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Full genome sequence of a Sathuvachari virus strain isolated in the southwestern-most archipelago of Japan

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Abstract

Two virus strains, tentatively designated as ON-6/P/05 and ON-7/E/05, were isolated from blood samples of healthy cattle in the Yaeyama Islands, located in the southwestern-most region of Japan, in 2005. Ultrastructural observations of infected baby hamster (BHK-21) cells revealed that the viruses had features consistent with those of orbivirus. As with other orbiviruses, the viral genome consists of 10 double-stranded RNA segments. The full genome sequence of ON-6/P/05 was determined and shared high nucleotide and amino acid identities (90.07–98.22% nucleotide identity; 96.16–99.72% amino acid identity) with that of Sathuvachari virus (SVIV), a member of the species *Sathuvachari virus* of the genus *Orbivirus*, originally isolated from starlings collected in southern India in 1963. The sequence of segment two of ON-7/E/05 was identical to that of ON-6/P/05. The isolation of SVIV from cattle also indicated that the virus has a wider host range than previously thought. The potential pathogenicity of SVIV in domestic animals should be considered in future disease surveillance within its distribution range.

Keywords Arbovirus · Orbivirus · *Reoviridae* · Mosquito-borne · *Bos taurus*

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Introduction

Orbiviruses (genus *Orbivirus*, family *Reoviridae*) are arthropod-borne viruses (arboviruses) transmitted by mosquitoes, *Culicoides* biting midges, sandflies, and ticks [1]. Their virus particles consisting of seven structural proteins (VP1-7) are icosahedral and non-enveloped. Their double-stranded RNA (dsRNA) genome consists of 10 linear segments packaged within the particle and encodes at least three non-structural proteins (NS1-3) in addition to the structural proteins. It was recently found that a fourth orbivirus non-structural protein (NS4) was encoded by an open reading frame (ORF) in segment nine overlapping the larger ORF encoding VP6 [2]. VP2 (outer capsid 1: OC1) is an outer shell protein and a major determinant of serotype within the species. Twenty-two recognized and eight tentative species are currently included in the *Orbivirus* genus [3, 4] and segregate into three genetic groups that correspond to their respective arthropod vectors: namely, mosquitoes, *Culicoides*/sandflies, and ticks [5]. Some *Culicoides*-borne orbivirus species, such as *Bluetongue virus* (BTV), *Epizootic hemorrhagic disease virus* (EHDV), and *Palyam virus* (PALV), cause severe diseases in domestic ruminants. BTV and EHDV are globally

distributed throughout tropical and temperate zones, and ruminant diseases caused by these viruses are defined as notifiable transboundary diseases by the World Organization for Animal Health (OIE) because of their high economic impact [6, 7]. A PALV serotype, Chuzan virus, caused a large epizootic of bovine congenital abnormalities in Japan [8]. Several mosquito- and tick-borne orbiviruses are thought to infect domesticated ruminants, but little is known about their distribution, circulation, pathogenicity, and impact on the livestock industry [3].

In Japan, distributions of three orbivirus species borne by *Culicoides*, one by mosquitoes and one by ticks have been identified to date [9–14]. In 2005, two viruses with orbivirus-compatible features were isolated from apparently healthy cows raised on Ishigaki Island of the Yaeyama Islands located in the southwestern-most part of Japan. The two virus isolates were not identified using a previously described multiplex RT-PCR for detection of arboviruses that infect cattle [15]. In this study, genetic analysis was conducted for identification of these viruses. We also report a comparison of their genomic sequences with those of known orbivirus species.

Results and discussion

Heparinized blood samples were taken from 14 cows (Japanese black cattle) from a cowshed located on Ishigaki Island on October 3, 2005. One of them had delivered a malformed calf with arthrogryposis just before the blood sampling. The blood samples were separated into plasma and blood cells by centrifugation and the blood cells were washed three times with cold phosphate-buffered saline (PBS) and stored at − 80 °C as described before [16]. A monolayer of baby hamster kidney (BHK-21) cells in a 24-well plate was washed three times with Earl's solution, inoculated with the frozen–thawed samples, and incubated for 1 h at 37 °C. After the inocula were replaced with maintenance medium, the inoculated cultures were maintained at 37 °C in an atmosphere containing 5% CO_2, and the cultures were observed for cytopathic effects (CPEs) over 7 days. Two more blind passages were conducted in the same manner when no CPEs were observed. Two viruses (ON-6/P/05 and ON-7/E/05) were obtained from plasma and blood cells of different cattle, respectively. By transmission electron microscopy, spherical virus particles approximately 80 nm in diameter together with tubule structures were observed in the cytoplasm of the infected BHK-21 cells. These findings were consistent with features of members of the genus *Orbivirus*. Electrophoretic analysis of viral dsRNAs purified from the infected BHK-21 cells revealed that 10 dsRNA segments of different sizes were observed (Fig. 1): the seventh band from the top was stronger, consisting of co-migrating segments

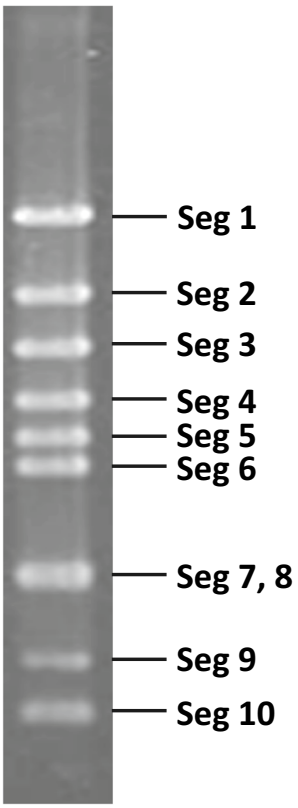

Fig. 1 Viral dsRNAs were extracted from the infected BHK-21 cells using TRIZOL reagent (Thermo Fisher Scientific, Waltham, MA, USA) as described previously [17]. The purified RNA was electrophoresed on 1% agarose and Tris-acetate–EDTA gel, stained with ethidium bromide and visualized with ultraviolet light

seven and eight on the gel. The full-length cDNAs of these segments were generated by the 'Full-Length Amplification of cDNAs' (FLAC) [17] and directly sequenced with the primers shown in the supplementary table. Full-length nucleotide sequences of all ten segments of ON-6/P/05 have been determined and submitted to the international database managed by the DNA Data Bank of Japan (DDBJ) under accession numbers LC382231-40. Identification of ORFs and translation to protein sequences were carried out with GENETYX ver. 10.1.3 (GENETYX, Tokyo, Japan). The total genome length was 18,831 bp, and each segment includes a single major ORF that encodes proteins equivalent to known orbivirus proteins (Table 1). All segments have five conserved nucleotides (GUUUA….) at their 5′ end and three conserved nucleotides (….UAC) at their 3′ end. The segment two sequence of ON-7/E/05 was identical with that of ON-6/P/05. Although all segments of ON-7/E/05 should be analyzed to exclude a possible occurrence of reassortment, two viruses probably share genetic features. The obtained sequences were compared with cogent sequences of other orbiviruses using the BLAST program in the GENETYX package. All segments shared significant

Table 1 Comparison between SVIV IAn-66411 and ON-6/P/05

Segment (encoded protein)	Segment size (bp)		No. of amino acids		Nucleotide identity (%)	Amino acid identity (%)
	IAn-66411	ON-6/P/05	IAn-66411	ON-6/P/05		
Seg 1 (VP1: Pol)	4015	4025	1321	1325	91.57	96.90
Seg 2 (VP3: T2)	2860	2861	919	920	96.85	99.45
Seg 3 (VP2: OC1)	2393	2391	777	777	97.49	98.58
Seg 4 (VP4: Cap)	1997	1995	644	644	98.09	99.06
Seg 5 (NS1: TuP)	1789	1788	562	562	98.04	98.93
Seg 6 (VP5: OC2)	1644	1642	522	522	90.07	98.08
Seg 7 (NS2: ViP)	1205	1203	366	366	98.50	99.72
Seg 8 (VP7: T13)	1188	1186	351	351	98.22	99.71
Seg 9 (VP6: Hel)	933	931	287	287	97.09	96.16
Seg 10 (NS3)	810	809	213	213	92.83	98.12
Total	18,834	18,831	5962	5967		

sequence identities with equivalent segments of Sathuvachari virus (SVIV) IAn-66411 of the species *Sathuvachari virus* (90.07–98.22% nucleotide identity; 96.16–99.72% amino acid identity) [18]. Phylogenetic trees constructed for the orbivirus proteins with MEGA7 [19] revealed that ON-6/P/05 and SVIV IAn-66411 grouped together most closely (supplementary figure). These findings clearly showed that ON-6/P/05 is a strain of SVIV. The database search also revealed that partial amino acid sequences of VP1, NS1, VP5, NS2, VP7, and VP6 of Tagtag virus (TAGV) isolated in Indonesia share significant homology with the equivalent segments of SVIV (92–100% amino acid identity) as shown in a previous study [18]. Partial sequences of VP1 and VP7 of Arakonam virus (ARAV) isolated in India showed 99% amino acid identity with those of SVIV. Because the sequences of VP2 (OC1) of TAGV and ARAV are still unknown, the serotype composition of the species *Sathuvachari virus* remains unclear. Encoded proteins of other known orbiviruses share low amino acid identities ($\leq$55%) with those of SVIV as described previously [18]. Segment nine of ON-6/P/05 has an additional down-stream ORF which codes for 96 amino acids and shares 93.7% identity with that of SVIV IAn-66411. The putative protein with approximately 12.3 kDa contains a coiled-coil domain in its N terminal half region. Potential monopartite (positions 15–24 and 61–73) and bipartite (positions 57–91) nuclear localization signals were identified in the putative protein. Although these features are shared by NS4 of orbiviruses, the potential NS4 of SVIV was highly variable from those of other orbiviruses (9.4–29.7%). Recent work indicated that BTV NS4 functions as an interferon antagonist [20]. Functional analysis for NS4 of other orbiviruses including SVIV would be necessary to clarify a determinant of virus virulence.

SVIV was first isolated in 1963 from starlings (*Brahminy myna*) collected in southern India [18]. Previous phylogenetic analysis indicated that SVIV is grouped with mosquito-borne orbivirus species although it is quite divergent from the other species in that group. The virus is most probably mosquito-borne as suggested by the phylogenetic analysis, but its transmission cycle remains unknown. Recently, a virus (CG LT392) isolated from an unidentified source collected in Vietnam over 50 years ago was identified as SVIV by metagenomics analysis [21]. It has been assumed that the species *Sathuvachari virus* consists of a single serotype. The current classification is consistent with the high sequence identity of VP2 (OC1) between ON-6/P/05 and IAn-66411, whose isolations are geographically and chronologically separated. However, to enhance our understanding of the intraspecies variation, full genome analyses of TAGV and ARAV are essential.

Our study shows that SVIV can spread beyond the tropical zone of southern Asia. In addition, the isolation of SVIV from cattle indicated that the virus has a wider host range than previously thought. The malformed calf delivered at the same cowshed where SVIV was obtained was suspected to be affected by Peaton virus of the genus *Orthobunyavirus* of the family *Peribunyaviridae*, because specific antibodies to the virus were detected from the pre-colostrum serum [22]. At present, SVIV has not been associated with clinical signs in animals. However, the impact of SVIV on wildlife and domesticated animals in its distribution range should continue to be assessed.

Incursions of arboviruses are often detected in the Yaeyama Islands earlier than in other parts of Japan [16]. The islands are in close proximity to established regions of various arboviruses. It is possible that airstreams from Southeast Asia support the long-distance transport of vectors infected with arboviruses to this region [23]. Further efforts for virus isolation/detection in the Yaeyama Islands will provide insights into the geographical distribution, genetic diversity, and transmission cycle of orbiviruses. The incidence rate of orbivirus infections is generally low,

and careful observations will be necessary to discern their involvement in animal diseases.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent is not required because no human participants were involved in this article.

Research involving human participants and/or animals This article does not contain any studies with human participants performed by any of the authors. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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