hosts.¹¹ Chicken and duck are susceptible to Japanese encephalitis virus (JEV) infection meanwhile pigs are the amplifying host. Poultry that live near pigs have higher chance to be infected by JEV which make them viral carrier.

Generally, direct person to person transmission of JEV does not or rarely occurs except through intrauterine transmission.¹² Certainly, the high propensity of *Culex tritaeniorhynchus* to feed on pigs and the high birth rate with rapid turnover of the pig population favor the hypothesis of the pig as being the main amplifying host for JEV leading to human JE infection cases.^{9,13} Pigs are the most efficient amplifying hosts for dissemination of JEV, capable of exhibiting up to 9 logs of viremia.^{2,14} It appears that a high level of JE incidence in humans is associated with the presence of pigs as amplifying hosts.^{9,13} Clinically, JEV field infections have been associated with increased rates of stillbirth and abortion in pigs,^{15,16} whereas in poultry JEV infection does not show any clinical sign.¹⁷

This study was conducted to detect the prevalence of antibody against JEV in ducks, chickens and pigs in Bali. Additionally, comparison of antibody against JEV in ducks that kept near pigs and ducks kept without pigs was also evaluated.

MATERIAL AND METHODS

Experimental Design and Data Collection

Two strategies were used in this study. In the first strategy, blood samples of ducks, chickens and pigs were collected in different regencies in Bali Province. The serum then tested serologically for the presence of antibody against JEV to obtain serological evidence for the presence of antibodies against JEV in various household animals such as ducks, chickens and pig in Bali, Indonesia. In the second strategy, blood samples were collected from ducks kept in household with and without pig population to ascertain the role of pig in the transmission of JEV. Two villages namely Carangsari villages in Badung regency and Peguyangan villages in Denpasar City were selected in purposive sampling for this study. In Carangsari village, sera samples of ducks were collected from households where ducks were raised with pigs nearby. Conversely, duck's serum samples

that reared with no pig around were obtained from Peguyangan Village.

Blood Collection and Sera Preparation

The blood samples for the first strategy were obtained at 2006, and for the second strategy at 2007. Blood samples were obtained from the brachialis vein of ducks or chickens and from auricular or jugular vein of pigs. Blood samples were left at 37°C for 1 hour and then at 4°C overnight. Sera samples were then separated from the clot by centrifuge at 2000 g for 15 minutes, heat inactivated at 56°C for 30 minutes, and stored at -20°C until used.

Enzyme Linked Immunosorbent Assay

The 96-wells ELISA microplate were coated for 16 hours at 4°C with the concentrated inactivated JEV vaccine (Nakayama strain) with concentration 0,5 mg/100 ml/well diluted in carbonate-bicarbonate coating buffer (0.1 M carbonate buffer, pH 9.6). After being incubated at 4°C for 16 hours, the microwells was washed three times with the Elisa washing butter (0.01% Triton-X-100 within the PBS). All the wells within the micro plate were then blocked by 200 ml of 3% skim milk within the PBS. The microplate was incubated for one hour at 37°C and was washed three times as described above. The duck sera were then added to each wells which was tested using the dilution 1: 200 (1 ml serum: 200 ml 3% skimmilk in PBS). After being incubated for one hour at 37°C and being washed three times, the anti-duck IgY-(KPL-USA) labelled with horse radish peroxidase (HRP) was added to the wells. Then the microplate was incubated again for one hour at 37°C before being washed three times as described above. Anti-duck IgY-HRP (diluted 1:500), anti-chicken IgY-HRP (diluted 1:3000) and anti-pig IgG-HRP (diluted 1:3000) were added for detecting JEV antibodies in respectively in ducks, chickens and pigs. One hundred ml of substrate solution (0.04% OPD and 0.003% H₂O₂ within Phosphate Citrate buffer) was added to each well and was incubated at a dark room. Finally, 50 ml stop solution (H₂SO₄ 6 N) was added to each wells. The optical density of the substrate solution was read in Elisa plate reader using 490 nm filter. Cut-off titer was determined in each ELISA plate as the mean+ 6 times SD of negative controls.

Data analysis

The seroprevalence of JEV antibodies in ducks, chickens and pigs presented as descriptive data as described in the first strategy. Statistical analysis was then conducted to determine the role pigs and ducks in the transmission of JEV. The data of OD higher than cut-off value and the total number of sera were tabulated using Statistical Product and Service

Solution (SPSS) Ver. 22 Software. Pearson correlation test was conducted to analyze the correlation of the antibody titer among those animals. Then, Pearson's chi-square test was conducted to evaluate the statistical pattern of seroprevalence between two conditions of ducks and pigs in households.

RESULTS

In this study, serum samples of ducks, chickens and pigs were examined for the presence of JEV antibodies. In the first strategy, the total sera samples of ducks, chickens and pigs were 121, 196, and 202 respectively. The presence anti-JEV antibodies are summarized in the [table 1](#). Of the sera samples tested by ELISA, 25 (20.6%) out of 121 sera samples of ducks, 72 (36.7%) out of 196 sera samples of chickens and 65 (32.2%) out of 202 sera samples of pigs were positive antibodies against JEV ([Table 1](#)).

In the second strategy to examine the role of pigs as amplifying host of JEV, 99 sera samples of ducks from 2 villages consisting of 50 sera samples of ducks in households raising ducks together with pigs (Carangsari village) and 49 sera samples of ducks in households raising ducks with no pigs nearby (Peguyangan village) were examined. The result showed that no significant different was found on the percentage of JEV positive and negative antibodies between the two villages ([Table 2](#)).

JEV antibody prevalence of two different species was analyzed for their association using Pearson correlation test. No correlation in JEV antibody between chicken and duck ($r=0.18$), duck and pig ($r=0.26$) as well between pig and chicken ($r=0.81$) ([Fig 1a, b and c](#)).

DISCUSSION

Bali is unique place in Indonesia inhabited by mostly non-Muslim people who have accustomed to raise pigs in their backyards or nearby the house especially in the rural community. Such pigs are usually kept in a simple stall or leashed under a tree or house canopy. In addition to keeping pigs, Balinese people also have free-roaming ducks and chickens. The presence of the animals such as poultry and pigs in a household increases the risk of human from contracting the zoonotic disease such as JE. Although, the direct transmission of the disease from animals to human and vice versa, is difficult to occur, the presence of vector such as mosquitoes make the transmission can occur very easily.

Based on our study (unpublished data), there are five major mosquito's species found in Bali Province, namely *Culex tritaeniorhynchus*, *Cx. quinquefasciatus*, *Cx. fuscocephala*, *Anopheles vagus*, and *Aedes albopictus*.

In this study, there is no difference in prevalence of the JE antibody between duck samples taken out of households raising pigs and ducks in the Carangsari village with those samples of ducks with no pigs around in Peguyangan village. One possibility might be there are other sources of infection other than swine or there might be infected vector moving from Carangsari to Peguyangan village as these two villages are well connected to each other. The principal vector species of JEV is *Culex tritaeniorhynchus*,¹⁸ were found in these two villages. The possibility of transmission source other than swine should also be evaluated, considering domestic poultry produce high enough viremia for an extended period of time to serve as a possible alternative source of JEV infection and transmission to humans. Efficient transmission of JEV to mosquitoes likely occurs from young viremic chicks and ducklings.¹⁷

The presence of anti-JEV antibodies in chickens, ducks and pigs as described above clearly shows that JEV infection occurs among those animals. The JEV infection in those animal is likely to take place naturally as they were never been vaccinated with the JEV vaccine. It is likely support the incidence of JE cases in human as has been shown by Kari et al.¹⁹ Although no isolation work was conducted during this study, the ELISA result suggested that those animals might have been exposed to the virus. The natural transmission cycle of JEV involves an

Table 1 JEV antibody prevalence in ducks, chicken and pig in Bali Province

Regency	Prevalence of JEV antibody*		
	duck	chicken	pig
Jembrana	0 (0/15)	24 (6/25)	40 (12/30)
Tabanan	4.8 (1/21)	39.4 (13/33)	37.5 (12/32)
Badung	9.1 (1/11)	26.1 (6/23)	37.5 (9/24)
Denpasar	50 (3/6)	4.3 (1/23)	100 (3/3)
Gianyar	31.0 (9/29)	42.9 (9/21)	40.7 (11/27)
Bangli	30.8 (4/13)	53.3 (16/30)	7.5 (3/40)
Klungkung	20 (1/5)	41.2 (7/17)	18.2 (4/22)
Karangasem	-	-	45.8 (11/24)
Buleleng	28.6 (6/21)	58,3 (14/24)	-
Total	20.7 (25/121)	36.7 (72/196)	32.2 (65/202)

*numbers in parenthesis indicates number of positive sample divided by total sample tested

Table 2 Prevalence of JEV antibody of duck kept with pig and without pig

Condition of duck	Positive	Negative	Total	p-value
Kept with pig	26 (52%)	24 (48%)	50 (100%)	
Kept without pig	27 (55.1%)	22 (44,9%)	49 (100%)	p=0.767
Total Prevalence	53 (53.5%)	46 (46.5%)	99 (100%)	

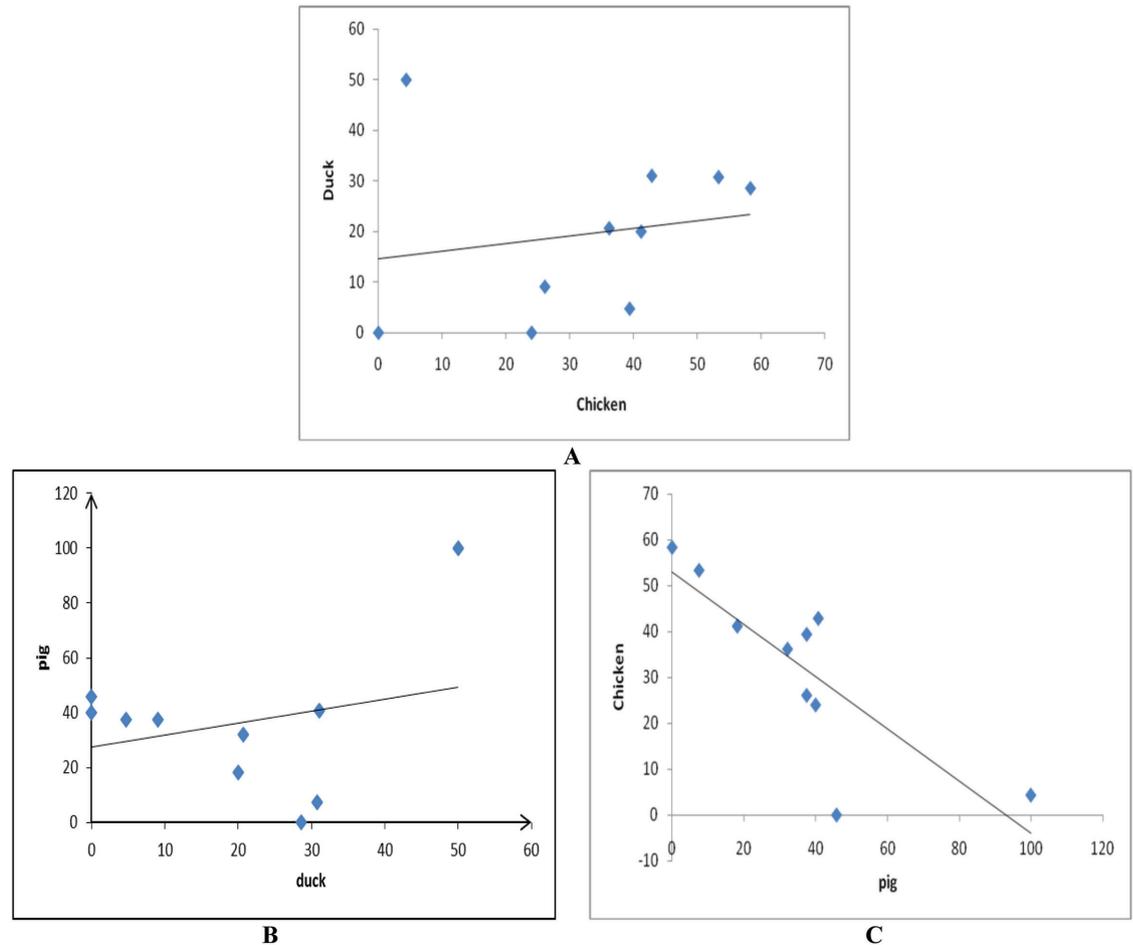


Figure 1 Correlation between JEV antibody titer in chicken and duck (A), duck and pig (B) and pig and chicken (C)

enzoonotic (sylvatic) mosquito-bird-mosquito and/or mosquito-pig-mosquito cycle.¹³ The transmission of JEV among animals and human is likely to occur via the bites of mosquitoes of especially *Culex sp.*^{3,4} The availability of infected *Culex sp.* should be monitored to avoid the outbreak of JEV in human. When an infected mosquito bites a healthy individual, it may lead to a nonspecific febrile illness or a severe meningoencephalomyelitis illness.²⁰

Japanese encephalitis virus (JEV) infection has been demonstrated in many animals such as ducks, chickens and pigs in Bali. The existence of the JEV antibody in those animals showed that those animals were infected by the JEV and was likely due to natural infection as they were never vaccinated with the JEV vaccine. Since pig is the amplifying host of JEV, infectivity of ducks reared in households with pigs and ducks in households without pigs were compared. The result clearly showed that there was no significant difference between ducks kept by pig owner and ducks in households in Bali without pigs, suggesting that there was no increase of the chance for JEV infection when ducks are kept near pigs.

CONCLUSION

Various domestic animals have contracted JEV infection in Bali. The rates of seroprevalence in Bali duck, chickens, and pigs were varied and no significant difference in seroprevalence of JEV antibody between duck kept with pig and those kept without pig.

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