Jpn. J. Infect. Dis., 72, 115–117, 2019

Short Communication

Neutralization Potency of Sera from Vietnamese Patients with Japanese Encephalitis (JE) against Genotypes I and V JE Viruses

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SUMMARY: Japanese encephalitis virus (JEV) is classified into 5 genotypes (GI, GII, GIII, GIV, and GV), and the GI and GIII strains are the most widely distributed in JE endemic areas. In recent years, GV JEV has been detected in China and Korea, suggesting that GV JEV may invade other JE endemic areas, including Vietnam, and that more attention should be paid to the JEV strains circulating in these areas. In this study, we investigated the neutralization ability of the sera collected from 22 Vietnamese patients with JE who lived in northern Vietnam against the GI and GV JEV strains. In most cases, the ratios of the titer against GV to that against GI (GV:GI) were equal to or less than 1:4. However, the titer against GV JEV was equivalent (1:1) to that against GI JEV in only a few cases, and no serum had a ratio higher than 1:1. Thus, our results did not show convincing evidence that GV JEV was emerging in northern Vietnam in 2014.

Japanese encephalitis (JE) is a central nervous system disease caused by infection by Japanese encephalitis virus (JEV), and it represents a significant public health problem in Asia. There are an estimated 68,000 cases of JE per year, resulting in 15,000 fatalities (1). JEV is a causative agent of acute encephalitis syndrome in Vietnam, with several hundred cases of JE annually. In 2014, 741 JE cases with 36 deaths were reported in the northern area of Vietnam (2). Humans are infected with JEV, which belongs to the genus *Flavivirus* in the family Flaviviridae, mainly through bites by *Culex* mosquitoes. JEV is classified into 5 genotypes, GI, GII, GIII, GIV, and GV, based on its genomic sequence (3). The GIII strain was the most widely distributed in JE endemic areas until the 1990s. However, the major genotype has begun to change from the GIII to GI strain since the early 1990s in most JE endemic areas (4–6). GI has been the only genotype detected in northern Vietnam since 2004 (7). It was suggested that GI JEV strains circulating in recent years are better able to replicate in mosquitoes than GIII strains (5). The first GV JEV (Muar strain) was isolated from a patient with encephalitis in Malaysia in 1952; however, no other GV JEV has been found in over 50 years (3). In 2009, GV JEV (strain XZ0934) was identified in *Culex* mosquitos in China, and the infectious virus was also isolated (8). The genomes of GV JEV were also detected in mosquitoes in 2010 in Korea (9). Moreover, all the JEV genomes detected in mosquito pools collected in Korea in 2012 were those of GV JEV (10). These findings raise the possibility that GV JEV may be emerging in other endemic areas, including northern Vietnam. Therefore, it is necessary to monitor the dynamics of circulating JEV strains in JE endemic areas.

It is quite difficult to detect the JEV genome in specimens from patients with JE. The amino acid sequence of the JEV E protein differs by 3% between GI and GIII JEV; however, the difference ratio in the amino acid sequence of the JEV E protein increases to 8–10% between the GI and GV strains and between the GIII and GV strains (3,11). Recently, we showed that the neutralization ability of mouse sera immunized with a GIII JE vaccine against the GV Muar strain was less than those against the GI and GIII JEV strains (11). These findings indicate that the antigenicity of GV JEV may be different from those of GI and GIII JEV, raising the possibility that the neutralization test can be used to differentiate between the GV JEV and other genotypes (GI and GIII) in patients with JE. In this study, we examined the neutralization ability of sera collected from patients with JE in northern Vietnam in 2014 against GI and GV JEV to evaluate the possibility that GV JEV was spreading in northern Vietnam in 2014.

This study was approved by the Institutional Review Board of NIHE, Vietnam (IRB-VN01057-23/2015, September 14, 2015). We used sera from 22 Vietnamese patients with JE (Table 1), who all lived in Son La province in northern Vietnam. The serum samples were subjected to an anti-JEV IgM antibody detection ELISA

Received June 4, 2018. Accepted September 28, 2018.
J-STAGE Advance Publication October 31, 2018.
DOI: 10.7883/yoken.JJID.2018.232

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Table 1. Neutralization titers of the sera from Vietnamese patients with JE against the GI Mie/41/2002 and GV Muar strains (experiment 1)

Patient No.	Age	Gender	Days after onset	JE Vaccination history	$PRNT_{50}$		Muar: Mie/41
					Muar (GV)	Mie/41/2002 (GIa)	
5819	40	Male	3	Unknown	160	160	1:1
5855	22	Male	12	ND*	1,280	2,560	1:2
5856	6	Female	9	ND	80	2,560	1:32
5857	5	Female	13	ND	5,120	10,240	1:2
5859	2	Male	6	ND	2,560	5,120	1:2
5862	8	Male	6	ND	40	320	1:8
5864	16	Male	17	Unknown	320	2,560	1:8
5865	10	Male	6	ND	640	5,120	1:8
5879	32	Female	13	Unknown	5,120	5,120	1:1
5880	2	Male	11	Unknown	40	320	1:8
5882	2	Female	10	ND	1,280	2,560	1:2
5898	2	Female	7	ND	640	2,560	1:4
5899	19	Female	7	ND	320	2,560	1:8
5905	19	Male	9	ND	80	640	1:8
5919	10	Female	2	ND	160	640	1:4
5936	34	Male	3	Unknown	160	640	1:4
5942	25	Male	7	ND	80	320	1:4
5956	11	Female	15	ND	320	2,560	1:8
5959	6	Female	2	ND	320	2,560	1:8
6043	26	Female	3	ND	160	320	1:2
6051	21	Male	6	ND	80	1,280	1:16
6114	20	Female	4	ND	20	40	1:2
Median	—	—	—	—	240	2,560	—
Mode	—	—	—	—	160	2,560	—
GMT**	—	—	—	—	273	1,202	—

*: ND, not done.
**: GMT, geometric mean titer.

Table 2. Neutralization titers of the sera from Vietnamese patients with JE against the GIa Mie/41/2002, GIb Mie/51/2005, and GV Muar, and recombinant rJEV-E^{XZ0934}-M41 (experiment 2)

Patient no.	$PRNT_{50}$				Muar: Mie/41	Muar: Mie/51	E^{XZ0934}: Mie/41	E^{XZ0934}: Mie/51
	Muar (GV)	rJEV-E^{XZ0934}-M41	Mie/41/2002 (GIa)	Mie/51/2006 (GIb)				
5819	40	40	80	80	1:2	1:2	1:2	1:2
5856	10	40	1,280	640	1:128	1:64	1:32	1:16
5859	320	1,280	2,560	1,280	1:8	1:4	1:2	1:1
5905	40	40	640	640	1:16	1:16	1:16	1:16

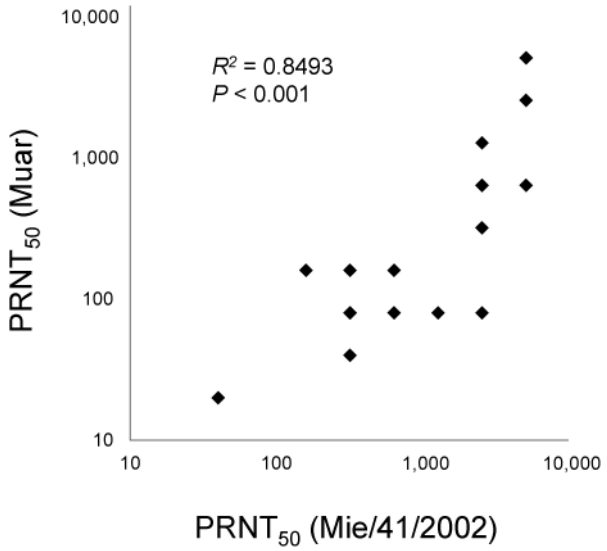

Fig. 1. Correlation of the neutralization titer ($PRNT_{50}$) of the sera from Vietnamese patients with JE against the GI Mie/41/2002 strain with that against the GV Muar strain. Spearman rank correlation coefficient was calculated and coefficient of determination (R^2) and statistical significance (P) were indicated.

using the GIII JEV antigen as described previously (12), and all sera were positive for anti-JEV IgM (data not shown). The onset dates of the patients with JE were between May and August in 2014. No JEV genome was detected in the patient specimens using the genome amplification method. The plaque reduction neutralization test (PRNT) was conducted using GV strain Muar, GI subgenotype a (GIa) strain Mie/41/2002, GI subgenotype b (GIb) strain Mie/51/2006, and a recombinant JEV rJEV-E^{XZ0934}-M41, which contains the majority of the E region of GV strain ZX0934 in the backbone of the Mie/41/2002 genome, as described previously (11). Briefly, each JEV solution was mixed with 2-fold serial dilutions of the sera, and the mixtures were then incubated. Vero cell monolayers were inoculated with each mixture and incubated; then, overlay medium containing 1% methylcellulose was added, and the cells were cultured for 5 days. The neutralization titer (50% PRNT titer, $PRNT_{50}$) of each specimen was defined as the reciprocal of the highest dilution level that resulted in a 50% reduction of the plaque number relative to that of the non-serum control.

The $PRNT_{50}$ of the patient sera against GIa Mie/41/2002 ranged between 40 and 10,240, whereas that against GV Muar ranged between 20 and 5,120 (Table 1). The median, mode, and geometric mean titer of the $PRNT_{50}$ against Mie/41/2002 were 2,560, 2,560, and 1,202, respectively, whereas those against Muar were 240, 160, and 273, respectively. The $PRNT_{50}$ against Muar correlated with that against Mie/41/2002, indicating that the neutralizing ability of each patient serum showed a similar trend for both challenge viruses even though the neutralization titer did not match in most samples (Fig. 1). The ratios of the titer against Muar to that against Mie/41/2002 (Muar:Mie/41) were also calculated. Fourteen (63.6%) and 6 (27.3%) out of 22 sera had a ratio equal to or less than 1:4 and equal to or less than 1:2, respectively. In 2 sera (9.1%), the titer against Muar was equivalent (1:1) to that against Mie/41/2002. No serum sample had a ratio higher than 1:1. The $PRNT_{50}$ of the 4 patient sera against GIb Mie/51/2006 and rJEV-E^{XZ0934}-M41, together with Muar and Mie/41/2002, were also measured (Table 2). The $PRNT_{50}$ values against Mie/51/2006 resembled those against Mie/41/2002, while the titers against rJEV-E^{XZ0934}-M41 were more similar to those against Muar.

In this study, the $PRNT_{50}$ values of the sera collected from the patients with JE in northern Vietnam against the GI and GV JEV strains were determined. The

$PRNT_{50}$ values against GI JEV were higher than those against GV JEV in most patients with JE tested in this study. Thus, our results did not provide convincing evidence that GV JEV was emerging in northern Vietnam in 2014 and suggest that GV JEV may not have been spreading there. However, it might not be possible to apply the neutralization test to estimate the genotype of patients suspected of being infected, because it is not known whether sera from patients infected with GV JEV have a higher $PRNT_{50}$ to GV JEV than to GI JEV. Additional studies comparing specimens collected from patients confirmed to be infected with GV JEV with those from patients confirmed to be infected with GI JEV are needed to evaluate the ability of the neutralization test to discriminate between these strains. GV JEV has not been detected in Vietnam; however, continuous and careful monitoring of GV JEV must be undertaken.

Acknowledgments This research is supported by the Japanese Initiative for Global Research Network on Infectious Diseases and the Research Program on Emerging and Re-emerging Infectious Diseases of the Japan Agency for Medical Research and Development (AMED) under Grant Number 18fm0108001.

Conflict of interest None to declare.

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