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Detection of Japanese Encephalitis Virus Genome in Ryukyu Wild Boars (*Sus scrofa riukiuanus*) in Okinawa, Japan

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There have been reports that wild boars have antibodies against Japanese encephalitis virus (JEV), and it has been suspected that wild boars might serve as an amplifier for transmission of JEV to humans (1-3). However, there have been no reports of isolation of JEV or detection of the JEV genome (JEV-RNA) in wild boars. In this study, we detected JEV-RNA from Ryukyu wild boars (*Sus scrofa riukiuanus*) in Okinawa Prefecture, Japan. This is the first reported detection of JEV-RNA from wild boars.

Blood samples were collected from 99 wild boars in the northern area of Okinawa Island and 27 wild boars on Iriomote Island from 1997 to 2005 (Fig. 1). The samples were centrifuged at 3,000 rpm for 10 min and the serum specimens were stored at −30°C. The sera were later examined for JEV antibody (3). Sixty-one samples (35 from northern Okinawa Island and 26 from Iriomote Island) were negative for JEV IgM and IgG antibodies (3), but only 50 of these 61 negative samples were used in the present study because the amounts of 11 samples collected from Okinawa Island were insufficient for the extraction of viral RNA.

Viral RNA was extracted from 140 μl of serum by QIAamp Viral RNA Mini kit (Qiagen, Tokyo, Japan). Viral RNA was reverse-transcribed and PCR-amplified by the One-Step RT-PCR kit (Qiagen) with primers for the E gene of JEV reported by Kuwayama et al. (4): i.e., JEen37s-first and JEen329c-first. PCR products were nested PCR-amplified by TaKaRa EX Taq (Takara Bio Inc., Shiga, Japan) with primers reported by Kuwayama et al. (4); i.e., JEen98s-second and JEen301c-second. A PCR-product of 194 nt is expected using these primers. Amplification products were separated by electrophoresis on 2% (w/v) agarose gel, and stained with ethidium bromide.

The JEV genome was detected in one of 50 wild boar serum samples. The positive serum was collected from a wild boar caught in Okinawa Island in May, 1998 and estimated to be 5 years old. Isolation of the infectious virus was attempted by inoculation of the serum into suckling mouse brains and onto Vero cells, but the infectious virus was not isolated. The serum had been hemolyzed, stored at −30°C, and frozen and thawed several times. These procedures may be the reason for the failure to isolate the virus.

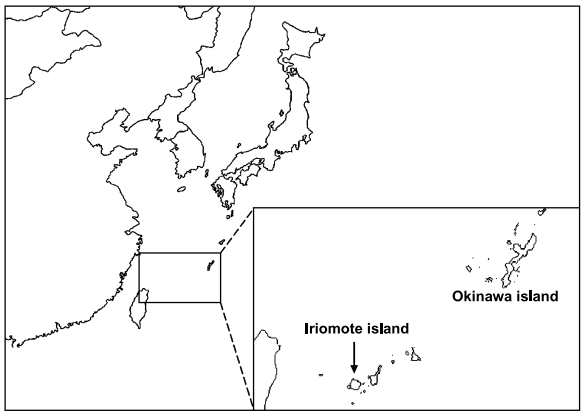

Fig. 1. Location of Okinawa Island and Iriomote Island where wild boar blood samples were collected.

The nucleotide sequence of the PCR product was then determined. JEV-RNA was reverse-transcribed and PCR-amplified with three primer sets; JE955f reported by Nerome et al. (5) and JE1606r (5′-GACYTYGAMCCCACGGTCAT-3′), JE1556f (5′-GAGTGGAYTRAACACTGAAGCG-3′) and JE2101r (5′-TCYATCTCVAHCAGCACCTTTG-3′), JE1970f (5′-CCYTGYAAAATTCCGATTGTCTC-3′) and JE2536r reported by Nerome et al. (5). The PCR product was second PCR-amplified with the same primer sets and sequenced using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, Calif., USA). The nucleotide sequence was compared with previously reported JEV sequences. The result indicated that the new JEV-RNA (JEV/wb/Okinawa/1/1998, DDBJ/EMBL/GenBank accession no. AB306941) belonged to genotype I. The sequence showed high levels (99.4%) of nucleotide homology with that of JEV/sw/Okinawa/285/2003 (AB238693) isolated from a pig on Okinawa Island in 2003 (Fig. 2).

We previously detected the antibody to JEV in Ryukyu wild boars captured in the northern area of Okinawa Island (3). In the present study JEV-RNA was detected in one of these serum samples. The nucleotide sequence of the detected JEV-RNA showed high homology with that of JEV previously isolated from swine on Okinawa Island. Detection of JEV-RNA and the JEV-specific antibody in wild boars sup-

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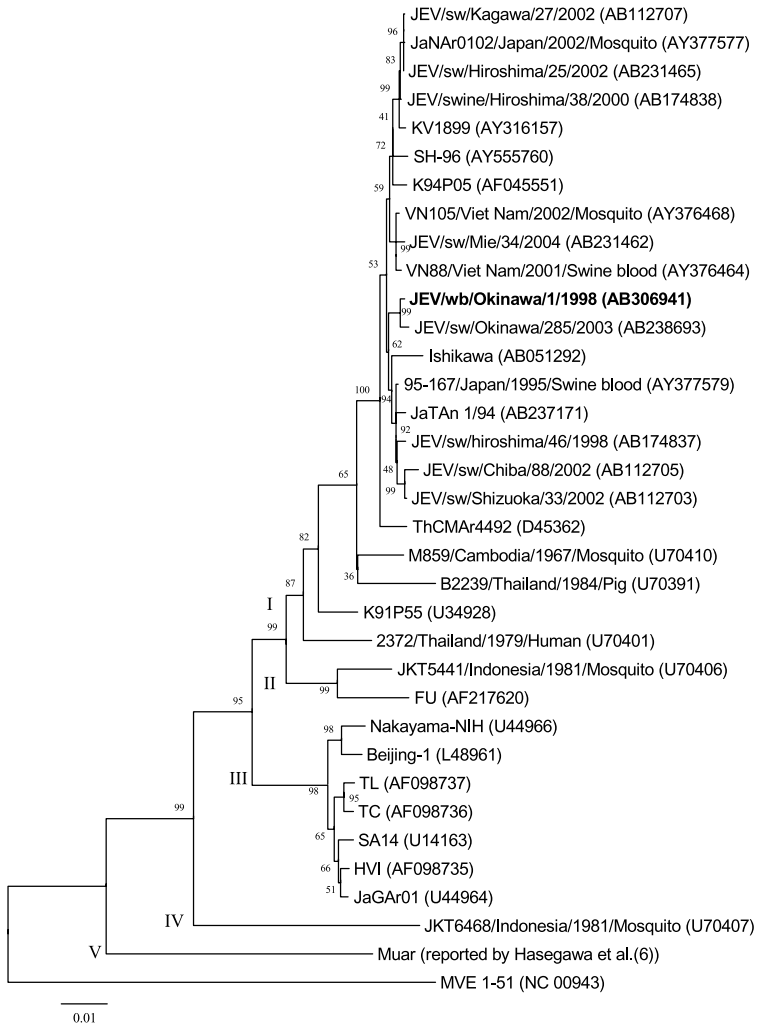

Fig. 2. Phylogenetic tree of 34 JEV strains and Murray Valley encephalitis (MVE) 1-51 strain constructed by the neighbor-joining method based on the nucleotide sequence of the E gene. JEV/wb/Okinawa/1/1998 obtained in this study is shown in bold type. I-V are genotypes indicated by Solomon et al. (7). Bootstrap support values given as a percentage of 1,000 replicates are indicated at each node.

ports the hypothesis that wild boars may play an important role as a reservoir and/or an amplifier of JEV. However, it should be stressed that further studies are still needed to confirm this hypothesis.

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