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Short Report: Investigation of Dengue and Japanese Encephalitis Virus Transmission in Hanam, Viet Nam

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Abstract. This study investigated whether a large dengue epidemic that struck Hanoi in 2009 also affected a nearby semirural area. Seroconversion (dengue virus-reactive immunoglobulin G enzyme-linked immunosorbent assay) was high during 2009 compared with 2008, but neutralization assays showed that it was caused by both dengue virus and Japanese encephalitis virus infections. The findings highlight the importance of continued Japanese encephalitis virus vaccination and dengue surveillance.

Dengue is an emerging health problem in northern Viet Nam, and in Hanoi, the capital, the largest recorded outbreak occurred in 2009, when 16,175 clinical cases were reported.[1] Clinical dengue incidence in northern Viet Nam is highest in urban Hanoi, and transmission commences around July, several months after the end of winter, and ceases in late December,[1] coinciding with changes in vector abundance.[2] Dengue cases have been detected sporadically in other northern provinces of Viet Nam,[3] and 11% of dengue patients presenting to a Hanoi hospital during 2008 had come from other provinces.[4] The extent to which this finding reflects local transmission or infections acquired during travel to endemic areas, such as Hanoi, is not clear. Serology is an important tool for understanding dengue virus (DENV) transmission, because a variable and sometimes large proportion of infections can be asymptomatic.[5] Therefore, we used serology to investigate DENV infection and transmission in a semirural commune approximately 60 km from Hanoi.

A cohort of 270 households was selected randomly from a semirural commune in Hanam province. Blood samples were collected in December of 2007, December of 2008, June of 2009, and April of 2010[6] (i.e., outside the typical dengue season). The research was approved by the institutional review board of the National Institute of Hygiene and Epidemiology and the Oxford Tropical Research Ethics Committee, University of Oxford. All participants provided written informed consent. DENV-reactive immunoglobulin G (IgG) in sera was detected by indirect enzyme-linked immunosorbent assay (ELISA) using plates coated with purified DENV-1, -2, -3, and -4 virions (Panbio E-DEN01G; Alere, Brisbane, Australia). As per the manufacturer's instructions, sera with absorbance values < 0.9 times the cutoff for the kit were classified as negative, sera with values > 1.1 times the cutoff were classified as positive, and sera with intermediate values were classified equivocal. ELISA seroconversion was defined as a change from negative to positive. Both Japanese encephalitis virus (JEV) and DENV circulate in northern Viet Nam, and there is substantial cross-reactivity between flavivirus antibodies.[7,8] Therefore, a subset of sera was assessed using the plaque reduction neutralization test (PRNT) with DENV-1, -2, -3, and -4 and JEV antigens performed as described previously.[9]

In total, 606 participants provided blood samples in April of 2010; 29 of those participants had equivocal DENV IgG ELISA results and were excluded. Of the remaining 577 participants, 240 (41.6%) participants were male, and the median age was 31 years (interquartile range [IQR] = 13–45); 205 (35.5%) participants were DENV IgG-seropositive, and 372 (64.5%) participants were seronegative. Seroprevalence increased with age (Figure 1), and seropositive participants were significantly older than seronegative participants (median = 49 years, IQR = 32–59 years versus median = 25 years, IQR = 13–40 years, $P < 0.001$). Earlier samples were assessed to determine the proportions that seroconverted during 2008 or 2009 or were already seropositive at baseline in 2007; 3 participants did not have an earlier serum sample, and 13 participants had equivocal results, leaving 561 participants who could be assessed for seroconversion. In total, 143 of 561 (25.5%) participants were already seropositive at baseline, and 418 participants were seronegative, of whom 3 (0.7%, 95% confidence interval [95% CI] = 0.1–2.2%) participants seroconverted during 2008 compared with 43 of 415 (10.4%, 95% CI = 7.6–14.0%) participants seroconverted during 2009. Participants who seroconverted during 2009 were significantly younger (median = 32 years, IQR = 17–47 years) than participants who were already seropositive (median = 54 years, IQR = 42–63 years, $P < 0.001$). However, the proportion of children ages 10 years or less who seroconverted was very low (Figure 1).

Virus neutralization assays were performed on paired sera from participants who seroconverted and had sufficient sera remaining (31/46). The median age of this subset of participants was 31 years (IQR = 17–47 years), similar to the age of all seroconverters. DENV-1 and JEV but not DENV-2, -3, or -4 reactive antibodies were detected in post-infection sera from seroconverters by PRNT assay when titers were based on a 70% reduction in plaques (Table 1). Notably, 24 of 31 ELISA seroconverters had detectable JEV PRNT70 titers in pre-conversion sera (i.e., sera that were negative in DENV IgG ELISA) (Table 1). Similarly, when a random subset of 23 of 372 participants who remained DENV IgG-seronegative in 2010 was assessed, 10 (43%) participants were found to have detectable JEV PRNT70 titers ranging from 16 to

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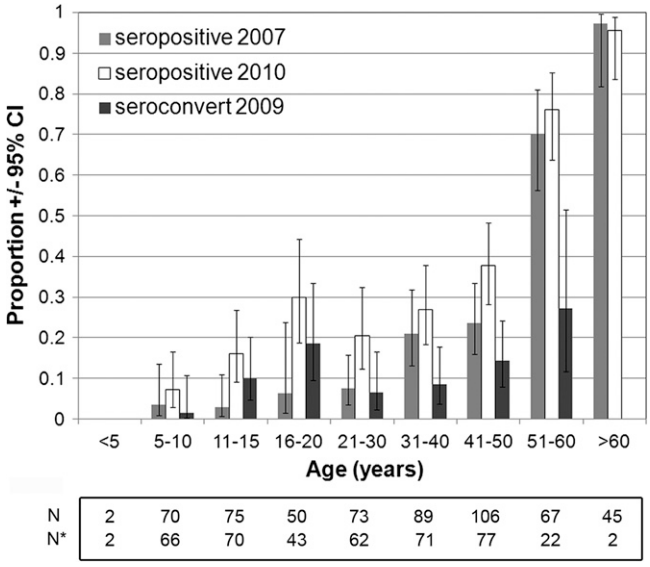

FIGURE 1. DENV IgG seroprevalence and seroconversion according to age. Results are shown as the proportion of participants that were DENV IgG-seropositive in either December of 2007 (gray bars) or April of 2010 (white bars). Also shown is the proportion of participants who seroconverted during 2009 (seronegative in December of 2008 and seropositive by April of 2010; black bars). The numbers shown below the chart are the denominator for each age group (*N*) and the denominator for seroconversion (*N**; i.e., the number in each age group that was seronegative at baseline).

34 (data not shown). This result suggests that prior JEV infection was common and not consistently detected by the DENV IgG ELISA. In contrast, all sera that were negative in DENV IgG ELISA also had undetectable PRNT70 titers against all DENV serotypes.

There are no widely accepted criteria for using PRNT to infer the infecting flavivirus or serotype of DENV infections. For example, one study considered infection to be primary if the PRNT70 titer was ≥ 30 for only one serotype (considered to be the infecting virus),[10] whereas another study considered that a PRNT50 titer ≥ 10 to only one virus was sufficient to infer primary infection.[11] Both studies classified multitypic PRNT profiles as secondary infections and seroconversion as secondary infection if the PRNT profile changed from monotypic to multitypic, but they did not attempt to infer the secondary infection serotype.[10,11] However, a statistical model combining PRNT data and polymerase chain reaction (PCR) -positive acute DENV infections from a prospective cohort study showed that the infecting DENV serotype could be inferred correctly from pre- and post-infection PRNT titers in 68% of cases, with slightly higher accuracy in primary infections than secondary infections.[12] The probability of a given DENV serotype being the infecting virus was inversely correlated with homotypic PRNT titer in pre-infection samples and positively correlated with homotypic PRNT titer in post-infection samples.[12] Here, we have inferred the infecting virus on the basis of (1) a PRNT70 titer ≥ 30 in the post-infection serum when pre-infection PRNT70 values were < 10 for all viruses or (2) the greatest rise in PRNT70 titer between pre- and post-infection samples (with minimum two-fold increase) when the pre-infection PRNT titer was ≥ 10 for at least one virus but < 30 for all viruses. Titer rises that did not meet these criteria were considered possible infections, and the absence of any titer rise was considered a false-positive seroconversion by DENV IgG ELISA. Using these criteria, 5 of 30 (17%) ELISA seroconversions during 2009 were defined as DENV-1 infection and 4 of 30 (13%) ELISA seroconversions during 2009 were defined as JEV infection (Table 1). It was not possible to differentiate between JEV and DENV in one case. The one seroconversion during 2008 that was tested by PRNT was defined as JEV infection; 7 (23%) ELISA seroconversions in 2009 were classified as possible JEV infections on the basis of PRNT titers, and 14 of 30 (47%) ELISA seroconversion seemed to be false-positive seroconversions (most of whom had pre- and/or post-infection ELISA values close to the negative or positive cutoff, respectively) (Table 1). This finding suggests that the ELISA cutoff values were not stringent enough for defining DENV seroconversion in this study. A similar study conducted in Brazil found that 35% of dengue IgG ELISA converters did not convert in PRNT50 assay with DENV and yellow fever antigens.[13]

PRNT profiles were also examined for a random subset of 19 of 143 participants who were seropositive in DENV IgG ELISA at baseline in December of 2007. Profiles were increasingly multitypic with increasing age, indicating that older seropositive participants have probably experienced more than one flavivirus infection (Table 2). The highest PRNT titers were most often against JEV (*N* = 8) followed by DENV-1 (*N* = 5) and DENV-2 (*N* = 4). Virus surveillance data indicate that DENV-1 and DENV-2 have been the most prevalent DENV types circulating in Hanoi since 1998 (Mai Quynh Le, personal communication).

We set out to determine if a large dengue epidemic that struck Hanoi in 2009 also affected a semirural area about 60 km away. Although 10% of cohort participants seroconverted in DENV IgG ELISA during 2009, virus neutralization assays indicated that only 17% of those seroconversions represented true DENV infections. This result equates to an overall estimated DENV infection incidence of 1.7% in cohort participants during 2009, assuming the same distribution of PRNT profiles in the one-third of seroconverters who did not have sufficient sera remaining for PRNT. All DENV infections were caused by DENV-1, the predominant serotype detected in Hanoi during 2009. The virus neutralization data also indicated that another 13% of seroconversions in 2009 were caused by JEV infection. A monotypic JEV neutralizing antibody PRNT profile was also common in baseline sera from participants across all ages (Tables 1 and 2), indicating exposure to JEV in preceding years. Together, the findings indicate that both DENV-1 and JEV circulated in Hanam in 2009, with an increased incidence of both infections in the cohort compared with the previous year. Similarly, the number of clinical dengue cases reported to the Hanam Preventive Medicine Center was higher in 2009 than previous years: 45 cases in 2009 compared with < 10 cases/year between 2001 and 2008. This result represents an incidence of 5.7 cases per 100,000 people in Hanam compared with 345 cases per 100,000 people in Hanoi during the 2009 outbreak.[1] The proportion of participants who said that they traveled outside Hanam at least one time per month was only 5.9% (95% CI = 4.5–7.6%), and none traveled on a weekly or daily basis. It is, therefore, likely that increased detection of both dengue cases and infections in Hanam during 2009 reflects some local transmission, but additional investigation of population movements between Hanam and Hanoi is required to be conclusive.

TABLE 1

DENV and JEV PRNT titers in pre- and post-dengue IgG ELISA conversion sera

			Dengue		PRNT 70*						PRNT 90*			
			IgG ELISA†		JEV			DENV-1			JEV		DENV-1	
Age (years)	Sex	Inference	Pre	Post	Pre	Post	Ratio	Pre	Post	Ratio	Pre	Post	Pre	Post
54	F	DENV-1	0.58	3.29	26	29	1.1	–	151	30.2	14	11	–	87
31	F	DENV-1	0.19	2.82	19	17	0.9	–	38	7.6	–	10	–	26
45	F	DENV-1	0.29	2.43	14	35	2.5	–	30	6.0	–	15	–	19
16	M	DENV-1	0.61	3.76	22	19	0.9	–	31	6.2	11	–	–	14
12	M	DENV-1	0.32	1.28	11	–	0.5	–	22	4.4	–	–	–	12
36	M	DENV-1 or JEV	0.17	3.72	10	73	7.3	–	31	6.2	–	14	–	19
14	M	JEV	0.24	2.34	–	1,053	210.6	–	–		–	339	–	–
18	M	JEV	0.26	1.80	13	126	9.7	–	–		–	70	–	–
5	**F**	JEV	0.37	1.52	–	114	22.8	–	–		–	52	–	–
24	M	JEV	0.69	1.60	29	64	2.2	–	–		16	30	–	–
12	F	JEV	0.23	2.67	–	33	6.6	–	–		–	11	–	–
49	M	Possible JEV	0.38	1.28	45	90	2.0	–	–		23	49	–	–
40	M	Possible JEV	0.55	1.16	32	70	2.2	–	–		16	14	–	–
35	F	Possible JEV	0.64	1.75	38	50	1.3	–	–		18	14	–	–
46	F	Possible JEV	0.68	1.24	64	101	1.6	–	–		30	25	–	–
15	F	Possible JEV	0.11	1.41	–	12	2.4	–	–		–	–	–	–
19	M	Possible JEV	0.12	1.26	–	11	2.2	–	–		–	–	–	–
19	F	Possible JEV	0.30	1.54	20	34	1.7	–	–		10	–	–	–
43	F	False	0.10	2.50	–	–		–	–		–	–	–	–
51	M	False	0.14	4.35	37	12	0.3	–	–		19	12	–	–
19	F	False	0.38	1.11	32	32	1.0	–	–		16	19	–	–
46	M	False	0.50	1.17	38	16	0.4	–	–		18	–	–	–
16	M	False	0.75	1.33	36	20	0.6	–	–		13	–	–	–
30	F	False	0.76	1.51	15	13	0.9	–	–		–	–	–	–
12	F	False	0.80	1.63	34	30	0.9	–	–		16	12	–	–
26	F	False	0.81	1.57	19	–	0.3	–	–		11	–	–	–
41	M	False	0.81	1.21	12	–	0.4	–	–		–	–	–	–
54	F	False	0.82	1.88	–	–		–	–		–	–	–	–
43	F	False	0.82	1.46	49	–	0.1	–	–		17	–	–	–
17	F	False	0.83	1.36	115	82	0.7	–	–		38	36	–	–
49	F	False	0.90	1.24	59	26	0.4	–	–		28	10	–	–

Results are shown for 31 participants, including 30 participants who seroconverted by dengue-reactive IgG ELISA during 2009 and 1 participant who seroconverted by dengue-reactive IgG ELISA during 2008 (bold). DENV-2, -3, and -4 PRNT titers were all below 10. F = female; M = male.

*Results are shown as reciprocal PRNT titers calculated using two-point linear regression. Sera with no detectable neutralizing activity at the lowest dilution (1:10) are indicated by – and were assigned a titer of five for the purpose of calculating ratios.

†Dengue-reactive IgG ELISA units calculated as the ratio of sample absorbance to kit calibrator value.

Data on virological surveillance in mosquitoes and pigs in the study region are scant. A study of mosquitoes in the northern provinces of Viet Nam, including Hanam, in 2002 and 2004 indicated that DENV vectors *Aedes albopictus* and *Ae. aegypti* represented < 0.1% of mosquitoes.[14] The main vector for JEV, *Culex tritaeniorhynchus*,[15] was abundant,[14] consistent with their habit of feeding on wading birds and domestic mammals, particularly pigs present in rural rice fields.[16] JEV could not be isolated from mosquitoes in that study, but another study isolated JEV from *Cx. tritaeniorhynchus* in neighboring Hatay province in 2006.[15] Pig densities are around 400 head/km^2 in the Red River Delta and Hanam, representing the highest densities in Viet Nam.[17] Several studies in Viet Nam show that the majority of pigs have JEV antibodies.[18,19]

JEV is characterized by high rates of subclinical infection. Between 1 in 25 and 1 in 1,000 infected people develop illness in unvaccinated populations, and only 1 in 2 million people develop illness in vaccinated populations.[20] JEV vaccine administration started in the study district in 2003, when 95% of 1- to 5-year-old children received two doses of vaccine and had a third dose 1 year later (Viet Nam EPI data, NIHE). Syndromic surveillance data collected by the Hanam Preventive Medicine Center indicated that viral encephalitis notifications decreased after JEV vaccine was introduced from 35 cases in 2003 to between 6 and 8 cases in 2007, 2008, and 2009.

TABLE 2

PRNT70 titers for a randomly selected subset of participants who were seropositive in DENV IgG ELISA at baseline

Age (years)	DENV-1	DENV-2	DENV-3	DENV-4	JEV
7	–	–	–	–	42*
22	–	–	–	–	127*
36	–	25*	–	–	24†
42	–	–	–	79*	40
44	–	–	–	24†	32*
44	–	117*	–	–	20†
45	42*	–	–	10	–
57	30	–	–	47	110*
60	131*	–	–	–	57
68	22	55*	–	27	12†
68	–	32*	–	–	27
72	–	26	–	–	41*
76	17†	19	67*	16‡	25
77	–	15†	–	–	17*
77	15†	–	–	17†	19*
77	29	23	13†	13†	105*
78	165*	–	11†	83†	39
86	203*	–	–	–	25
91	43*	18	11†	12†	22

Sera from 2007 (baseline seropositive participants: 9 male and 10 female). Results are shown as reciprocal PRNT70 titers (– indicates ≤ 10) calculated using two-point linear regression.

*The virus giving the highest titer for each individual.

†Titers were calculated to be < 10 when the neutralizing percentage was increased to 90% (i.e., PRNT90 titer < 10).

JEV vaccine introduction had also been associated with a decrease in the proportion of acute encephalitis cases that are JEV-positive.[8,14] Although JEV vaccination provides protection against clinical illness, there may be less effect on infection risk, which was exemplified in Japan, where 3–17% of the population were infected annually in the pre-vaccine era compared with 0.2–10% since vaccination was introduced.[20] The inferred proportion of participants in this study with JEV infection during 2009 was 1.4% (i.e., 13.3% of 10.4%).

In conclusion, this study found evidence of DENV infection in a semirural community nearby Hanoi during 2009. Local dengue transmission may have occurred but at a low level compared with Hanoi. JEV infections were also detected, and there was evidence of considerable past JEV exposure. Together, these findings highlight the importance of continued JEV vaccination and dengue surveillance.

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