

## SHORT COMMUNICATIONS

# Detection of Zika Virus Antibodies in Retrospective Serum Samples from Suspected Dengue Cases in Sarawak, Malaysian Borneo

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## ABSTRACT

Sarawak, a state in Malaysian Borneo, has never reported a case of Zika virus (ZIKV) infection nor conducted any seroprevalence studies on the virus. This study aimed to provide a serological insight into ZIKV in Sarawak. 212 sera samples collected from a dengue surveillance study conducted from 2007 to 2011 were retrospectively analyzed. Samples negative for both dengue and Japanese encephalitis virus by RT-PCR were screened by immunoblots assay for ZIKV prM protein. Positive samples were further subjected to 50% plaque reduction neutralization test (PRNT50) for confirmation. 20 (9.4%) sera samples were positive for ZIKV prM protein but not against prM of dengue and Japanese encephalitis virus. These 20 samples were further subjected to PRNT50, and six samples (2.8%) showed possible seropositivity for ZIKV. This preliminary investigation provides serological evidence of ZIKV infection in Sarawak and highlights the importance of conducting surveillance programs for ZIKV in this dengue-endemic state. *Malaysian Journal of Medicine and Health Sciences* (2024) 20(1):374-377. doi:10.47836/mjmhs.20.1.46

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samples of dengue suspected cases collected for a dengue surveillance study in Kuching, Sarawak from 2007 to 2011.

## INTRODUCTION

ZIKV is a member of the *flavivirus* family and is closely related to other viruses such as DENV, JEV, yellow fever and West Nile viruses. Although it generally causes mild illness, the re-emergence of ZIKV has been associated with microcephaly and other neurological disorders (1), leading to the declaration of a Public Health Emergency of International Concern by WHO in 2016 (2). While most neighboring Southeast Asian countries have reported sporadic cases and limited outbreaks of ZIKV (3), Malaysia has reported very few cases to date, with only eight cases diagnosed as of 2018 (4). Sarawak, a Malaysian Borneo state, has never reported any cases of ZIKV, although two autochthonous cases were reported in its neighboring state of Sabah in 2016 (5). One possible explanation for the lack of reported cases in Sarawak is high pre-existing levels of population immunity to ZIKV. However, no seroprevalence study on ZIKV has ever been conducted in Sarawak. This study aims to provide a preliminary insight into the serological evidence of ZIKV in Sarawak by analyzing retrospective

## MATERIALS AND METHODS

### Serum samples

212 serum samples were tested in this study. These retrospective febrile patients' samples were part of dengue surveillance study in Kuching, Sarawak from year 2007 to 2011 and were stored under optimal conditions. These samples were selected based on (i) tested negative by RT-PCR for both DENV and JEV in the surveillance study; (ii) sufficient volume available ( $\geq 300 \mu\text{l}$ ). The used of archived samples is approved by the university's medical research ethics committee (FME/22/69).

### Viruses and cell lines

The virus strains used in in this study were MR 766 (ZIKV), FSS 13025 (ZIKV), P6 740 (ZIKV), D2 NGC (DENV-2) and JEV Nakayama (Japanese encephalitis virus). All the viruses were propagated in C6/36 *Aedes albopictus* mosquito cell line. Neutralization test were done against MR 766 and D2 NGC virus strains using Vero cell line (African green monkey epithelial kidney cells).

### Preparation of infected cell lysate, western blotting and immunoblots assay

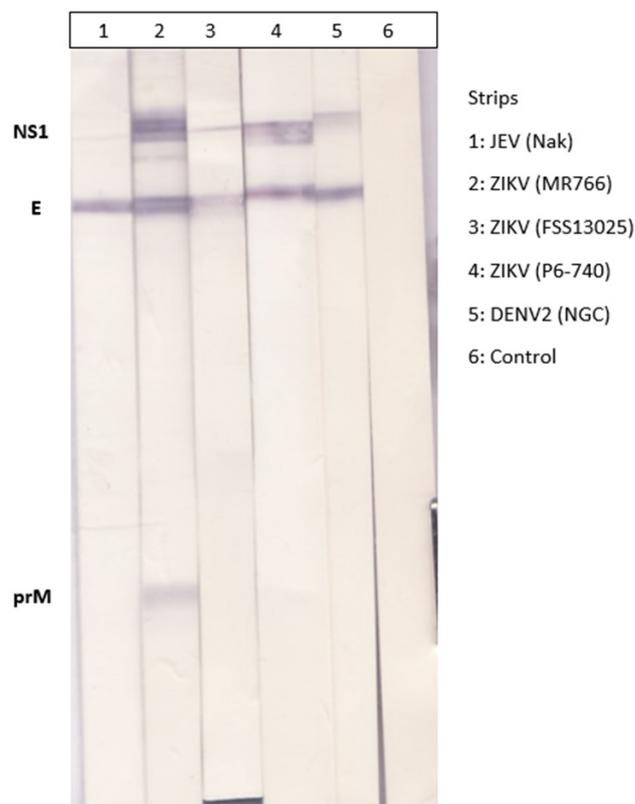
The assay was essentially performed as described previously (6). The methods used in the aforementioned study was used in preparing the infected cell lysates, the western blotting and the immunoblots assay for this investigation. In the immunoblots assay, the membrane strips were probed with serum diluted at 1:200 dilution overnight. The secondary antibody used in the assay is anti-human IgG HRP (Dako P214). All the protocol is as described previously.

### Plaque reduction neutralization test (PRNT50)

The PRNT50 was performed as originally described by deMadrid and Potterfield (7) using Vero cells and semi solid carboxymethylcellulose overlay. Four fold serial dilutions (dilution at 1:10, 1:40, 1:160, 1:640, 1:2560) of heat inactivated sera were incubated together with either ZIKV or DENV for 1 hour at 37°C in duplicate in 24-well plates (TPP, Switzerland). The cell suspension was then added and allowed to adhere for 3 hours at 37°C before addition of overlay. The plates were incubated for 6 days before the monolayer stained with naphthalene black and the plaques were counted.

## RESULTS AND DISCUSSION

This short study was conducted on retrospective samples that were available at the author's laboratory. A total of 788 sera samples were collected for a dengue surveillance study from the year 2007-2011 in the capital city of Sarawak, Kuching. All samples that were previously tested negative by RT-PCR for both dengue and Japanese encephalitis with available remaining sample volume of  $\geq 300 \mu\text{l}$  were included in the analysis. 212 samples fits the criteria and were screened by immunoblots assay. Samples with positive response to the prM protein of either strains of ZIKV (MR 766, FSS 13025 and P6 740) but not to DENV and JEV (Fig. 1) were determined as probable ZIKV infection. Serologic cross-reactivity between Flavivirus is well documented which making determination of ZIKV seroprevalence in dengue-endemic areas such as the situation in Malaysia and similarly in Sarawak, difficult. The cross-reactive epitopes of Flavivirus are mainly found on the viral E protein. The E protein has been shown to be most homologous among the flaviviruses and serological cross-reactivity is common among flaviviruses (8). Study by Priyamvada and colleagues (9), reported that the E protein of ZIKV and DENV2 share ~54% amino acid sequence identity. This explains the highly cross-reactive positivity of the E antigen that is also observed in this study (Fig. 1). Other study had also reported high protein identity, although to a lower similarity to DENV2, to other arthropod borne viruses such as West Nile virus, Yellow Fever virus and Tick-borne encephalitis virus (10). Study has also shown that in early DENV infection, cross-neutralization of ZIKV is detectable. The response is more specific only after more than 6



**Figure 1: Representative Immunoblot strips showing clear detection of prM of ZIKV (MR766) but not prM of DENV and JEV. Strips contain viral antigens separated by SDS-PAGE under non reducing without heating conditions.**

months post-infection (11). To overcome this limitation, we employed the method of detecting specific response to the prM protein of ZIKV prior to neutralization test to ZIKV and DENV. According to Cardoso and colleagues (6), antibodies against the prM protein of JEV do not cross-react with the prM of DENV or WNV therefore it is conceivable that detection of specific response to prM is a useful tool in differentiating antibody responses to different flaviviruses. By this approach we reporting a 9.4% (20 samples) with positive response to prM of ZIKV and not to DENV and JEV. Six samples (2.8%) showed possible neutralization to ZIKV; these are samples that produced 50% plaque reduction dilution at  $\geq 1:40$  or 4-fold greater than DENV titer (Table I). This data is the first available serological evidence of ZIKV from Sarawak, Malaysian Borneo. The low seropositivity does not agree with the high-level pre-existing immunity in the community theory. This study is also in line with the finding of other studies in Malaysia that show low seroprevalence rates of Zika virus (4,12,13). On the other hand, a study conducted in a neighboring state, Sabah, had shown an increased in the incidence of ZIKV infection (14). Therefore, in order to understand better the serostatus of ZIKV in Sarawak, further study with a larger sample number and more recent samples collection is required, these are among the clear limitations of this study. Another limitation to this study is, the limited amount of serum available which limit the neutralization study with other flaviviruses only to DENV. Ideally if

**Table 1: ZIKV specific prM antibody detected in 20 samples and seropositive in 6 of the samples**

Sample	Year of collection	Immunoblots assay (prM)					PRNT50		Interpretation
		ZIKV (FSS 13025)	ZIKV (MR 766)	ZIKV (P6 740)	DENV-2	JEV	MR 766	DENV-2	
<b>DKA7/9/1</b>	<b>2007</b>	+	++	+	-	-	<b>1:160</b>	<b>1:10</b>	<b>ZIKV</b>
DKB7/8/15	2007	-	+	-	-	-	1:10	1:10	ND
DKB7/21/15	2007	+	+	+	-	-	1:10	1:10	ND
<b>DKB7/22/15</b>	<b>2007</b>	-	++	-	-	-	<b>1:160</b>	<b>1:40</b>	<b>ZIKV</b>
<b>DKB7/23/15</b>	<b>2007</b>	+	++	+	-	-	<b>1:160</b>	<b>1:10</b>	<b>ZIKV</b>
DKB7/25/15	2007	+	+	-	-	-	1:40	1:40	ND
DKC7/4/1	2007	-	+	-	-	-	<1:10	<1:10	ND
DKA8/25/15	2008	-	+	-	-	-	<1:10	<1:10	ND
DKA8/32/15	2008	+	+	+	-	-	1:10	1:10	ND
<b>DKB8/5/15</b>	<b>2008</b>	+	+	+	-	-	<b>1:160</b>	<b>&lt;1:10</b>	<b>ZIKV</b>
DKB8/16/15	2008	-	+	-	-	-	1:10	1:10	ND
DKC8/3/1	2008	-	+	+	-	-	1:10	1:10	ND
DKC8/7/1	2008	+	+	-	-	-	<1:10	<1:10	ND
DKD8/12/1	2008	-	+	-	-	-	<1:10	<1:10	ND
<b>DKA10/12/1</b>	<b>2010</b>	+	++	+	-	-	<b>1:640</b>	<b>1:10</b>	<b>ZIKV</b>
<b>DKA10/18/1</b>	<b>2010</b>	-	+	-	-	-	<b>1:40</b>	<b>&lt;1:10</b>	<b>ZIKV</b>
DKA10/53/1	2010	-	+	-	-	-	<1:10	<1:10	ND
DKA10/65/1	2010	+	+	-	-	-	1:10	1:10	ND
DKC11/1/1	2011	-	+	+	-	-	<1:10	<1:10	ND
DKC11/4/1	2011	-	+	-	-	-	<1:10	<1:10	ND

++ = positive for prM; + = weak positive for prM; - = negative for prM; ND = not determined

the amount of sample permitted, PRNT50 with other flaviviruses such as JEV will give a more comprehensive result. Nevertheless, this preliminary finding warrant a surveillance study of ZIKV infection in Sarawak, to assess the seroprevalence and seroconversion of ZIKV in this Borneo State of Malaysia.

## CONCLUSION

In conclusion, this study highlights the potential presence of Zika virus (ZIKV) in Sarawak, Malaysian Borneo, with a seroprevalence of 9.4% based on the detection of specific responses to the prM protein of ZIKV. However, the low seropositivity does not support the theory of high-level pre-existing immunity in the community. The results also underscore the challenges of determining ZIKV seroprevalence in dengue-endemic areas due to the well-known serologic cross-reactivity between flaviviruses. The study emphasizes the importance of continued surveillance of ZIKV in Sarawak, as misdiagnosis may occur due to overlapping clinical presentations with other tropical diseases such as dengue. Further research is needed to better understand the epidemiology of ZIKV in Sarawak and to inform public health strategies for preventing and controlling the spread of this emerging infectious disease.

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