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## DETECTION OF JAPANESE ENCEPHALITIS VIRUS & WEST NILE VIRUS IN SUSPECTED POPULATION IN PAKISTAN

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### Abstract

**Objectives:** To investigate the presence of IgM and IgG in the sera of patients screened for Dengue and Chikungunya during Dengue outbreak 2015-2019 in Karachi, Pakistan.

**Methods:** Japanese encephalitis virus (JEV) and West Nile virus (WNV) are two neglected viral infections in Pakistan. A sero-survey was conducted to assess the exposure of the population to JEV and WNV in Pakistan. The patients screened negative for Dengue and Chikungunya but had acute undifferentiated febrile illness during Dengue outbreak 2015-19 in Karachi, Pakistan were included in the study. Total 115 human sera were subjected to ELISA for detection of IgM and IgG antibodies produced in response to West Nile virus and Japanese Encephalitis virus.

**Results:** Our results highlighted that the overall prevalence of Japanese Encephalitis and West Nile Virus in screened population was 76.52%. The sero-positivity for Japanese encephalitis virus was found in 50 samples whereas; the prevalence of West Nile virus (WNV) was detected in 38 samples. However, 21 samples with co-circulation were indicated in our results.

**Conclusion:** In conclusion, this is an alert for physicians to suspect WNV and JEV infection in patients with a dengue-like illness especially those patients who are dwelling or traveling to and from Pakistan. Therefore, there is a need of standardized diagnostic facilities and surveillance system at national level to avoid misdiagnosis and rule out the actual prevalence rate in Pakistan.

**Keywords:** Co-circulation, Chikungunya, Dengue, Japanese encephalitis virus, Sero-positivity, West nile virus

## INTRODUCTION

Arbovirus infection is one of the neglected viral infections worldwide. Mostly, viruses from three families including *Togaviridae*, *Flaviviridae* and *Bunyaviridae* are significantly prevalent cause several emerging and reemerging diseases in South East Asia (1). *Japanese encephalitis virus* (JEV) and *West Nile virus* (WNV) belong to the Japanese encephalitis (JE) antigenic complex of viruses within the family *Flaviviridae*. These viruses are transmitted chiefly by *Culex* sp. in different parts of South East Asia. These viruses are sustained in environment by a mosquito–bird–mosquito transmission cycle where birds act as natural amplifying reservoir and mammals serve principally as blind alley hosts as momentary viraemia is insufficient to infect *Culex* mosquitoes (2, 3). Asymptomatic infection is usually observed in humans as a result of pathogenesis of these viruses.

JEV causes symptomatic as well as asymptomatic infections. The ratio of symptomatic infections is low presenting mainly encephalitis and meningitis (symptomatic to asymptomatic infections estimated to be 1:25-1:1000). Therefore, it could not be ignored. The annual incidence of clinical disease varies both across and within endemic countries, ranging from <1 to >10 per 100 000 population or higher during outbreaks. A literature review estimates nearly 68 000 clinical cases of JE globally each year, with approximately 13600 to 20400 deaths (4). JE primarily affects children. Most adults in endemic countries have natural immunity after childhood infection, but individuals of any age may be affected. Surveillance studies in 24 countries shows that ~ 67,900 JE cases have aroused annually, with only 10,426 cases accounted in 2011 (5-8). The fatality rate in case of symptomatic JE infections is 30%, however up to 50% survivors presents neurologic or psychiatric



consequences. In Pakistan, JE prevalence was first confirmed in the early 1980s by a sero-epidemiological investigation. During 1992, there was a JE epidemic in which cerebrospinal fluid was analyzed by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) to confirm acute encephalitis cases (5-8).

WNV causes fever and neuroinvasive disease. Globally, JE is endemic and different outbreaks of WNV disease have been reported in Europe, America, Africa, and parts of Asia. In India, antibodies against WNV were first detected in human serum samples from Bombay in 1952 and later in South Arcot district of Tamil Nadu. Pakistan reported serological evidence in humans and vector competence for WNV in 1982. Currently, WNV has been identified in horses and humans in Pakistan and its neighboring countries, Iran, Afghanistan, and India as a cause of human encephalitic disease (5, 9-20).

Pakistan is at a constant risk of suffering Arbovirus outbreaks and epidemics due to having favorable climate for vector, poor socio-economic status, poor health and hygienic environment, and geographical placement among Arbovirus endemic neighbors in the global map. Furthermore, Pakistan has recently experienced different epidemics and case reports of Arboviral infections, notably *Crimean-Congo hemorrhagic fever virus*, WNV, *Chikungunya virus* (CHIKV), and *Dengue viruses* (DENVs) (8, 21-23). *Culex* (competitive vector) is found in large quantity, especially in comparison with *Anopheles* and *Aedes*, which account for significantly lower portions of mosquito fauna in Karachi (27, 28). Unfortunately, there is no surveillance system for Arbovirus infections at national level and hence, little is known about epidemiology of JEV and WNV. We have limited local information of vectors circulating, reservoirs, trends, correlating parameters of transmission cycle. Few studies have been reported but these are too localized and foreign funded studies. Due to aforementioned facts, Pakistan is urgently needed national surveillance data base and effective implementation of preventive and control strategies. Therefore, a sero-survey was conducted to estimate the presence of JEV and WNV infections in Pakistan.

## METHODOLOGY

This surveillance study was based on the samples collected from patients with acute undifferentiated febrile illness enrolled in different health care facilities and hospitals located in Karachi, Pakistan during Dengue outbreak 2015-19 (9). Blood samples (3-5 ml) were collected in vacutainers and transported to the Lab, in cold chain at 2-8°C. Sera were then separated out and stored in aliquots at -80°C. Total of 105 febrile patients previously screened negative for Dengue and Chikungunya but presenting with rash, headache, arthralgia, myalgia, gastrointestinal distress, acute hemorrhagic fever, acute flaccid paralysis, encephalitis, and/or unexplained fever. CDC (Arboviral Diseases, Neuroinvasive and Non-neuroinvasive 2015 Case Definition) guideline of Arboviral diseases was followed for the selection of patients with clinical symptoms (24). The presence of Dengue virus infection was previously done by NS1 antigen, anti-Dengue IgM and anti-Dengue IgG antibodies ELISA. Whereas, screening for Chikungunya virus was previously carried out through RT-PCR, anti-Chikungunya IgM & anti-Chikungunya IgG ELISA. Demographic data was also collected from the patients including age and sex. In addition, 10 samples from healthy individuals were also included in this study as negative control. All the samples were processed in BSL-2 facilities.

All sera were subjected for ELISA to detect IgM and IgG antibodies produced in response to West Nile virus and Japanese Encephalitis virus. The Anti-West Nile IgM, Anti-West Nile IgG, Anti-Japanese Encephalitis IgM and Anti-Japanese Encephalitis IgG antibodies were detected by using EUROIMMUN ELISA, Medizinische Labordiagnostika AG, Germany (EI 2663-9601 M, EI 2663-9601 G) kits following the manufacturer's instructions. The results are classified as positive, negative and equivocal according to the manufacturer's instructions. All tests were run in duplicate. ELISA reader (ELX 800, Biotek) was used to measure the optical density at 450nm wave length.

## RESULTS

Total 115 samples were analyzed in this study to rule out the seroprevalence of Japanese encephalitis virus (JEV) and West Nile virus (WNV). Out of 115 samples the healthy individuals were 10 and found negative for IgM and IgG antibodies against both of the Arboviruses. The sero-positivity for

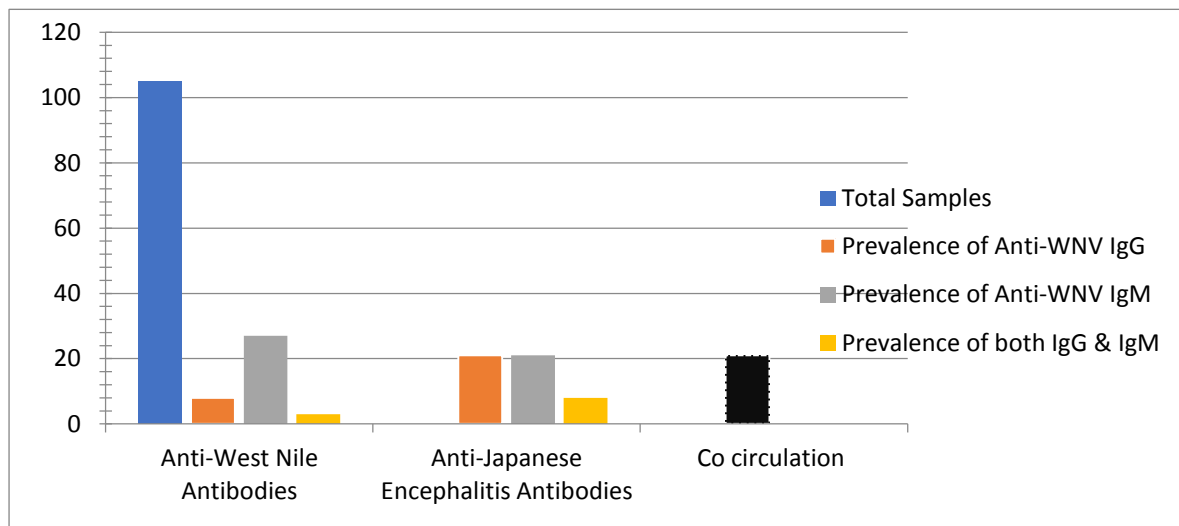
Japanese encephalitis virus was found in 50 samples in which 21 samples had only anti- Japanese encephalitis IgM antibodies and anti- Japanese encephalitis IgG antibodies were detected in 21 samples. However, both types of antibodies were identified at the same time in 08 patients (Table I).

While investigating the samples for West Nile virus (WNV), total of 38 samples were positive by presenting anti-West Nile IgM antibodies alone in 27 samples and anti-West Nile IgG antibodies alone in 08 samples. In addition, 03 samples had both IgM and IgG antibodies against West Nile virus (Fig. 1).

Overall, 76.52% of study population was positive in which 43.47% individuals showed prevalence of Japanese encephalitis virus (JEV) and only West Nile virus (WNV) was prevalent in 33% individuals. Furthermore, 18% individuals presented the co-prevalence of both Arboviruses. No significant difference was noticed in percentage positivity in different age groups for both JEV and WNV.

**Table I.** Seroprevalence of Antibodies against Japanese encephalitis virus & West Nile virus in Pakistan during 2015-19

Antibodies against Arbovirus	Positivity in samples (n)	
	Japanese encephalitis virus (JEV)	West Nile virus (WNV)
IgM antibodies only	21	27
IgG antibodies only	21	08
IgM and IgG antibodies both	08	03
<b>Total</b>	<b>50</b>	<b>38</b>



**Fig. 1.** Results obtained during the study pertaining to prevalence of Antibodies against WNV & JEV

## DISCUSSION

Prevalence of JEV and WNV infections in Pakistan is very little known at national level. It has become necessary to understand the extent of the infection to plan and implement the intervention measures. Pakistan is a Dengue and Chikungunya endemic country. Keeping in view, it became very difficult to categorize the introduction of WNV and JEV in Pakistan as the symptoms of Dengue, Chikungunya, Japanese encephalitis virus and West Nile virus are quite similar (9, 14, 25). They share same ecological conditions, seasonality and vectors. Since there was no documentation of the outbreak and risk of JE and WEV in this region at national level, it was thus necessary to have baseline information on the seroprevalence of these viruses in Pakistan. Our study result indicated that the overall seroprevalence of JEV and WNV in targeted Pakistani population is 76.52%. The percentage for the prevalence of JEV is 43.47% and higher than WNV which is 33%. However, 18% co-circulation was indicated in our results. Therefore, it has become necessary to understand the extent of the infection in the area to plan and implement the control measures. Diagnosis and appropriate treatment of arboviral disease is challenging in areas where these pathogens co-circulate. Laboratory confirmation remains a major challenge, and RT-PCR is not very useful in diagnosis of WNV or JEV as viremia occurs 3–4 days before the onset of clinical symptoms. Given that

DENV, JEV, and WNV are flaviviruses, there is serological cross-reactivity and absolute confirmation requires the most cumbersome and highly skilled plaque reduction assay. This type of infrastructure is lacking in many labs and medical facilities in Pakistan.

The limitation of this study was that patient serum was tested only via ELISA for the detection of these viruses due to the less volume of serum samples as these had been utilized for Dengue and Chikungunya screening. Due to the availability of inadequate kits and reagents, only limited numbers of samples were analyzed for the same. Despite this limitation, our results are the indication of JEV and WNV circulation in Pakistan by the presence antibodies against these viruses and in accordance to the other studies in Pakistan (9-14, 25, 26). Pakistan is endemic with the vectors of these viruses and the climatic and sanitation conditions are supportive of active propagation and dissemination of vectors and consequent risk of an epidemic outbreak of these viruses. Active disease surveillance and preventive strategies are not available in Pakistan but are urgently needed at regional and national levels to prevent such from happening in timely manner. Moreover, there is as a great need for the standardization of diagnostic assays and procedures, more accurate diagnostic assays, and renovated diagnostic algorithms for Pakistan and other countries where JEV and WNV circulates with other flaviviruses. While development of surveillance networks, diagnostic assays, and standardized procedures can be overwhelming for developing countries, it is rapidly becoming apparent that the long-term costs to public health are greater in the face of flavivirus disease. Given the high seroprevalence of flaviviruses, reinstatement of DEWS or similar program should be seriously considered for Pakistan. Regardless, the findings of this study are an alert for physicians to suspect WNV and JEV infection in patients in a dengue-like illness in patients inhabiting or traveling to and from Pakistan.

## CONCLUSION

In conclusion, this is an alert for physicians to suspect WNV and JEV infection in patients in a dengue-like illness in patients inhabiting or traveling to and from Pakistan. Therefore, there is a need of standardized diagnostic facilities and surveillance system at national level to avoid misdiagnosis and rule out the actual prevalence rate in Pakistan.

### Limitations of the study:

The major limitation of current work was found to be a single technique e.g. ELISA with which patient sera were tested for the detection of these viruses due to the less volume of serum samples as these had been utilized for Dengue and Chikungunya screening. Due to the availability of inadequate kits and reagents, only limited numbers of samples were analyzed for the same. Despite this limitation, our results are the indication of JEV and WNV circulation in Pakistan by the presence of antibodies against these viruses and in accordance to the other studies in Pakistan (9-14, 25, 26).

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