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Seroprevalence of Japanese encephalitis in animal populations

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Abstract
Background: Japanese encephalitis virus (JEV) is a mosquito borne flavivirus and is a significant zoonotic pathogen. Geographically it is present in most of the Asian countries and some Pacific areas.
Objective: The purpose of this study was to investigate the sero-prevalence of Japanese encephalitis antibodies in animal populations.
Materials and Methods: Sera samples from different species of animals were collected from different places of Tamil Nadu and Puducherry. A total of 1502 sera samples from pig 470, cattle 705, sheep 200 and horse 127 were collected.
Results: In this study a total of 1375 sera samples (470 from pig, 705 from bovine and 200 from sheep) were screened by IgG ELISA for Japanese encephalitis virus antibodies. Out of which 104 (22.1%) from pig and 30 (15%) from sheep were positive for JEV antibodies. None of the bovine samples were positive by ELISA against JEV antibodies. The horse serum samples numbering (127) sent to National research centre on Equine, Hisar, Haryana out of which 10 (7.87%) samples were positive by Haemagglutination Inhibition test (HI).
Conclusion: These study gives a knowledge regarding circulation of JEV antibodies in animal population, thus implies that JEV may be a risk for transmission in future to humans living in urban areas of Chennai and outside Chennai.

Keywords: Japanese encephalitis virus, zoonotic, haemagglutination inhibition test (HI)

Introduction
Japanese encephalitis virus (JEV) is one of the emerging zoonotic diseases which is globally spread to larger geographical area. Discovered 125 years ago JE has spread widely in the 20th century. Almost half of the human population now lives in countries where the disease is endemic It is one of the most common mosquito transmitted disease, causing encephalitis worldwide (Weaver and Reisen, 2010) [1]. It belongs to the family of Flaviviridae which consists of three other genera; pestivirus, hepacivirus and flaviviruses (MacLachlan and Dubovi, 2011; Unni *et al*., 2011) [2, 3].
In India the existence of JEV was first reported serologically in 1954 (Smithburn *et al*., 1954) [4]. However the disease was first recognized in Vellore (North Arcot district), Tamil Nadu in 1955. Since then epidemics of JE in different states have been recorded (Dhillon and Raina 2008; Kabilan *et al*., 2004) [5, 6]. The incidence of disease increases in rainy season both in tropical and temperate climate (Saxena *et al*., 2009) [7]. The large geographic area in which JEV is present comprises both temperate and tropical regions. It occurs in annual epidemics or endemic form in many Asian countries (Campbell *et al*., 2011; Hanna *et al*., 1996, Hanna *et al*., 1998; CDC, 2013) [8-11]. Postulated explanations for JE expansion are bird migration, certain irrigation projects, animal smuggling, global warming and rice plantations creating a favourable environment for vector proliferation.
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) [12]. Pig acts as important amplifier host for the virus, while birds can also be involved in its amplification. The virus causes reproductive losses in swine and encephalitis in horses. Humans become infected only coincidentally and are dead end host.
JEV is associated with reproductive failure in swine (Lim *et al*., 2007) [13] and also causes fever, headache aseptic meningitis change of consciousness convulsions, vomiting and encephalitis (Yang *et al*., 2007) [14]. In horses most JEV infections remain sub-clinical and the only symptom may be fever and a short period of lethargy (Misra and Kalita, 2010) [15].

There is no specific treatment of JE and only supportive care is provided to patient. Hence prevention of JE is considered as an important intervention in JE. Basically there are three ways of preventing and controlling the spread of arboviruses like JEV reduction of vectors and their habitats, reduction of amplifying hosts/reservoirs and through different measures to protect individuals from being infected (Tiroumourougane *et al.* 2002; Saxena and Dhole, 2008; Dutta *et al.*, 2010) [16, 17, 18]. Viruses belonging to genus *Flavivirus* represent some of the most important emerging or re-emerging pathogenic agents that cause disease in humans (Solomon and Mallewa, 2001) [19].

Serology plays an important role in confirming the diagnosis. Detection of antibodies can be achieved by different methods the most common being haemagglutination inhibition (HI) and enzyme-linked immunosorbent assays (ELISA). Both of these methods can be 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) [20].

The purpose of this study was to investigate the sero-prevalence of Japanese encephalitis antibodies in animal populations in Chennai and outside Chennai. This information can highlight the risk that poses to humans living in urban areas.

Materials and Methods

Study area for collection of sera samples

Sera samples were collected from different places of Tamil Nadu and Puducherry for the period January 2015- March 2016 as mentioned below in Table-1.

Collection of sera samples

A total of 1502 blood samples were collected from different species of animals and shown in (Table-2). 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: Collections of serum samples from different species of animals

Species	Place	Area of Collection
Pig	Organised farm	Thiruvarur, Nagapattinam
		Kattupakkam (University research farm)
		Namakkal (Instructional Livestock farm)
	Unorganised farm	Puducherry (pig farm)
Cattle		Perambur slaughter house
	Unorganised farm	Thiruvallur
		Kanchipuram
	Organised farm	Namakkal (Instructional livestock farm)
Sheep	Perambur	Perambur slaughter house
Horse	M.V.C Hospital	Large animal ward
	Chennai	Police mount horse
	Organised farm	Hosur
	Anthiyur	Anthiyur sandy
	Chettinad	Private stud farm

Enzyme Linked Immunosorbent Assay

Sera samples of cattle, sheep and pig were subjected to ELISA as per the protocol mentioned in commercial kit by the manufacturer. A commercially available ELISA kits were procured from CUSABIO, China (for bovine and porcine) and YH Biosearch Laboratory, China (for sheep) screening of antibodies against JEV.

Haemagglutination Inhibition test

A total of 127 sera samples collected from horses were sent to National Research Centre on Equine, Hisar and Haryana for screening of antibodies against JEV by Haemagglutination Inhibition (HI) test. HI antibody titres ≥1:20 were considered positive.

Results

In this study serum samples were collected and screened for the presence of JEV antibodies. A total of 1502 serum samples (pig 470, cattle 705, sheep 200 and horse 127) were collected.

Enzyme Linked Immunosorbent Assay

ELISA was employed with 1375 (pig 470, bovine 705 and 200 sheep) sera samples to identify the serological status of JE in pig, cattle and sheep. Out of which 104 (22.1%) pig and 30 (15%) from sheep were positive respectively for JEV antibodies (Table 2; Fig 1).

Haemagglutination Inhibition test (HI)

The horse serum samples numbering (127) were sent to National Research Centre on Equine, Hisar, Haryana out of which 10 (7.87%) samples were positive by HI.

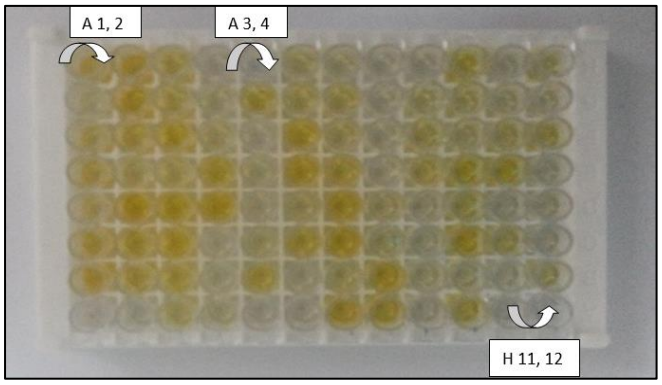
Titre value more than 1:20 is considered positive of JEV antibodies so in this study titre value of 1:40, 1:80 and 1:160 was found. Out of which 7 (70%) samples have a titre value of 1:40, 2 (20%) samples have a titre value of 20 and 1 (10%) have a titre value of 1:160 (Table 3).

Table 2: Sero-epidemiological study by doing IgG ELISA

Serum sample	No. of samples	Sample positive for IgG antibodies
Pig	470	104 (22.1%)
Bovine	705	0
Sheep	200	30 (15%)

Table 3: Haemagglutination Inhibition (HI) analysis of horse samples

Titres	No. of sample	Positive samples (%)
1: 40	7	**70**
1:80	2	**20**
1:160	1	**10**
Total	10	**100**



A 1, 2 – Positive Control
A 3, 4 - Negative Control
H 11, 12- Blank control
Other wells – Test sera

Fig 1: Enzyme Linked Immunosorbent Assay

Discussion

Japanese encephalitis virus (JEV) is one of the most common

mosquito transmitted pathogen causing encephalitis worldwide (Weaver and Reisen, 2010) [1]. Globally it is the most important cause of epidemic encephalitis and childhood mortality. In India 597,542,000 people live in JE endemic regions and approximately 1500- 4000 cases are reported annually (Kabilan *et al*., 2004) [6]. The disease epidemics are a regular occurrence during late summer and early winter months in various states of India. Keeping in view this study was done to find out the seroprevalence of JEV among animal populations.

Seroprevalence in different species of animals

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) [21].

Enzyme linked Immunosorbent assay (ELISA)

ELISA is a simple, sensitive and economical method that can be used for flavivirus detection. ELISA is a 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) [22, 23].

In this study Enzyme linked Immunosorbent assay was performed by using commercially available kit with 1375 serum out of which 470 serum samples from pig, cattle 705 and 200 from sheep of which 104 (22.13%) pig, 30(15%) sheep were positive and in cattle none of the sample was positive (Table 2).

The prevalence levels are in agreement with the observations of Yang *et al*. (2006) [24] who reported the seroprevalence of 21.7% from finishing pigs by I-ELISA.

Kumanan *et al*. (2002) [35] recorded a prevalence of 26.4% in Tamil Nadu. Acha and szyfrez, (2003) [26] opined that Tamil Nadu is endemic for JE and in such areas the seropositivity can go up to 100% in the pig population. Duong *et al.* (2011) and Lindahl, (2012) [27, 28] also found that more than 90% of the pigs older than 6 months kept in endemic areas often are seropositive to JEV.

Danze *et al*. (2014) [29] reported 32.6% seroprevalence of JE in pigs. Pant, (2006) [30] in Nepal reported a high seroprevalence of JE in pigs, ducks and horses viz., 48.11%, 26.79% and 50%.

JEV does not multiply in cattle therefore they may be safely used as sentinels (Ilkal *et al*., 1988) [31]. But longitudinal sero-epidemiological studies on cattle are scarce (Horimoto *et al*., 1987) [32].

High prevalence of JE in pigs indicates circulation of virus in endemic areas leading to epidemic encephalitis in children.

Haemagglutination inhibition test

HI remained one of the main diagnostic tests for many years for serodiagnosis of JEV antibodies. A total number 10 (7.87%) out of 127 serum samples of horse were tested positive for JEV with titre ranging from 1:40, 1: 80 and 1:160. Gulati *et al*. (2011) [33] also reported that out of 3,286 equines in 13 states in India, 10% were positive for JEV antibodies by serosurveillance doing HI test. Widjaja *et al.* (1995) [34] reported the prevalence of JEV antibodies by HI in horses of Java, Indonesia.

The JEV seroprevalence in horses we observed may have potential implications for the future spread of JEV in equine populations, resulting in increasing morbidity and mortality in these animals.

Conclusion

Sero-prevalence study showed that there is presence of JEV antibodies in animals which indicates that JEV is transmitting between mosquitoes to vertebrate hosts. With growing urbanization the importance of keeping animals in urban may act as potential source for vector borne as well as other zoonotic diseases which has to be addressed and more studies on vectors species present, its bio ecology and serosurveillance studies are needed to carried out in this line.

Diagnostics test such as ELISA and HI can be used for sero surveillance study. Detailed epidemiological studies with rapid Japanese encephalitis diagnosis enables estimation of the prevalence of JE in animals and the seroprotection levels of antibodies against JE is helpful to give clue about risk of transmission of diseases to other animals and humans.

Author's Contributions

M. Sekar and L. Gunaseelan have designed the study project as well as corrected the manuscript. Sonuwara Begum has done the research work, data compiling and manuscript preparation. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no conflict of interests.

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