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A first note on Japanese encephalitis virus isolation from *Culex quinquefasciatus* Say in Northern West Bengal

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ABSTRACT

Japanese encephalitis (JE) is endemic in many parts of India including the state of West Bengal. In West Bengal, the first major outbreaks of JE occurred in the districts of Bankura and Burdwan in 1973. The *Culex vishnui* subgroup of mosquitoes has been implicated as major vectors of JE. However in India, JE virus (JEV) has been isolated from 16 species of mosquitoes. During September 2011, JE cases were reported from four districts -Jalpaiguri, Darjeeling, Dinajpur and Cooch Behar of West Bengal (North). Adult mosquitoes were collected, identified, pooled and screened for JEV using antigen capture ELISA. Out of 279 mosquito pools tested, one pool of *Cx. pseudovishnui* and three pools of *Cx. quinquefasciatus* were found positive for JEV. The ELISA positive pools were further confirmed as JEV by insect bioassay (Toxo-IFA). Two pools of *Cx. quinquefasciatus* were confirmed as JEV. This represents the first report of JEV isolation from *Cx. quinquefasciatus* in West Bengal.

Keywords: *Cx. quinquefasciatus*, JEV, Toxo-IFA, West Bengal

1. Introduction

Outbreaks of Japanese encephalitis (JE) have occurred in many states in India. In West Bengal, the major outbreak of JE took place in 1973 in the districts of Burdwan and Bankura where more than 700 cases and 300 deaths have been reported. Since then many outbreaks have been reported. Every year sporadic cases are continuously being reported from different districts of West Bengal [1].

JE is a viral disease caused by JE virus (JEV). JEV is maintained in a zoonotic cycle, which can be both enzootic and epizootic. This cycle involves pigs as the major reservoir / amplifying host, water birds as carriers and mosquitoes as vectors. The *Culex vishnui* subgroup of mosquitoes consisting of *Cx. tritaeniorhynchus* Giles, *Cx. vishnui* Theobald and *Cx. pseudovishnui* Colless have been implicated as major vectors of JE. However in India, JEV has been isolated from 16 species of mosquitoes [2].

The filarial vector, *Cx. quinquefasciatus* Say is the most common domestic species in urban, semi urban and rural areas. Over the years, very large numbers have been processed for virus isolation. It has been shown to be capable of transmitting WN and JE viruses in the laboratory [3-4]. Experimental vertical transmission of JEV by *Cx. quinquefasciatus* was also demonstrated [5]. Two isolates of JE virus from *Cx. quinquefasciatus* was reported first time in Thailand [6]. In India, a single isolation of JEV was made from *Cx. quinquefasciatus* in Kolar district in 1986 [7].

In West Bengal JEV was not isolated from *Cx. vishnui* group during 1973 JE outbreak. This naturally raised questions about the mosquito species acting as vector of JEV in West Bengal. Therefore, in an effort to determine the vectors of JE in this area all species of mosquitoes collected between September 2011, January 2012 and May 2012 were processed for virus isolation, and the results of which are reported in this paper.

2. Materials and methods

2.1. Study area

West Bengal is located at the eastern region of India. Kolkata (formerly Calcutta) is the

capital of this State is also one of the metropolitan cities of India. The state has an area of 88752 Km^2. At January 2011, the population of the state is 91347736 of which 10112599 (11.07%) belong to the age group 0-6 years. Agriculture is the main economic source of this state. Except the northern hilly region, other parts of this state are warm and humid for the maximum time of the year. The main seasons are summer, monsoon, autumn, late autumn and winter. The summer lasts from mid – March to mid – June, with the temperature ranging from 38 to 45 ^oC. The monsoon arrives by the middle of June and lasts up to September.

During September 2011, JE cases were reported from four different districts (Jalpaiguri, Darjeeling, Dinajpur and Cooch Behar) of West Bengal (North) from first quarter onwards. A team visited West Bengal on three occasions (September 2011, January 2012 and May 2012) to investigate JE outbreak. Adult mosquitoes were collected around cattle sheds during dusk hours and from human dwellings (indoor resting) and outdoor resting places during day time 8-10 a.m. After collection samples were transported to the field laboratory, lightly anaesthetized with ether, species identified [8] and sorted on ice into pools of 1-50 specimens/pool. Unfed mosquitoes were pooled on the same day of collection, whereas engorged female mosquitoes were held for 48 h for digestion of blood meals before pooling.

Mosquito pools were stored at -80 °C until processed for virus detection and isolation as described [9]. Two systems were used:

2.2 Antigen capture ELISA

Monoclonal antibody 6B4A-10 (reactive against all viruses in JE/WN/SLE/MVE complex) was used as capture antibody and monoclonal antibody peroxidase conjugate SLE MAB 6B6C-1 (reactive against all flaviviruses) as detector antibodies (supplied by Dr. T.F. Tsai, Centers for Disease Control and Prevention, Fort Collins Co., USA). A mosquito pool was considered ELISA positive if its optical density (OD) value is $\geq$ mean + 4SD of the six normal pools.

2.3 Insect bioassay

Toxorhynchites splendens mosquito larvae were inoculated with ELISA positive pools intracerebrally, incubated for 7-10 days at 29 °C and then tested by the indirect immunofluorescence assay (IFA) on head squeeze preparations (Toxo-IFA). Smears were tested with JEV specific monoclonal antibody, MAB 112 (supplied by Dr. Kimura Kuroda, Tokyo Metropolitan Institute of Neurosciences, Japan), and detected by FITC conjugated anti-mouse immunoglobulin (Dakoppats, Denmark).

3. Results & Discussion

Culex quinquefasciatus stood first as the most abundant species in indoor and outdoor collections in the present study. A total of 3,063 mosquitoes divided into 279 pools were processed. Table 1. shows details of mosquito species processed for virus isolation.

These included 10 species of *Culex*, 6 species of *Anopheles*, 2 of *Mansonia* and 1 each of *Aedes* and *Armigeres*. Of 279 mosquito pools tested, one pool of *Cx. pseudovishnui* from Khudtrampalli village in Jalpaiguri district and three pools of *Cx. quinquefasciatus* collected one pool each from Sitharayan colony, Sushorta Nagar & and Matigara Bazar were found positive for JEV by antigen capture ELISA. The ELISA positive pools were further confirmed as JEV by insect bioassay (Toxo-IFA) using JE specific monoclonal antibodies. Two pools of *Cx. quinquefasciatus* were confirmed as JEV by Toxo-IFA (Fig. 1).

Culex tritaeniorhynchus has been considered the principal vector of JEV in India [9]. The isolation of JEV other than the major vector *Cx. tritaeniorhynchus* prompted the study of the transmission potentiality of other species in West Bengal. The possible role of *Cx. quinquefasciatus* mosquitoes as a transmitter of JEV in West Bengal has been discussed in this the present study. Two isolates of JE virus from *Cx. quinquefasciatus* was reported for the first time in Thailand [6]. In India, a single isolation of JEV was made from *Cx. quinquefasciatus* in Kolar district in 1986 [7]. Two isolates of WN virus from this species have been obtained from Manjri, near Pune. Because of its high prevalence in many areas and its predilection for human blood, this species may have a potential for the dissemination of human diseases caused by WN virus.

Table 1: JE virus infection in mosquitoes collected from West Bengal

Sl. No	Species	No. positive	No. of pools	No. of mosquitoes
1	*Ae. albopictus*	0	6	6
2	*An. barbirostris*	0	1	1
3	*An. pallidus*	0	2	3
4	*An. peditaeniatus*	0	3	20
5	*An. stephensi*	0	1	1
6	*An. subpictus*	0	8	36
7	*An. vagus*	0	12	84
8	*Ar. subalbatus*	0	12	54
9	*Cx. bitaeniorhynchus*	0	8	10
10	*Cx. fuscocephala*	0	7	18
11	*Cx. gelidus*	0	41	662
12	*Cx. infula*	0	1	1
13	*Cx. nilgiricus*	0	1	1
14	*Cx. pseudovishnui*	1	7	11
15	*Cx. quinquefasciatus*	3	77	1211
16	*Cx. tritaeniorhynchus*	0	52	837
17	*Cx. vishnui*	0	15	36
18	*Cx. whitmorei*	0	2	11
19	*Ma. annulifera*	0	10	20
20	*Ma. uniformis*	0	13	40
	Grand Total	4	279	3063

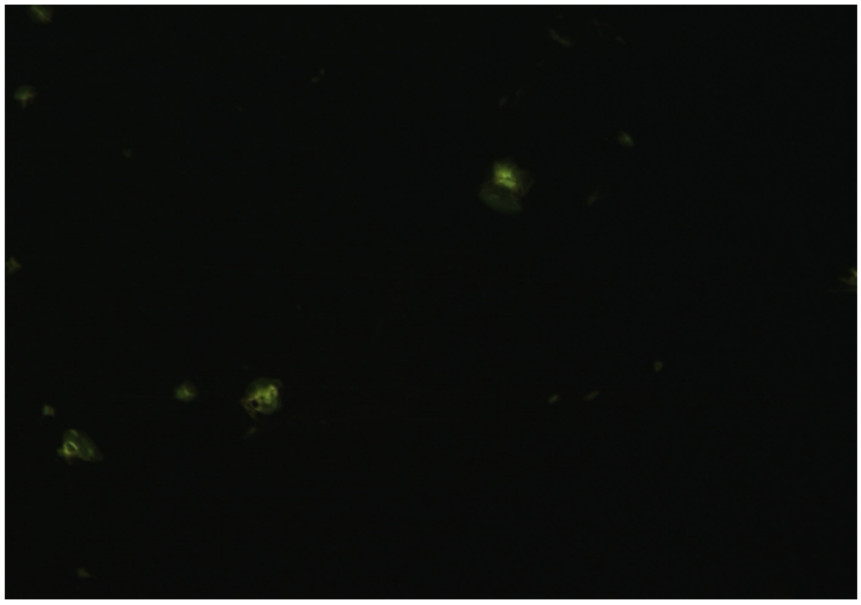

Fig 1: Toxo-IFA showing JE virus positive in *Cx. quinquefasciatus* collected from West Bengal

A strain of Wanowrie virus was isolated from this species in Manjri, Pune district, Maharashtra. It is strongly anthropophilic (53.2–62.7%); 7–14.7% cattle feeding and 1.5% feeding on pigs were also observed.

During the first epidemic of JE in 1973 in Asansol of Burdwan district, West Bengal, JEV has been isolated one each from *Cx. vishnui*, *An. barbirostris* and *An. hyrcanus*. JEV isolation in Bankura district, West Bengal from *An. hyrcanus* (2 isolates) and one each from *Cx. bitaeniorhynchus* and *Cx. epidesmus* and from a pool of *Cx. pseudovishnui*, a filterable agent unrelated to JEV was also isolated [10]. But so far there was no report on JEV isolation from *Cx. pseudovishnui* and *Cx. quinquefasciatus* in northern West Bengal.

4. Conclusion

In the present study, *Cx. quinquefasciatus* was found in good numbers and was present in all types of collections. In view of the lack of data on virus susceptibility further studies are necessary to assess its significance in the epidemiology of JE at present. The present study calls for further studies investigations on the ecology and vector potential of *Cx. quinquefasciatus*. As research progress, the role of the various proven and suspected mosquito vectors will become clearer and no doubt many new agents and cycles involving hitherto unsuspected mosquito species will be revealed.

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