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# The emergence in Japan of *Sathuperi virus*, a tropical Simbu serogroup virus of the genus *Orthobunyavirus*

Brief Report

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**Summary.** In 1999, two viruses were isolated from blood samples of sentinel cattle in the Western part of Japan. The physiochemical and morphological properties of these viruses indicated that they belonged to the family *Bunyaviridae*. Sequence analysis of the S segment indicates that the two viruses are closely related to *Sathuperi virus* (SATV). The N-terminal 168 amino acid of the G2 protein of the M segment was highly homologous with that of SATV (98.2%). Given these results, we conclude that the newly isolated viruses are closest to SATV, which was initially isolated in India and Nigeria over 30 years ago.

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The Simbu serogroup of the genus *Orthobunyavirus* of the family *Bunyaviridae* consists of 25 antigenically related viruses that are transmitted by *Culicoides* biting midges and mosquitoes [1]. The virion contains three segments of single-stranded, negative-sense RNA, termed large (L), medium (M) and small (S) after their size [5]. The L segment codes for the L protein, that is, the viral transcriptase-replicase; the M segment encodes the polyprotein, that is, the precursor to the viral envelope proteins G1 and G2, together with a non-structural protein, NSm; and the S segment encodes the nucleocapsid (N) and non-structural (NSs) genes in overlapping reading frames. Several viruses of the Simbu serogroup are recognized as important human and animal pathogens. *Oropouche virus* (OROV) causes

human febrile illness in tropical areas of South America, and *Akabane virus* (AKAV) and Aino virus (AINOV) are responsible for bovine epidemic abortion and stillbirth and congenital defects of calves in Japan and Australia.

In 1999, two viruses were isolated from sentinel cattle in Okayama Prefecture (34.17 to 35.21 N, 133.16 to 134.24 E) in the western part of Honshu Island, Japan [6]. The physiochemical properties of these viruses and the results of examination by transmission electron microscope indicate that they belong to the family *Bunyaviridae*. In a dot immunobinding assay conducted by Yoshida and Tsuda [16], these viruses were reacted with monoclonal antibodies to the nucleocapsid protein of AKAV, showing that they belong to the Simbu serogroup. Neutralization tests indicate that two viruses were closely related. However, the viruses are not neutralized with specific antibodies to AKAV and AINOV in cross-neutralization tests, suggesting that these viruses differ from known viruses in Japan. From September to November of 1999, seroconversion (35.3% of 68) of sentinel cattle to the virus was detected throughout Okayama Prefecture and seroprevalence was observed in areas neighboring this region. However, no antibodies to the virus were detected in sera collected from sentinel cattle from 1995 to 1998 [6], indicating that the virus was new in this region in 1999.

Each virus of the Simbu serogroup is identified on the basis of cross-neutralization tests. However, it is difficult to prepare the complete set of reference viruses of the Simbu serogroup and their corresponding antibodies in each laboratory. Recently, the nucleotide sequences encoding the nucleocapsid and partial G2 protein of Simbu serogroup viruses have become available [13], providing genetic information that can be useful in clarifying the genetic position of the newly isolated viruses within the Simbu serogroup. Herein, to identify the isolated viruses, the nucleotide sequences of the S and partial M segments were determined and compared with those of other Simbu serogroup viruses.

The viruses were isolated on September 29, 1999, at Yoshii-cho (34.36 to 34.44 N, 133.21 133.29 E) in Okayama Prefecture from the plasma of sentinel cattle housed in same cowshed [6]. They were provisionally named OY-1/P/99 and OY-2/P/99, and were passaged 6 and 4 times, respectively, on a hamster lung cell line (HmLu-1). Viral RNA was extracted from the supernatant of infected cells using a High Pure Viral RNA kit (Roche Diagnostic, Mannheim, Germany). Reverse transcription-polymerase chain reaction (RT-PCR) was carried out using a Titan One Tube RT-PCR kit (Roche). To amplify the S segment, primers AINO-5′ (5′ AGTAGTGTGGCTCCAC 3′) and AINO-3′ (5′ AGTAGTGTACTCCACTAT 3′) [10] were used for the reaction. cDNA synthesis was conducted for 30 min at 50 °C. The mixture was then heated at 94 °C for 2 min to stop the reverse transcription. The PCR conditions applied were 30 cycles of 30 sec at 94 °C, 30 sec at 42 °C, and 1 min at 68 °C, followed by 7 min of extension at 68 °C. The M segment was amplified using the primer BUN-5′M (5′ GCTCTAGAAGTAGTGTGCTACC 3′) described previously [15]. cDNA synthesis was carried out for 30 min at 48 °C and the mixture was then heated at 94 °C for 2 min to stop the reverse transcription. The PCR conditions applied were 10 cycles of 30 sec at 94 °C, 30 sec at 40 °C and 3.5 min at 68 °C, followed by 40 cycles of 30 sec at 94 °C, 30 sec at 50 °C,

and 3.5 min at 68 °C, increasing the time by 5 sec per cycle. Finally, the reaction mixture was incubated for 7 min at 68 °C. The PCR product was separated by electrophoresis in a 1% TAE agarose gel and the precise band was extracted from the gel using a QIAquick Gel Extraction Kit (Qiagen, Tokyo, Japan). The product was ligated into a pGEM-T Easy vector (Promega, Madison, WI, U.S.A.) and amplified in JM109 bacterial cells provided by the manufacturer. The plasmid was purified with Wizard Plus Minipreps DNA Purification Systems (Promega), and its sequence was determined by the dideoxynucleotide chain termination method using a Model 4200 automated DNA sequencer (LI-COR, Lincoln, NE, U.S.A.). To minimize sequence errors, we sequenced at least 3 clones containing cDNA of each segment.

The cDNA of the S segments of OY-1/P/99 and OY-2/P/99 was amplified by RT-PCR and their respective nucleotide sequences were determined. Both sequences were found to have a sequence of 807b and proved to be 100% identical, indicating that the two viruses are the same. The sequence data has been deposited in GenBank under the accession numbers AB125371 for OY-1/P/99. The sequence contains two overlapping open reading frames (ORFs) potentially encoding 233 and 91 aa of N and NSs proteins, respectively. The determined sequence was compared with other sequences using a Basic Local Alignment Search Tool (BLAST) search (National Center for Biotechnology Information, National Institute of Health, Bethesda, MD). It was found that the sequence is homologous to the N ORF of Simbu serogroup viruses. Additionally, the percentage of identity between OY-1/P/99 and Simbu serogroup viruses was obtained using the GAP program of the University of Wisconsin Genetics Computer Group (UWGCG) package [4] (Table 1). The N ORF of OY-1/P/99 shows high identity with that

**Table 1.** Nucleotide and amino acid sequence identities among N ORF sequences of OY-1/P/99 and Simbu serogroup viruses

| Virus | OY-1/P/99 | SATV | SHAV | DOUV | AINOV | PEAV | AKAV |
|---|---|---|---|---|---|---|---|
| | Nucleotide sequence identity (%) | | | | | | |
| OY-1/P/99 | – | 97.6 | 93.0 | 91.1 | 78.3 | 77.8 | 78.1 |
| SATV | 99.1 | – | 92.6 | 93.0 | 78.1 | 77.8 | 78.7 |
| SHAV | 97.0 | 97.9 | – | 91.1 | 75.8 | 77.3 | 77.4 |
| DOUV | 97.0 | 97.9 | 95.7 | – | 77.1 | 77.1 | 77.8 |
| AINOV | 80.7 | 79.8 | 78.1 | 78.5 | – | 91.3 | 77.3 |
| PEAV | 81.1 | 81.1 | 79.4 | 79.8 | 98.3 | – | 76.8 |
| AKAV | 80.3 | 80.3 | 79.4 | 79.0 | 82.4 | 82.8 | – |
| | Amino acid sequence identity (%) | | | | | | |

*SATV* Sathuperi virus, strain I-11155, Genbank accession no. AF362403; *SHAV* Shamonda virus strain An5550, Genbank accession no. AF362404; *DOUV* Douglas virus strain CSIRO 150, Genbank accession no. AF362393; *AINOV* Aino virus, Genbank accession no. M22011; *PEAV* Peaton virus strain CSIRO110 Genbank accession no. AY048678; *AKAV* Akabane virus strain OBE-1, Genbank accession no. AB000851

of *Sathuperi virus* (SATV; 97.6% in nt; 99.1% in aa), *Shamonda virus* (SHAV; 93.0% in nt; 97.0% in aa), and Douglas virus (DOUV; 91.1% in nt and 97.0% in aa). However, the N ORFs of AKAV, AINOV and Peaton virus (PEAV), prevalent in Japan, are relatively distant from that of OY-1/P/99 (about 80% both in nt and aa). A phylogenetic tree based on N ORF sequences was generated by neighbor-joining analysis [14] using Kimura's two-parameter formula (Fig. 1) and shows the closest relationship between OY-1/P/99 and SATV.

RT-PCR with the primer BUN-5′M resulted in a 4.0 kb fragment from OY-1/P/99 and the PCR product was cloned to the plasmid vector. The G2 coding region of the cDNA was partially sequenced and compared with other Simbu serogroup viruses reported previously [13, 15]. The nucleotide sequence (GenBank accession number AB125372) potentially encoded the N-terminal of the G2 protein. The N-terminal 168 amino acid sequence of OY-1/P/99 was highly homologous (Fig. 2) with that of SATV (98.2%) and DOUV (95.2%), but differed significantly from that of the other Simbu serogroup viruses (less than 75% identity). We therefore conclude that the isolated viruses are most similar to SATV.

SATV was initially isolated in 1957 from mosquitoes in India [3], and was later obtained from cattle and biting midges in Nigeria [2, 9]. It has previously been detected only in tropical regions. There have been no reports on isolating the virus for about 30 years, and no report has yet been published that associates it with any disease in humans or animals. It was reported that SATV is serologically and genetically related to DOUV isolated in Australia [8, 13], and SATV was later isolated from cattle in Okayama Prefecture of western Japan [6]. No illness was observed in the cattle from which the viruses were isolated. No prevalence of SATV has been observed since 2000, indicating that SATV is not yet established in Japan. It also suggests that no efficient vector species is constantly available in Japan.

In addition to SATV, several bovine arboviruses have recently emerged in Japan. A new variant of Ibaraki virus of the genus *Orbivirus* of the family *Reoviridae* was prevalent in the main islands of Japan and caused a severe outbreak of abortion and stillbirth in cattle in 1997 [11, 12], and in 1999, PEAV was newly isolated in the southern part of Japan [10]. It is thought that these bovine arboviruses are transmitted by *Culicoides* biting midges and that the spread of the viruses is related to the wind-born dispersal of the midges. It is unlikely that these viruses are present year-round in Japan because epidemics of the viruses are not observed in winter. It is probable that the infected midges are brought over the East China Sea by the seasonal window in summer, when they cause the epidemics in Japan [7]. Emerging and re-emerging vector-born diseases have recently begun to occur frequently and are spreading throughout the world. Their appearance and resurgence may be associated with global warming and ecological changes that increase vector activities. The emergence of bovine arboviruses including SATV may be good illustration of this phenomenon, although the introduction route of the viruses remains unclear. In order to determine the present distribution of these viruses in temperate regions like Japan, it is necessary to develop and implement a sensitive surveillance system for detecting exotic vectors and arboviruses from

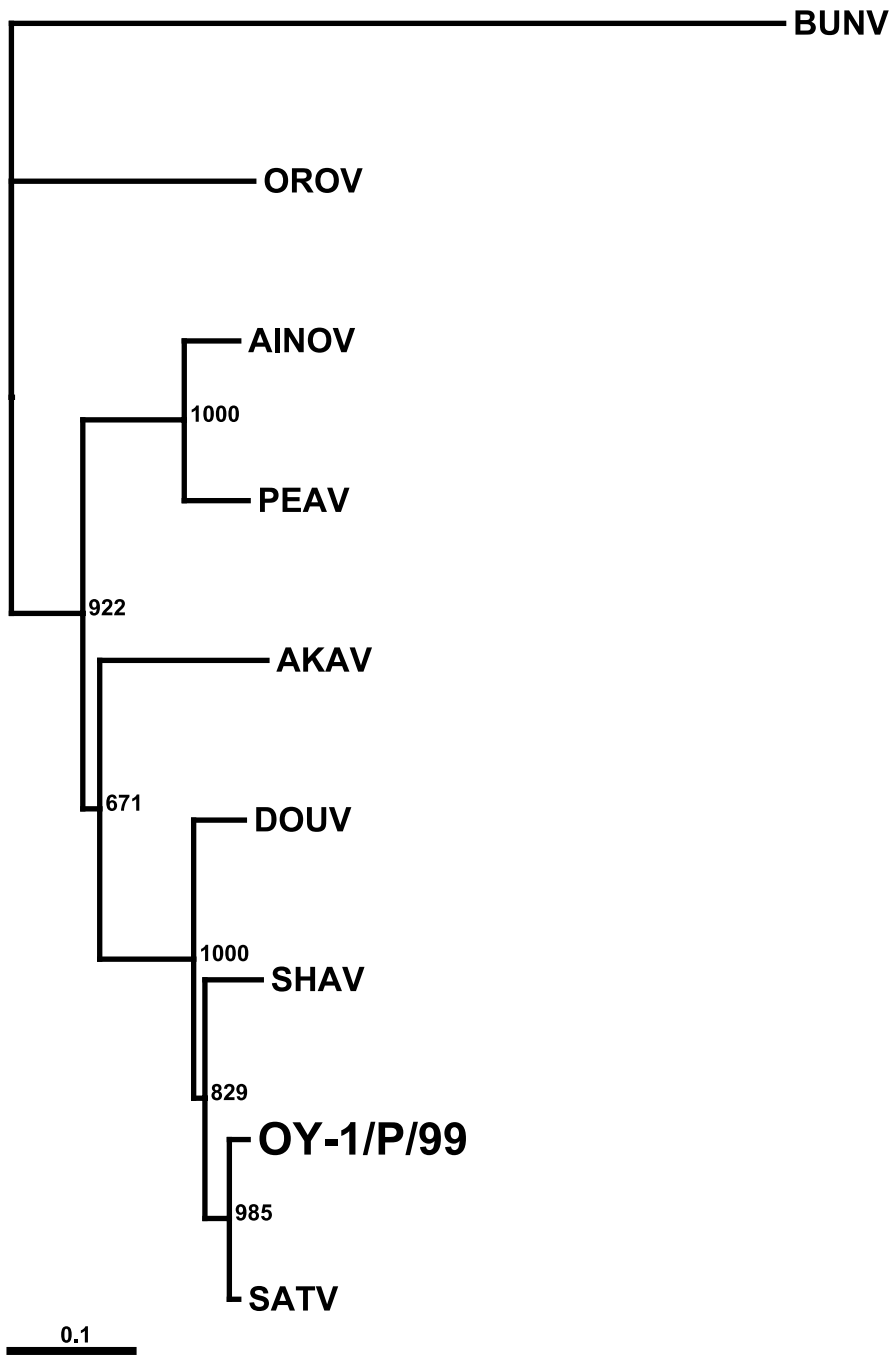


**Fig. 1.** Phylogenetic analysis of OY-1/P/99 and other Simbu serogroup viruses based on their N ORF nucleotide sequences. The N ORF sequence of *Bunyamwera virus* (BUNV; GenBank accession number NC_001927) was used as an outgroup to root the tree. For the N ORF sequences, those listed in Table 1 and the following published sequence was used: OROV TRVL9760 (AF164531). Numbers above the internal nodes indicate the bootstrap values obtained with 1,000 replicates. Scale bar represents 10% nucleotide sequence divergence

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OY-1/P/99       MLLNIILVLNLAYSAFTLPLKEGTRGSRCFLNGELVKTVNTSKVVSECCVKDDISIIKSN
SATV            .......I.....L.............................................
DOUV            ....NV.I.....L.............................................

OY-1/P/99       SEHYKSGDRLAAVIKYYRLYQVKDWHSCNPIYDDHGSFMILDIDNTGTLIPKMHTCRVEC
SATV            ...........................................................A..
DOUV            ....R.............I.................V.....................

OY-1/P/99       EISLNKDTGEILLNSYRINHYRISGTMHVSGWFKNKIEIPLENTCESI
SATV            ...........................................
DOUV            ..A........................................
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**Fig. 2.** Alignment of the N-terminal 168 amino acid sequence of OY-1/P/99, SATV and DOUV. Amino acid sequences of SATV and DOUV were obtained from a previous study [13]. Amino acid sequences identical to those of OY-1/P/99 are indicated by dots

tropical and sub-tropical areas. The present study demonstrates the necessity of such a system to predict and decrease the risks of arboviral diseases in these regions.

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