Veterinary Microbiology 179 (2015) 197–203

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Isolation and characterization of a novel Rhabdovirus from a wild boar (*Sus scrofa*) in Japan

Kouji Sakai[a,1], Katsuro Hagiwara[b,1], Tsutomu Omatsu[c,1], Chinami Hamasaki[d], Ryusei Kuwata[d], Hiroshi Shimoda[d], Kazuo Suzuki[e], Daiji Endoh[f], Noriyo Nagata[g], Makoto Nagai[c], Yukie Katayama[c], Mami Oba[c], Ichiro Kurane[h], Masayuki Saijo[h], Shigeru Morikawa[i], Tetsuya Mizutani[c,*], Ken Maeda[d,**]

[a] *Department of Virology 3, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashimurayama, Tokyo 208-0011, Japan*
[b] *Virus Unit, School of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi, Bunkyodai, Ebetsu, Hokkaido 069-8501, Japan*
[c] *Research and Education center for Prevention of Global Infectious Diseases of Animals, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu-shi, Tokyo 183-8509, Japan*
[d] *Laboratory of Veterinary Microbiology, Joint Faculty of Veterinary Medicine, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515, Japan*
[e] *Hikiiwa Park Center, 1629 Inari-cho, Tanabe 646-0051, Japan*
[f] *Department of Veterinary Radiology, School of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi, Bunkyodai, Ebetsu-shi 069-8501, Japan*
[g] *Department of Pathology, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashimurayama, Tokyo 208-0011, Japan*
[h] *Department of Virology 1, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashimurayama, Tokyo 208-0011, Japan*
[i] *Department of Veterinary Science, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashimurayama, Tokyo 162-8640, Japan*

ARTICLE INFO

Article history:
Received 23 January 2015
Received in revised form 15 May 2015
Accepted 18 May 2015

Keywords:
Rhabdovirus
Novel virus
Wild boar (*Sus scrofa leucomystax*)
Rapid determination of viral nucleic acid sequences (RDV)

ABSTRACT

A novel rhabdovirus was isolated from the serum of a healthy Japanese wild boar (*Sus scrofa leucomystax*) and identified using the rapid determination system for viral nucleic acid sequences (RDV), next-generation sequencing, and electron microscopy. The virus was tentatively named wild boar rhabdovirus 1 (WBRV1). Phylogenetic analysis of the entire genome sequence indicated that WBRV1 is closely related to Tupaia rhabdovirus (TRV), which was isolated from cultured cells of hepatocellular carcinoma tissue of tree shrew. TRV has not been assigned to any genus of Rhabdoviridae till date. Analysis of the L gene indicated that WBRV1 belongs to the genus Vesiculovirus. These observations suggest that both TRV and WBRV1 belong to a new genus of Rhabdoviridae. Next-generation genome sequencing of WBRV1 revealed 5 open reading frames of 1329, 765, 627, 1629, and 6336 bases in length. The WBRV1 gene sequences are similar to those of other rhabdoviruses. Epizootiological analysis of a population of wild boars in Wakayama prefecture in Japan indicated that 6.5% were positive for the WBRV1 gene and 52% were positive for WBRV1-neutralizing antibodies. Furthermore, such viral neutralizing antibodies were found in domestic pigs in another prefecture. WBRV1 was inoculated intranasally and intraperitoneally into SCID and BALB/c mice and viral RNA was detected in SCID mice, suggesting that WBRV1 can replicate in immunocompromised mice. These results indicate this novel virus is endemic in wild animals and livestock in Japan.

1. Introduction

The Japanese wild boar (*Sus scrofa leucomystax*) is increasingly important in the spread of viruses and bacteria to domestic animals and humans in Japan. Numerous viruses have been detected in wild boar, including hepatitis E virus, Aujeszky's disease virus, porcine reproductive and respiratory syndrome virus, porcine parvovirus, classical swine fever virus, African swine fever virus, swine vesicular virus, swine influenza virus, and porcine circovirus type 2 (Meng et al., 2009; Montagnaro et al., 2010; Vengust et al., 2006; Vicente et al., 2002). In addition, we have recently isolated sapelovirus-like virus (wild boar sapelo

* Corresponding author at: Research and Education Center for Prevention of Global Infectious Diseases of Animals, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo 183-8509, Japan.
Tel.: +81 42 367 5749; fax : +81 42 367 5742.
** Corresponding author at: Laboratory of Veterinary Microbiology, Joint Faculty of Veterinary Medicine, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515, Japan.
E-mail addresses: tmizutan@cc.tuat.ac.jp (T. Mizutani), kmaeda@yamaguchi-u.ac.jp (K. Maeda).
[1] These authors contributed equally in this study.

http://dx.doi.org/10.1016/j.vetmic.2015.05.013
0378-1135/

virus-like virus 1; WBSV1) (Abe et al., 2011) and have detected antibodies to Aujeszky's disease virus, canine distemper virus, Japanese encephalitis virus, and hepatitis E virus in wild boars in Japan (Hara et al., 2014; Kameo et al., 2012; Mahmoud et al., 2011; Ohno et al., 2009). Human habitation of suburban areas and deforestation for agricultural purposes may increase the chance of wild boar contact with domestic animals and humans. The wild boar is thus considered to play a crucial role in the transmission of infectious agents to domestic animals and humans in Japan.

We developed a system for the rapid determination of viral nucleic acid sequences (RDV) to detect and identify viruses in infected culture supernatants (Mizutani et al., 2007). This system does not require a molecular cloning step. We have previously discovered numerous novel viruses, including bat adenovirus 1 (Maeda et al., 2008), bat *Hipposideros diadema* herpesvirus 1 (Watanabe et al., 2009), bat betaherpesviruses 2, (Watanabe et al., 2010), Ostrich reovirus 1 (Sakai et al., 2009), mosquito Phasi Charoen bunyavirus (Yamao et al., 2009), mosquito Omono River totivirus (Isawa et al., 2010), Japanese eel endothelial cells-infecting polyomavirus-like virus (Mizutani et al., 2011), wild boar sapelovirus-like virus 1 (Abe et al., 2011), and goat enterovirus (Omatsu et al., 2014). Information regarding unknown viruses that infect wild boars is valuable for taking measures to prevent virus transmission from wild boars to domestic animals and humans. The RDV method and next-generation sequencing technology are powerful tools for the identification of such viruses. In this study, we isolated viruses from wild boars and determined their nucleic acid sequences using RDV and next-generation sequencing.

2. Materials and methods

2.1. Virus isolation

We collected serum samples from 50 wild boars captured in Wakayama prefecture from 2008 to 2009 in Japan. These sera were inoculated into mosquito C6/36 cells. At 14 days post inoculation (d.p.i.), the supernatants of C6/36 cells were inoculated into monkey kidney Vero cells.

2.2. Sera

Sera of pigs were collected in slaughter facility from August to December, 2008. The slaughter house was located over 500 kilometers from Wakayama prefecture. All sera were stocked at −20 °C until use and inactivated at 56 °C for 30 min just before virus-neutralizing test.

2.3. Virus replication kinetics

The isolated virus was inoculated on Vero cells, which were seeded on 6 well plates (5.0×10^5 cells per well) as a multiplicity of infection (moi) of 0.002, 0.02, 0.2 and 2. After 1 h adsorption, the inoculum was removed, the cells were washed twice with Dulbecco's Modified Eagle's medium (DMEM) without fetal calf serum (FCS), and then 2 ml of DMEM containing 10% FCS was added. The supernatants were collected every 12 h, and virus titers were determined by plaque assay. These experiments were duplicated and the average was calculated.

2.4. Rapid determination of viral RNA sequences (RDV) system

We used RDV (version 2.1) to identify viral nucleic acid sequences from the supernatant of Vero cells inoculated with the serum collected from wild boar No. 08–13 (Sakai et al., 2007). In briefly, the culture supernatant was treated with DNase I and RNase A, followed by RNA extraction using the Total RNA Isolation Mini Kit (Agilent Tech. Inc., Santa Clara, CA, USA). The RNA solution was amplified using a whole transcriptome amplification system (WTA; Sigma–Aldrich, Saint Louis, MO, USA) as the first step of creating a cDNA library. As described in our previous report (Sakai et al., 2007), we used AmpliTaq Gold LD (Applied Biosystems, Foster City, CA, USA) to obtain a high yield of WTA products. After the amplicon was digested with *Alu*I, the EcoRI-NotI-BamHI adaptor (Takara Bio Inc., Shiga, Japan) was ligated to the DNA fragments. The second cDNA library was then amplified using AmpliTaq Gold PCR Master Mix (Applied Biosystems) using specially designed primer sets (Sakai et al., 2007). In this study, we used the primer H1-1 primer as a forward primer and H9-1 to

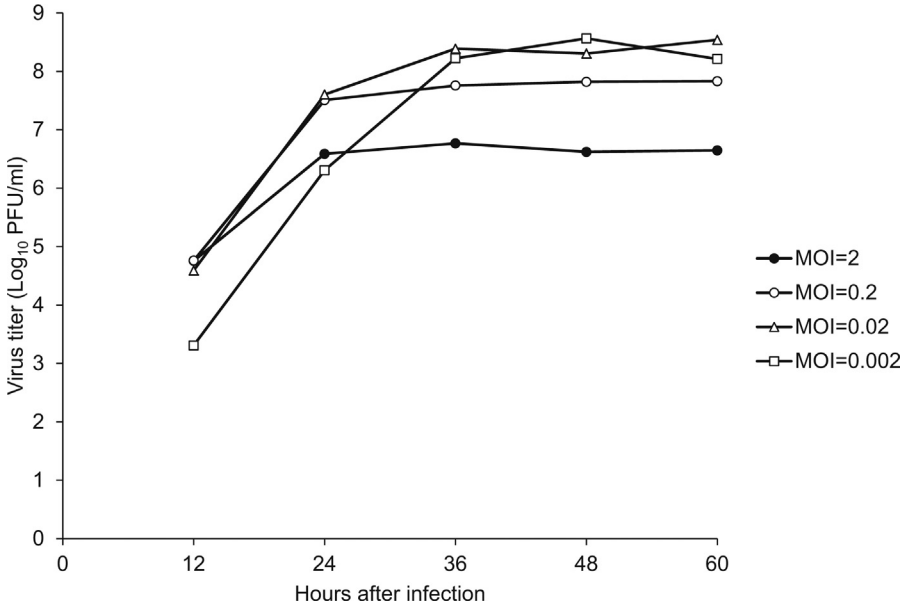

Fig. 1. The isolated virus replication kinetics. The isolated virus was inoculated on Vero cells as MOI of 0.002, 0.02, 0.2 and 2. The supernatants were collected every 12 h, and virus titers were determined by plaque assay.