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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating 2017: 5.03
TPI 2017; 6(9): 524-526

www.thepharmajournal.com
Received: 15-07-2017
Accepted: 16-08-2017

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Detection of Japanese encephalitis viral antibodies from swine

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Abstract
Background: Japanese encephalitis (JE) is a viral disease of zoonotic importance and one of the leading causes of viral encephalitis in Asian countries.
Objective: The aim of this study was to detect Japanese encephalitis viral antibodies from swine in the suburbs of Chennai and outside Chennai to find evidence of the presence of JEV circulation and exposure in urban areas by using indirect ELISA.
Methodology: A total of 470 serum samples from swine were collected and indirect IgG commercial available kit was used for the detection of JEV antibodies.
Results: The overall presence of JEV antibodies was 104 (22.1%).
Conclusion: Sero-positivity study showed that there is presence of JEV antibodies in swine population which indicates that JEV is transmitting between mosquitoes to vertebrate hosts. Diagnostics test such as ELISA can be used for sero surveillance study.

Keywords: Japanese encephalitis, antibodies, surveillance, sero-positivity

Introduction
Japanese encephalitis (JE) is a viral disease of zoonotic importance and one of the leading causes of viral encephalitis in Asian countries. It is the most common mosquito-transmitted pathogen causing encephalitis worldwide (Weaver and Reisen, 2010) [1].
The main transmission cycle for JEV is via mosquito vectors mainly *Culex* species. Natural cycle of JEV maintained via pig-mosquito-pig and bird-mosquito-bird circulation (Hurk *et al*., 2009) [2]. Pig acts as important amplifier host for the virus, while birds can also be involved in its amplification. Pigs serve as amplifying hosts and humans and horses are dead end hosts for the virus. The distribution of Japanese encephalitis is linked mostly to irrigated rice production and pig rearing. The virus causes reproductive losses in swine and encephalitis in horses.
Serology plays an important role in confirming the diagnosis. Detection of antibodies can be achieved by different methods the most common being enzyme-linked immunosorbent assays (ELISA). ELISA is a recent and promising immunodiagnostic tool for detecting antigen as well as antibodies in humans and animals. IgM capture ELISA is most widely accepted standard for serodiagnosis of JE in human (Solomon *et al*., 1998; Ravi *et al*., 2006) [3, 4].
The aim of this study was to detect Japanese encephalitis viral antibodies from swine in the suburbs of Chennai and outside Chennai to provide the evidence of the presence of JEV circulation and exposure in urban areas by using indirect ELISA.

Material and Methods
Study area for collection of sera samples
Sera samples were collected from different places of Chennai and Puducherry as mentioned in Table-1 for the year 2015 from University and private pig farm.
A total of 470 blood samples were collected as shown in Table-1. 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 serum was harvested in 1.5 ml storage vials and stored at -20 ˚C until further processing.

Table 1: Collection of serum samples

Species	Area of Collection	Total number
Pig	Thiruvarur, Nagapattinam	342
	Kattupakkam (University research farm)	90
	Namakkal (Instructional livestock farm)	23
	Puducherry	15
Grand Total		470

Enzyme linked immunosorbant assay
A commercially available ELISA kits were procured from CUSABIO, China (porcine). Serum samples of pigs were subjected to ELISA as per the protocol mentioned in commercial kit by the manufacturer.

Results
JEV serosurveillance was employed with a total of 470 pig sera to identify the serological status of JE in pig by ELISA. Out of which 104 (22.1%) sera were positive for JEV IgG antibody.

Discussion
JE remains endemo-epidemic in several Asian countries. Every year outbreaks among humans and domestic animals are reported in different regions of the world. Sero diagnosis is primarily used for JEV surveillance.
Serology remains the gold standard for diagnosis of JE infection. Even in the best laboratory facilities 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)[5]. ELISA is a simple, sensitive and economical method that can be used for flavivirus detection. In India serodiagnosis for JE in swine is done by using ELISA. 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) [6].
In this study Enzyme linked immnuosorbent assay was performed by using commercially available kit to 470 sera samples from pigs collected from different places of Tamil Nadu, of which 104 (22.13%) samples were positive. The prevalence levels are in agreement with the observations of (Yang *et al*., 2006) [7] who reported the sero-positivity of 21.7% from finishing pigs by I-ELISA. Different parts of country reported 12 to 44 per cent of JE antibodies in pig populations (WHO, 2006) [8].
Khan *et al*., (2011) [9] reported the presence of JEV in Arunachal Pradesh from human clinical samples during 2005-2010. Sero-epidemiological study in Pasighat, East Siang district in 2005 reported positive cases from human clinical samples (40%) and from porcine samples (80%) was detected. Kumanan *et al*., (2002) [10] 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 can go up to 100% in the pig population.
Duong *et al*., (2011) [11] and Lindahl *et al*., (2012) [12] also found that more than 90% of the pigs older than 6 months kept in endemic areas often are sero-positive to JEV.
However (Dhanze *et al*., 2014) [13] reported 32.6% seroprevalence of JE in pigs. Pant *et al*., (2006) [14] in Nepal reported a high seroprevalence of JE in pigs, ducks and horses viz., 48.11%, 26.79% and 50%.
High prevalence of JE in pigs indicates circulation of virus in endemic areas leading to epidemic encephalitis which is one of the leading causes of encephalitis in India.

Conclusion
This study gives an insight that there is presence of JEV antibodies in pigs in Chennai and outside Chennai which indicates that JEV is transmitting between mosquitoes to vertebrate hosts. Diagnostic test such as ELISA can be used for sero surveillance study of JEV.

Author's Contributions
MS and LG have designed the study project as well as corrected the manuscript. SB has done the research work, data compiling and manuscript preparation. All authors read and approved the final manuscript.

Acknowledgments
The authors acknowledge the help and facilities provided by the Department of Veterinary Public Health and Epidemiology, Madras Veterinary College, Chennai for the completion of this research.

Competing Interests
The authors declare that they have no competing interests.

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