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Isolation and Molecular Comparison of Japanese Encephalitis Virus in Ishikawa, Japan

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In the past several years in Japan, only a few cases of Japanese encephalitis (JE) have been reported. It is thought that the decrease of JE cases is due mainly to vaccination and mosquito control. Another possibility is a change in the pathogenicity of JE virus (JEV). To discuss these points, it is essential to isolate new virus strains and investigate them at the molecular level.

Over the last several years, we have examined swine antibody against JEV by means of Western blot assay using JEV-E protein expressed in *Escherichia coli* and confirmed the increase of anti-JEV titer in the summer season (1,2). We isolated the viruses from swine mononuclear cells and the mosquito, *Culex tritaeniorhynchus*. The mosquitoes were collected by the dry ice trapping method in Ishikawa Prefec-

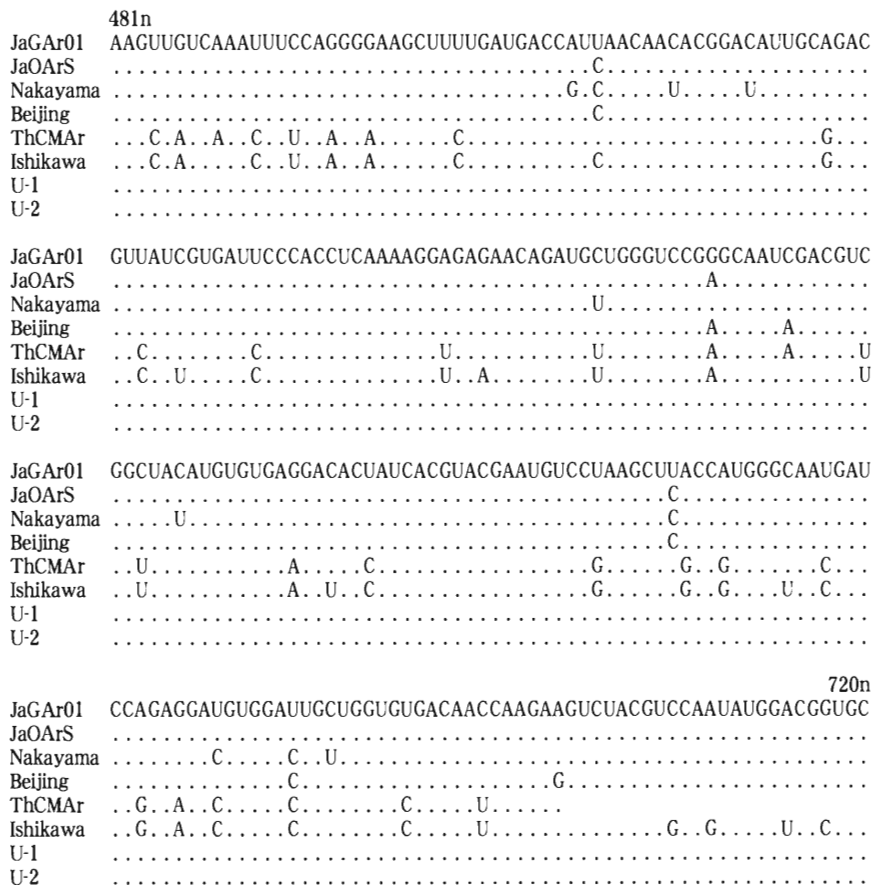


Figure. Comparison of nucleotide sequence of prM region among JEV strains. ThCMAR is Thailand strain (5).

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ture. In 1998, 1999, and 2000, we caught 200, 68, and 98 mosquitoes, respectively, at the same place and in the same season, i.e., in the middle of August. By the virus isolation method using Vero cells, we derived two JEV isolates, U1 and U2, from the mosquitoes only in 1998. We sequenced the genomic RNA of the strains using the direct sequencing method employing RT-PCR product. New JEV isolate-U1 and -U2 genomic RNAs show a nucleotide sequence similar to Japanese isolates, genotype III (Figure). Although the infectious virus was not isolated, another JEV-specific RT-PCR product was detected from the mosquito sample in 1999, and indicated the similarity to the Ishikawa strain isolated from swine mononuclear cells (1994). The amino acid sequences of the structural protein region of the Ishikawa strain showed several unique substitutions and clearly differed from other usual Japanese isolates including JaGAR01 (3) and Nakayama. The Figure indicates the difference in the prM region sequence. In addition, we found that the Ishikawa strain genomic RNA had several unique sequences including the deletion of 13 nucleotides at the 3' NCR (data not shown). These data may indicate that the Ishikawa strain belongs to a different subtype, the genotype group I, which contains Thailand JEV strains ThCMAR4492 and 6793 (4,5). Their multiplication pattern in cultured cells and their immunogenicity were almost the same as the JaGAR01 strain, a typical Japanese isolate (data not shown).

These data including the antibody assay of swine sera in Ishikawa indicate that JEV virulent types are distributed over a wide area of Japan, even though JE rarely occurs. In addition, on the basis of the sequence data of the Ishikawa strain and the recent report by Ma et al. (6), it is likely that the change in the distribution of JEV strains in Japan might have

occurred at around 1993-1994.

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