



SHORT COMMUNICATION

Prevalence Status of Antibodies of Japanese Encephalitis in Pigs in Peri-Urban Area of Chennai

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ABSTRACT

Japanese Encephalitis (JE) is a mosquito borne viral zoonotic disease caused by the Japanese Encephalitis Virus (JEV) of *Flaviviridae* family and it is one of the leading causes of acute encephalitis syndrome in Asian countries. This study was conducted between November 2018 and February 2019 to understand the dynamic status of sero-prevalence of Japanese encephalitis in pigs in peri-urban areas of Chennai using indirect ELISA. A total of 241 sera samples of pigs was collected from peri-urban areas of Chennai and subjected to indirect IgG capture commercial ELISA kit for screening for JEV antibodies. The overall prevalence of JEV antibodies was found as 35.48% which showed the circulation of JEV antibodies in swine population in peri-urban areas of Chennai indicating that pigs still act as amplifier hosts for vectors for the transmission of the disease in Peri-urban areas of Chennai.

Keywords: Japanese Encephalitis, Pig, seroprevalence, ELISA, Chennai

Japanese encephalitis virus is the most common mosquito-transmitted pathogen causing encephalitis worldwide (Weaver and Reisen, 2010). In Asia, it is the leading cause of viral encephalitis and prevalent in eastern and southern Asia, covering a region with a population of over three billion (Ghosh and Basu, 2009).

Natural cycle of JEV exists in a zoonotic cycle between mosquitoes and pigs and/or water birds, maintained via pig-mosquito-pig and bird-mosquito-bird circulation (Hurk *et al.*, 2009). *Culex tritaeniorhynchus* mosquito is a primary vector for JE transmission that breeds in paddy fields and drainage ditches, thus making the disease a major concern in rice growing areas.

Pigs are thought to represent the most significant host in the supply of virus in blood for infection of feeding mosquitoes. Human get infection through bite of infected mosquitoes. High viraemic state observed in pig responsible for the perpetuation of the disease in rural

settings without displaying any overt clinical signs except abortion and still birth in pregnant sows (Guerin and Pozzi, 2005). JE is commonly diagnosed by detection of specific antibodies in serum. Currently ELISA has widely accepted and used for diagnosis of JEV in both animals and humans. Manual ELISA (Enzyme-linked immunosorbent assay) assays and commercially available IgM ELISA kits are mostly used for the diagnosis of Japanese encephalitis in swine (Litzba *et al.*, 2010). Nearly 50% of JE survivors suffered permanent and irreversible neurological sequel, resulting in a heavy burden to public health and society (Campbell *et al.*, 2011).

The purpose of this study is to understand the current status of seroprevalence of Japanese encephalitis in pigs in peri-urban areas of Chennai.

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The study was carried out in peri-urban areas of Chennai. A total 241 samples were collected from pig in peri-urban areas of Chennai showed in Table 1. The study focused on the high-risk areas of Japanese encephalitis and other factors like paddy cultivation area, migratory birds and stagnated water were also considered. Sample collection was conducted from November 2018 to February 2019 to determine the current status of JEV in Pigs.

Table 1: Collection of serum samples

Species	Area of Collection	Total number	Grand total
Pig	PGRIAS, Kattupakkam	44	241
	University research farm, Madhavaram	32	
	Velachery and Perungudi	26	
	Ambattur and Maduravoyal	29	
	Alandur	37	
	Vadapalani	31	
	Poonamallee	42	

A pro-coagulation tube (4 ml) was used to collect blood from ear vein of each pig. After collection, the blood was allowed to clot at room temperature for 30 min before placing in an ice box. All the blood samples were centrifuged at 2000 rpm for 15 min and then serum was harvested in 1.5 ml storage vials and stored at -20°C until further processing.

Serological detection of antibodies was performed using a commercially available indirect pig Japanese Encephalitis (JE) Antibody (IgG) ELISA Kit CUSABIO, China. Sera samples were subjected to ELISA as per the protocol mentioned in commercial kit by the manufacturer. Samples were duplicated and micro plates were read at wavelength of 450 nm within 10 min of adding the stop solution. Test is validated if OD value of Negative Control must less than 0.1 and the OD value of Positive Control must no less than 0.6. Calculation was done as per manufacture protocols.

$$S/P = (OD_{sample} - OD_{negative}) / (OD_{positive} - OD_{negative})$$

- While $S/P \geq 0.25$: Positive
- While $S/P < 0.25$: Negative

Serosurveillance was employed with a total of 241 pig sera to identify the serological status of JE in pig by ELISA.

Out of which 85 (35.48%) sera were positive for JEV IgG antibody.

Manual ELISA (Enzyme-linked immunosorbent assay) assays and commercially available IgM ELISA kits are mostly used for the diagnosis of Japanese encephalitis in swine (Litzba *et al.*, 2010). JEV cannot usually be isolated from the clinical specimens, probably because of the low circulating viral numbers and the rapid development of neutralizing antibodies (Solomon *et al.*, 2003). ELISA is used to detect IgG and IgM antibodies in serum or cerebrospinal fluid (CSF) taken from suspected host animals or infected individuals (Hall *et al.*, 2012).

A total 241 sera sample of pigs collected from peri-urban areas of Chennai, of which 85(35.48%) samples were positive. The prevalence levels are in agreement with the observations of Dhanze *et al.* (2014) who reported the sero-positivity of 32.6% of JE in pigs. Different parts of country reported 12 to 44 per cent of JE antibodies in pig populations (WHO, 2006). Serological survey of Prompiram *et al.* (2011) showed that 39% of pigs were seropositive for JEV in Thailand. Sonuwara *et al.* (2017) reported that there was 22.1 per cent seropositivity against JEV in pigin Chennai by ELISA.

Kumanan *et al.* (2002) recorded a prevalence of 26.4% in Tamil Nadu. Acha and szyfrez (2003) opined that Tamil Nadu is endemic for JE and in such areas, the sero-positivity could go upto 100% in the pig population.

CONCLUSION

This study showed that there is presence of JEV antibodies in pigs in peri-urban areas of Chennai which indicates that JEV is circulating among vertebrate hosts. Due to development of high-titered JEV viremias in pigs which the most important source of infection for mosquitoes that transmit JEV to humans. JE in pig can be controlled by vaccination, vector control and serodiagnosis by ELISA

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