Veterinary Microbiology 221 (2018) 1–7

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Experimental infection of BALB/c mice with a caprine *Pestivirus H* isolate

Hongfei Shi[a], Chaoliang Leng[a], Qian Xu[a], Zhujin Jiao[a], Hongling Shi[a], Shiyu Sun[b], Reng Qiu[a], Yunchao Kan[a,*], Lunguang Yao[a,*]

[a] Henan Provincial Engineering Laboratory of Insects Bio-reactor, China-UK-NYNU-RRes Joint Libratory of Insect Biology, Nanyang Normal University, Nanyang, PR China

[b] Liaoning Center for Animal Disease Control and Prevention, Shenyang, PR China

ARTICLE INFO

Keywords:
Pestivirus H
Pathogenesis
BALB/c mice
Immunofluorescent staining

ABSTRACT

To data, small animal *Pestivirus H* infection models have not been established. In order to develop a new infection model, BALB/c mice were inoculated with *Pestivirus H* strain HN1507. The virus-inoculated mice displayed nasal discharge and fever clinical signs. Histopathological changes in *Pestivirus H*-infected mice included alveolar septa thickening and alveolar atrophy in the lungs from 1 to 11 days post-inoculation (PI). Furthermore, we observed tracheal epithelial cell abscission and inflammatory cell infiltration in the tracheas from 1 to 9 days PI, infiltration of eosinophils in the spleens from 1 to 9 days PI, intestinal villi abscission and lysis of epithelial cells in the intestines from 1 to 11 days PI. The results of virus isolation showed that *Pestivirus H* replicated well in the lungs, tracheas, spleens, and intestines of infected BALB/c mice, and peak viral titers were observed 3 days PI. RT-PCR and immunofluorescence results were in agreement with the virus isolation results; however, the hearts of infected mice from 1 to 3 days PI were positive while virus isolation results were negative. To the best of our knowledge, this is the first study reporting *Pestivirus H* detection in BALB/c mice. Our findings indicated that *Pestivirus H* strain HN1507 was pathogenic to BALB/c mice and caused clinical signs and histopathological lesions in *Pestivirus H*-infected BALB/c mice.

1. Introduction

Pestivirus H, also referred to as HoBi-like pestivirus or bovine viral diarrhea virus 3 (BVDV-3) (Liu et al., 2009; Smith et al., 2017), is a single-stranded positive-sense RNA virus within the Pestivirus genus, which causes respiratory diseases in cattle under natural and experimental conditions (Decaro et al., 2011, 2013, 2012b, 2012c, 2012d) and impacts on cattle productivity (Decaro et al., 2016). The Pestivirus genus had included bovine viral diarrhea virus 1 (BVDV-1), bovine viral diarrhea virus 2 (BVDV-2), classical swine fever virus (CSFV) and border disease virus (BDV) (Simmonds et al., 2017) before 2017, now according to the Proposed revision to the taxonomy of the genus Pestivirus, family Flaviviridae, the Pestivirus genus includes Pestivirus A (original designation BVDV-1, Pestivirus B (BVDV-2), Pestivirus C (CSFV) and Pestivirus D (BDV), Pestivirus E (pronghorn pestivirus), Pestivirus F (Bungowannah virus), Pestivirus G (giraffe pestivirus), *Pestivirus H* (Hobi-like pestivirus), Pestivirus I (Aydin-like pestivirus), Pestivirus J (rat pestivirus) and Pestivirus K (atypical porcine pestivirus) (Smith et al., 2017). Pestivirus A (BVDV-1) and Pestivirus B (BVDV-2) are major viruses associated with a number of feedlot cattle diseases that cause reproductive disturbances and have immunosuppressive effects, leading to severe economic losses worldwide (Larson, 2015). Pestiviruses are not strictly host-specific, and natural infections with these two viruses have been reported in swine, sheep, goats, camels, alpaca and other wild ruminants (Goyal et al., 2002; Hamblin et al., 1990; Mishra et al., 2012; Nelson et al., 2008; Tao et al., 2013; Wentz et al., 2003). Experimental infections with the *Pestivirus H* in sheep and goats have also been described (Bauermann et al., 2015; Decaro et al., 2015, 2012b), but all strains described in these studies were isolated from bovines.

The isolation of the *Pestivirus H* in small ruminants was not been reported before 2016 in China. However, there were many cases of sheep and goats showing similar clinical signs to the *Pestivirus H* infection in 2015, and a virus strain showing a cytopathic effect in Madin-Darby bovine kidney cells, was isolated from a nasal swab collected from goats in Henan Province, China (Shi et al., 2016). The isolate was confirmed as a *Pestivirus H* by complete genome sequencing and named HN1507. Subsequently, *Pestivirus H* has been frequently detected in sheep and goats with respiratory diseases in Henan Provinces, and sequencing has identified the RNA of these strains were almost as similar to the RNA of the HN1507 stain. Nonetheless, the pathogenesis of HN1507 is not clear and needs further studies. To date, limited studies

* Corresponding authors.
E-mail addresses: kanyunchao@163.com (Y. Kan), lunguangyao@163.com (L. Yao).

https://doi.org/10.1016/j.vetmic.2018.05.016
Received 24 April 2018; Received in revised form 23 May 2018; Accepted 23 May 2018

on the pathogenesis of BVDV infection in BALB/c mice were conducted (Han et al., 2016; Seong et al., 2016, 2015). The establishment of a BALB/c mouse model to study *Pestivirus H* pathogenesis has not been reported. Therefore, experimental infection of specific pathogen-free BALB/c mice might be an ideal animal infection model for studies on the pathogenesis of new *Pestivirus H* isolates since the acquisition of suitable calves for experimental infection is not easy or economical. A practicable model of *Pestivirus H* infection would provide a basis for investigating the mechanisms of its pathogenesis and disease progression. Therefore, to address our objectives, BALB/c mice were inoculated with *Pestivirus H*, clinical characteristics were recorded during disease progression, and mice were ultimately euthanized and their tissues collected for analysis. Results showed that BALB/c mice appear to be susceptible to the *Pestivirus H*.

2. Materials and methods

2.1. Viruses and culture

Madin-Darby bovine kidney (MDBK) cells (purchased from the China Institute of Veterinary Drugs Control), in which no pestivirus was detected by PCR, according to published methods (Decaro et al., 2012d), were used to propagate virus. The *Pestivirus H* cytopathogenic strain HN1507, derived from a diseased goat was cultivated in MDBK cells for 72 h using 1% equine serum (Solarbio, China) DMEM and incubated at 37 °C, under 5% CO_2 conditions. Viral stock was stored at −80 °C for future use.

2.2. Animals

Specific pathogen-free (SPF) female BALB/c mice (aged 3 to 4 weeks) were purchased from a commercial breeder (Beijing Vital River Laboratory Animal Technology Company, China), and housed under controlled conditions in individually ventilated cages. All procedures were carried out in strict accordance with Chinese Law for the Care and Use of Animals and approved by the Office of Laboratory Animal Management of Liaoning Province, China (approval number: 17114). Every effort was made to minimize suffering during the study.

2.3. Experimental infection

Forty-two mice were used in this study. Half of these animals were each inoculated via the oronasal route with 10^5 TCID50 HN1507 in a volume of 50 μL as infection group and the other half of mice were each inoculated via the oronasal route with the same volume of supernatant from MDBK cells free of virus as control group. The animals were observed daily for clinical signs, and temperatures were also recorded daily. Immediately following euthanasia, samples of heart, liver, spleen, lung, kidney, trachea and intestines of the infected (3 animals) and control group (3 animals) were collected at 1, 3, 5, 7, 9, 11 and 14 days post inoculation (PI), then prepared for histopathological examination, immunofluorescent staining, virus isolation and real-time PCR. Because the volume of trachea in each mouse was small, the three tracheas of infected mice and control mice at each time point were tested as follows: one for virus isolation; one for histopathological test and immunofluorescent staining; and one for RT-PCR.

As a reference of clinical scoring system for bovine respiratory disease complex developed by veterinarians at the University of Wisconsin (McGuirk, 2008) a clinical scoring system for mice in this work was developed on the basis of activity level, nasal discharge and rectal temperature (each parameter scored from 0 to 3, where 0 is normal and 3 is the worst; maximum score of 9).

2.4. Virus isolation and detection of viral DNA by RT-PCR

At 1, 3, 5, 7, 9, 11 and 14 days PI three infected mice and three control mice were euthanized. The partial organs of heart, liver, spleen, lung, kidney, and trachea from each animal were removed and weighed, then homogenized separately in 1.5 ml eppendorf tubes containing 1 ml of MEM. Homogenates were centrifuged, and the supernatant was removed and assayed immediately. Virus was isolated by using MDBK cells as follows: serial 10-fold dilution of tissue homogenates were made in DMEM supplemented with 1% equine serum. Each dilution was inoculated into four wells of MDBK cells cultured in 96-well cell culture plates in a 5% CO_2 incubator at 37 °C for five to seven days. $TCID_{50}$ of tissue homogenates was calculated based on the Reed-Muench assay (Reed and Muench, 1938).

Total RNA was extracted from tissue homogenates using an EasyPure Viral DNA/RNA Kit (Transgen Biotech, China), according to the manufacturer's instructions. The RNA was resuspended in 20 μl DEPC-treated water and kept at −80 °C for further analysis. The 5′-UTR RT-PCR primers (Liu et al., 2009) were used for *Pestivirus H* detection according to the published protocol. Amplified products were separated by electrophoresis in 1.5% agarose gel in Tris–acetate EDTA buffer, stained in 0.5 mg/ml ethidium bromide, and viewed under ultraviolet light.

2.5. Histopathology and immunofluorescent staining

Tissue samples were fixed in 10% paraformaldehyde, embedded in paraffin wax, cut in 4 μm thick sections, and mounted on microscope slides. For a routine histological examination, the sections were stained with hematoxylin and eosin (H & E). The same protocol was used for section preparation for the immunofluorescent (IF) staining assay. The sections were treated using protocols published in previous reports (Dong et al., 2012; Shi et al., 2014). Primary antibody of rabbit polyclonal antibodies against E2 protein of *Pestivirus H* (developed in our laboratory) at a dilution of 1 in 100 was applied overnight at 4 °C. The sections were then washed with PBS (pH 7.4), and fluorescein isothiocyanate (FITC)–conjugated goat antibody against rabbit (Thermo Fisher Scientific, USA) was used at a dilution of 1:100 for 30 min. After three washes, sections were counterstained with DAPI (4′,6-Diamidine-2′-phenylindole dihydrochloride; 15 μg/ml) for 3 min. Finally, the slides were observed by a fluorescence microscopy (OLYMPUS BX53, Japan). The control sections were also examined.

HE-stained tissues were scored by an experienced veterinary pathologist who was blinded to this research on the basis of inflammation, tissue damage and affected area (each parameter scored from 0 to 3, where 0 is normal and 3 is the worst; maximum score of 9).

2.6. Statistical analysis

Results were expressed as means ± standard errors of the means (SEM), as generated by GraphPad Prism, version 6.0 (GraphPad Software, San Diego, CA).

3. Results

3.1. Clinical observation

The BALB/c mice in control group were clinically normal and their rectal temperatures remained normal (37.7 °C–38.5 °C). The BALB/c mice in the infection group inoculated with the *Pestivirus H* cytopathogenic strain HN1507 began exhibiting a few clinical signs at 2 days PI and consistent findings were nasal discharge from 2 to 10 days PI, mildly elevated rectal temperatures (39.2 °C–39.6 °C) were also observed from 2 to 11 days PI. During in the whole experimental infection period the median days for occurrence of fever and clinical signs were 9 days. The mean daily clinical scores of inoculated mice are shown in Fig. 1. The highest mean daily clinical score was obtained at 5 dpi. The mean of the highest daily score achieved for individual mice was 5.53.