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Discovery of genome of an immunodeficiency-associated virus-like virus from pig feces in Japan

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Abstract

Immunodeficiency-associated stool virus (IASV) is an unclassified virus, for which the only genome information is available from a patient infected by human immunodeficiency virus. In domestic animals, one report described detection of IASV-like virus in pig feces, whereas no nucleotide sequence information of this virus is currently available. Using deep sequencing method, we detected a DNA fragment homologous to IASV in several pig feces in Japan. The sequence of the PCR product in this sample had 70% homology to that of IASV. The infectious rate of the IASV-like virus was 72.9% among the 9 pig farms, from which the samples were collected. There was no clear correlation between the presence of IASV-like virus and the fecal characteristics.

Key Words: immunodeficiency-associated virus, pig, feces

Next-generation sequencing (NGS) has been used widely in various fields in biology and has led to the discovery of many viruses[2,3,7,11)]. We have also discovered novel viruses from the diarrhea samples of pigs and cattle, including a novel porcine rotavirus, astrovirus, kobuvirus, and posavirus[1,5,9,13)]. Recently, we detected the fur seal feces-associated circular DNA virus JPN1 (FSfaCV-J1) in pig feces, using a novel method for detecting single-stranded DNA viruses.[10)] Using this newly developed method and deep-sequencing analysis, the present study explored the presence of other novel single-stranded DNA viruses in pig fecal samples in Japan.

The fecal samples were collected from 85 piglets across 9 farms in Japan during January

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and February, 2014. Feces were taken from the anal of pigs as much as possible. Nucleic acids were extracted from the supernatant of a 10% suspension of feces in sterile phosphate-buffered saline, using the High Pure Viral Nucleic Acid Kit (Roche, Basel, Switzerland) and DNA Mini Kit (QIAGEN, Hilden, Germany). Four of the 85 DNA samples were pooled for deep sequencing analysis. The DNA samples were treated with duplex-specific nuclease (DSN), which digests double-stranded DNA and not single-stranded DNA[13] according to the protocol of our previous report[10]. Then, the Nextera XT DNA Sample Prep Kit (Illumina, San Diego, CA, USA) was used to construct the library for deep sequencing, according to the manufacturer's protocol. The DNA library was sequenced using a MiSeq bench-top sequencer (Illumina) with the MiSeq Reagent Kit v2 (50 cycles).

We obtained total 2,502,419 single reads, which were then de novo assembled into contigs using CLC Genomics Workbench 6.5.1. (CLC bio, Aarhus, Denmark). We obtained total 1,628 contigs, which were then analyzed with the BLASTn program on the NCBI Website. Among them, 5 contigs were homologous to the complete genome of immunodeficiency-associated stool virus (IASV) (Accession No. KJ003983.1) (cut off E-value $<$ 1.0E-10). The sequence of reads obtained from Miseq was deposited to DDBJ as a sequence read archive (SRA) (BioProject Accession No. PRJDB 6574).

IASV is an unclassified, circular, double-stranded DNA virus, with a genome length of 99,915 bp[4]. The virus was detected from the feces of a patient infected with human immunodeficiency virus, and its genome sequence derived from that study is the only one deposited in GenBank[4]. Although DNA fragments homologous to IASV have been found in the feces of pigs using NGS, no information about the viral nucleotide sequences is available[6]. The pathogenicity of IASV and the possibility of its zoonosis are unknown.

To further confirm the data obtained from NGS, we subjected each of the IASV-positive samples to PCR analysis. To this end, we designed PCR primers (IAS_F: 5'-TTGGAGTCCAGGCAA GGTTA-3'; and IAS_R: 5'-CCTGCAAGTTTACCT GTAGC-3') basis on the consensus nucleotide sequence obtained mapping of NGS reads to IASV. One microliter of DNA was subject to PCR amplification in a 25-μl reaction volume containing 1 μl of each primer (10 μM) and 12.5 μl of Premix Ex Taq Hot Start Version (TaKaRa Bio, Shiga, Japan). The PCR cycle conditions were an initial denaturation at 95°C for 2 min; followed by 35 cycles of 94°C for 30 sec, 57°C for 30 sec, and 72°C for 1 min; and a final extension at 72°C for 5 min. All of the 4 samples showed the expected size of the 789 bp-long PCR product, further confirming our NGS data.

We arbitrarily choose 1 PCR product from the 4 PCR products and performed direct sequencing analysis. The nucleotide sequence of the sample showed 70% homology to IASV found in the human sample[4] by BLASTn analysis. The virus carrying the detected sequence was designated as porcine feces-associated IASV-like virus (PfaIV) and its nucleotide sequence was deposited to DDBJ (Accession No. LC318452). The nucleotide sequences of IASV and PfaIV were aligned for visual comparison, and likewise their putative amino acid sequences (Fig. 1). The putative amino acid sequences of these two viruses had 67% homology according to the BLASTp algorithm. The maximum consecutive length of the consensus amino acid sequences of both viruses was 26 residues (Fig. 1). No results were obtained that predicted the function of this putative protein in the BLAST analysis.

To know prevalence of PfaIV in Japanese pig farms, each of the 85 samples was analyzed by PCR using IAS_F and IAS_R primers and the nucleotide sequences were determined with direct sequencing. Both diarrheal and healthy feces from 62 pigs out of the 85 pigs (72.9%) were positive for PfaIV (Table 1). Our previous study showed the possession rate of FSfaCV-J1 in pig feces was 76%, which is similar to that of PfaIV found in this study[10]. Both PfaIV and FSfaCV-J1 were

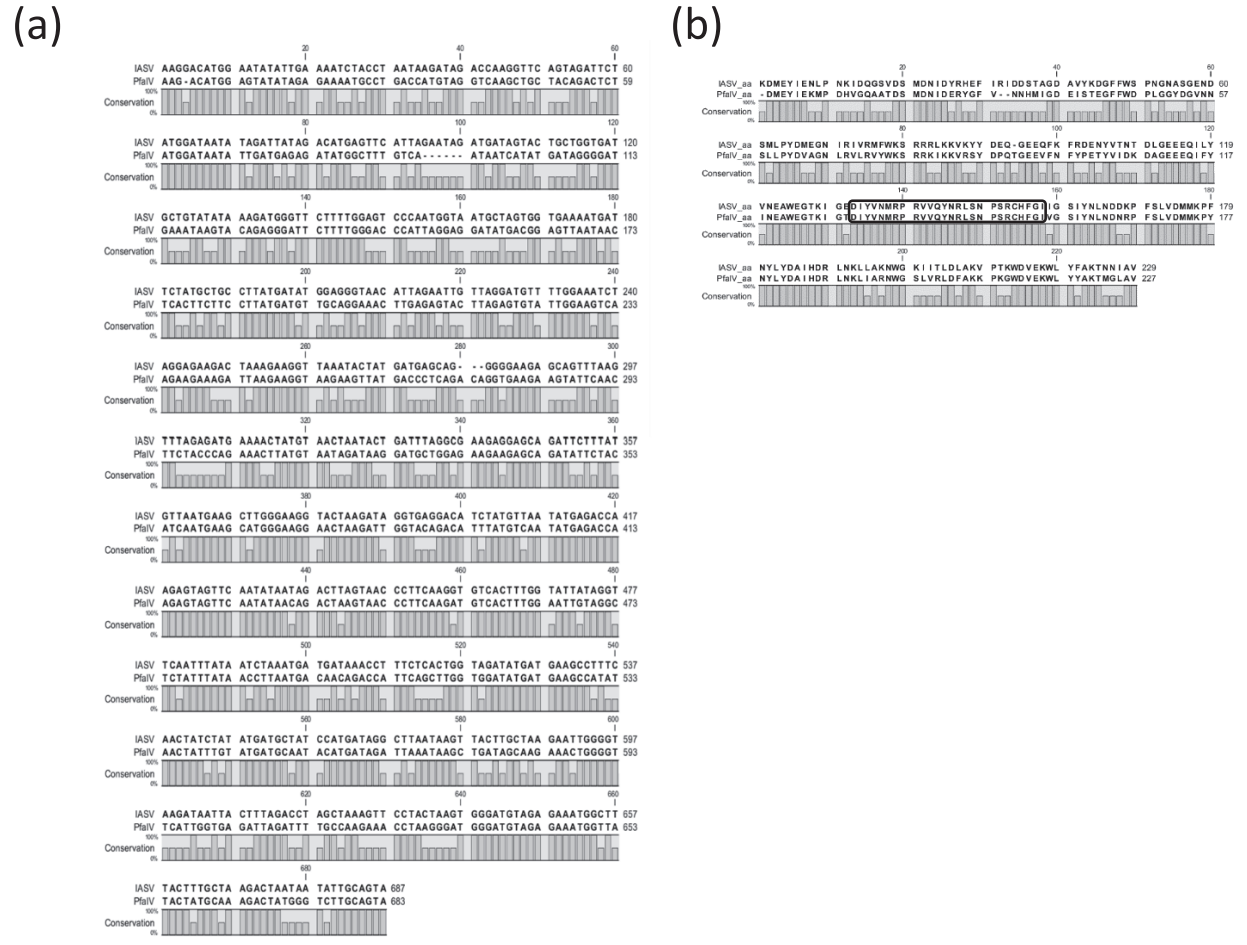

Fig. 1. Sequence alignment for comparison of immunodeficiency-associated virus (IASV) with porcine feces-associated IAS virus-like virus (PfaIV). (a) Alignment of the nucleotide sequences of IASV and PfaIV. (b) Alignment of the amino acid sequences of IASV and PfaIV. The open reading frame of IASV was found using the Find Open Reading Frame command of CLC Genomics Workbench with genetic code 1. The 26 consensus amino acid sequences are shown in the open box.

Table 1. Relationship between possession of the immunodeficiency-associated virus (IASV) gene-like fragment and the fecal characteristics

	Diarrhea/ Soft feces	Common feces	Total
IASV-like virus (+)	23	39	62
IASV-like virus (−)	9	14	23
Total	32	53	85

detected from the same piglets (data not shown); hence, there is a possibility that many of the piglets were coinfected with both viruses. No clear correlation was found between the fecal characteristics and the presence of PfaIV. However, further viral isolation studies are necessary to clarify the pathogenicity of PfaIV.

We detected PfaIV using NGS after digestion of the double-stranded DNA, implying the PfaIV genome to be a single-stranded DNA. However, IASV was deposited onto GenBank as a double-stranded DNA virus. Furthermore our additional studies suggest that the IASV-like virus was a double-stranded DNA virus. Briefly, IAS virus was confirmed to have the double-stranded structure because the restriction enzyme-treated sample did not make PCR product with a newly designed primer to fit the restriction enzyme site (data not shown). We think that a part of single-stranded DNA structure, which was formed during replication of PfaIV, was amplified at

NGS and PCR, leading to discovery of IASV-like sequence in our studies. In any case, further study is necessary to confirm it.

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