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Short Communication

Susceptibility of Indigenous and Transplanted Mosquito Spp. to Dengue Virus in Japan

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SUMMARY: Dengue fever, an acute, mosquito-borne, febrile illness caused by *Flavivirus* spp., is a problem in Africa, South and Southeast Asia, Latin America, and the Caribbean. A dengue outbreak occurred after nearly 70 years of absence or no detection, and then 158 autochthonous cases occurred in Japan from August to October 15, 2014. The most competent mosquito vectors for dengue virus transmission were *Aedes aegypti* and *A. albopictus*. Since *A. albopictus* is widely distributed across Japan and *A. aegypti* recently invaded Japan by airplane, we examined the susceptibility of these species to infection by dengue virus.

Dengue virus (DENV), which is transmitted by *Aedes* mosquitoes, is a global public health concern associated with an average of 390 million infections annually, 96 million of which are severe (1). In Japan, 249 cases of imported dengue fever were reported in 2013 (2). *A. albopictus* is present in Japan, and in August 2012, invasion of the yellow fever mosquito, *A. aegypti*, was reported at Narita International Airport (3) and then at Haneda International Airport in early to mid-2013 (personal communication). Moreover, in late August 2013, a single DENV infection was confirmed in a German individual travelling to Japan (4,5). In this study, we demonstrated the susceptibility of foreign (imported) and Japanese (indigenous) *Aedes* mosquito spp. to DENV infection. Although, *A. aegypti* has been compared with *A. albopictus* in DENV-infection experiments by Vazeille et al., a quantitative analysis of the salivary glands (SG), which are known to facilitate DENV transmission, was not performed (6). Furthermore, there are no reports of DENV1-transmission experiments in the literature (6). Furthermore, we must be careful while interpreting the results of our studies when using *A. albopictus* colonies with greater than F40 generations for DENV2-transmission experiments, as described by Vazeille et al. (6).

Although, DENV2 antigen has been detected in the SG of *A. aegypti*, a quantitative analysis failed to confirm these findings (7). In addition, a quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) analysis failed to show DENV1 transmission (8).

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For the DENV-transmission experiments, we used DENV1, D1 (11-120), isolated from the serum of a returnee from Bangkok, Thailand, and DENV2, D2 (11-122/1), isolated from the serum of a returnee from Bali, Indonesia (both provided by the Department of Virology I, National Institute of Infectious Diseases). The experimental mosquitoes were *A. aegypti*, LBN strain, collected in Los Banos, Philippines (2011); *A. albopictus* IKT strain, collected in Kawasaki, Kanagawa Prefecture, Japan (2008); *A. albopictus* EBN strain, collected in Ebina, Kanagawa Prefecture, Japan (2012); and *A. albopictus* HCM strain, collected in Ho Chi Minh, Vietnam (2013). Four- to five-day-old adult mosquitoes were orally infected with DENV by exposure to blood mixed with 10^5 copies/mL of DENV in artificial membrane feeders. After 1 h of feeding, the mosquitoes were maintained at 28°C for more than 20 days. To detect DENV, the presence of viral RNA and antigens in SG, midguts (MG), and other tissure speciments (CA) of blood-fed females was examined. Pools of every 10 tissues (SG, MG, and CA) were tested for the presence and quantity of DENV by using a modified SYBR Green RT-PCR assay (9). The presence of DENV in the SG and midgut was assessed using an immunofluorescence assay.

As shown in Fig. 1A, DENV1 was detected with greater frequency in the SG of *A. aegypti* than in the SG of other mosquito spp. However, *A. aegypti* showed lower susceptibility to DENV2 infection than did *A. albopictus* (Fig. 1B). In this experiment, the *A. aegypti* population showed higher susceptibility to the 11-120 strain of DENV1 than to the 11-122/1 strain of DENV2 (Fig. 1). We confirmed the presence of DENV1 in the MG and SG of *A. albopictus* EBN strain by immunofluorescence analysis (Fig. 2B,D,F). DENV1 was detected in secretory cells, but not in the secretory cavity of the SG. DENV1 was also detected in the SG of *A. albopictus* IKT strain (Fig. 2H). Furthermore, DENV1 was detected in the ovaries of *A. albopictus* HCM strain (Fig. 2J). Since vertical transmission of dengue virus

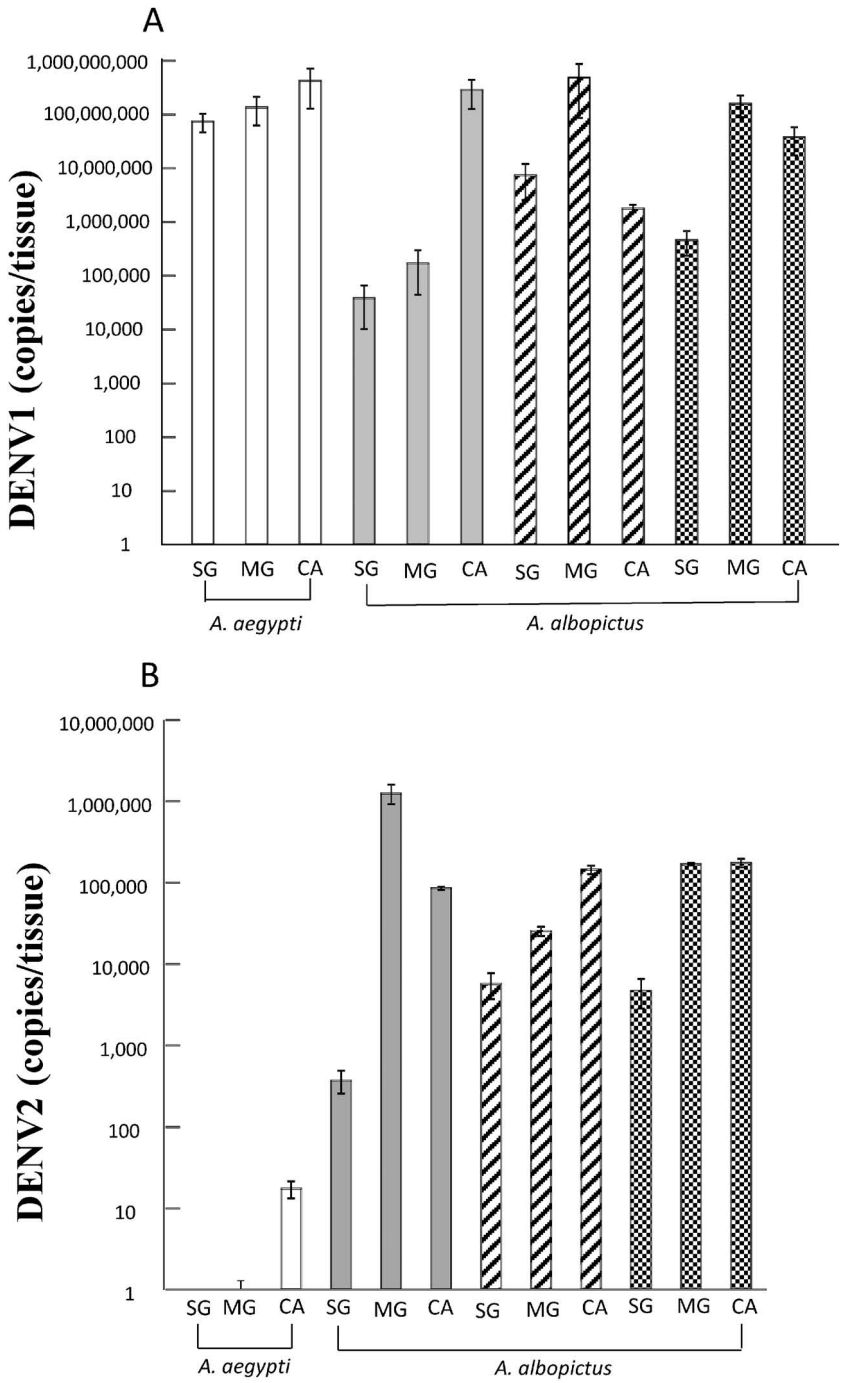

Fig. 1. Susceptibility of *Aedes aegypti* and *Aedes albopictus* to DENV1 and DENV2 infection. (A) The mosquitoes were fed on DENV1-infected blood and DENV1 genomic RNA was quantified by qRT-PCR. □, *A. aegypti* LBN strain (21 dpi); ■, *A. albopictus* IKT strain (21 dpi); ▨, *A. albopictus* EBN strain (23 dpi); ▩, *A. albopictus* HCM strain (22 dpi). (B) The susceptibility of DENV2 in *A. aegypti* and *A. albopictus*. The mosquitoes were fed on DENV2-infected blood and DENV2 genomic RNA was quantified by qRT-PCR. □, *A. aegpyti* LBN strain (21 dpi); ■, *A. albopictus* IKT strain (21 dpi); ▨, *A. albopictus* EBN strain (25 dpi); ▩, *A. albopictus* HCM strain (22 dpi). SG, salivary glands; MG, midguts; CA, carcass. The error bar represents standard deviation.

was reported in *A. aegypti* collected in Surabaya, Indonesia (2008–2011) (10), we surmised that vertical transmission of DENV1 could also occur in *A. albopictus*.

As shown in Table 1, *A. aegypti* LBN strain exhibited greater susceptibility to DENV1 strain 11-120 than to DENV2 strain 11-122/1. This differential susceptibility to a low infectious dose of dengue virus has been reported in 2 field populations of *A. aegypti* (11), suggesting the need to adopt numerous DENV strains in susceptibility testing. All 3 strains of *A. albopictus* from Japan and a foreign country were found to be susceptible, with greater susceptibility to the 11-120 strain of DENV1 than to the 11-122/1 strain of DENV2.

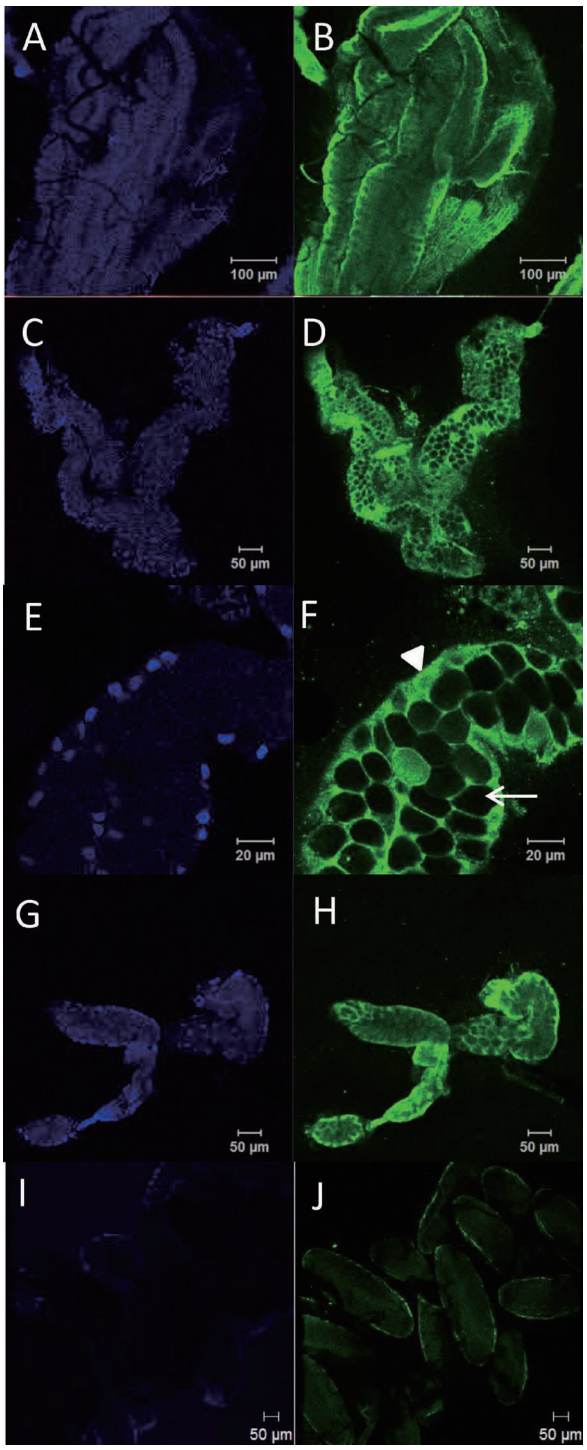

Fig. 2. (Color online) The midguts, salivary glands, and ovaries of *A. albopictus* infected with DENV1. (A–B) The midguts of *A. albopictus* EBN strain infected with DENV1 (25 dpi). A, Nuclei were stained blue with DAPI. B, Immunofluorescence assay (IFA) of viral load in an infected midguts. (C–F) The salivary glands of *A. albopictus* EBN strain infected with DENV1 (25 dpi). C and E, Nuclei were stained blue with DAPI. D and F, IFA of viral load in the infected midguts. The white arrow head represents the secretory cell. The white arrow represents the secretory cavity. (G–H) The salivary glands of *A. albopictus* IKT strain infected with DENV1 (21 dpi). G, Nuclei were stained blue with DAPI. H, IFA of viral load in the infected midguts. (I–J) Ovaries of *A. albopictus* HCM strain infected with DENV1 (22 dpi). I, Nuclei were stained blue with DAPI. J, IFA of viral load in the infected ovaries. Scale bars: (A, B), 100 μm; (C, D, G, H, I, J), 50 μm; (E, F), 20 μm.

Table 1. Susceptibility of foreign and domestic mosquitoes (*Aedes aegypti* and *Aedes albopictus*) to dengue virus infection

Days post-infection		21–25		
Tissue	Dengue virus	Salivary gland	Midgut	Carcass
Aedes aegypti LBN strain	Type 1	6	6	6
	Type 2	1	1	2
Aedes albopictus IKT strain	Type 1	6	6	6
	Type 2	2	6	6
EBN strain	Type 1	6	6	6
	Type 2	5	6	6
HCM strain	Type 1	6	6	6
	Type 2	4	6	6

6 >10,000 5 5,000–10,000 4 1,000–5,000 3 500–1,000
2 10–500 1 <10. (copies/tissue)

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Conflict of interest None to declare.

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