Veterinary Microbiology 192 (2016) 181–185

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Short communication

The pathogenicity of novel duck reovirus in Cherry Valley ducks

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ARTICLE INFO

Article history:
Received 1 April 2016
Received in revised form 14 July 2016
Accepted 22 July 2016

Keywords:
Novel duck reovirus
Pathogenicity
Innate immunity
Cherry Valley duck

ABSTRACT

The novel duck reovirus (NDRV) is an emerging, contagious infection. To better realize the pathogenic mechanism of NDRV in ducks, an infection experiment was conducted. The resulting data demonstrated that typical gross lesions were observed in the infected ducks. NDRV was able to replicate in various tissues, leading to these pathological lesions, especially on the liver and spleen. Real-time quantitative PCR showed that the expression of most innate immune-related genes was up-regulated and the antiviral innate immune response could be established in both the liver and spleen. This study indicates that NDRV is a pantropic virus. To resist viral infection, several pathogen recognition receptors can cooperatively recognize NDRV and initiate innate immunity, but the responses are different between different tissues. As far as we know, this is the first systematic investigation of the pathogenicity of NDRV in Cherry Valley ducks based on the host's innate immunity, and these data will provide new insights into the further study of the disease.

1. Introduction

In 2006, an infectious disease of Pekin ducks of unknown causative agent, characterized by the hemorrhagic spots and necrotic foci of the spleens and livers, was observed in Fujian, Guangdong, and Zhejiang, China (Liu et al., 2011). The various species of ducks could be infected, such as Pekin ducks, Muscovy ducks and domesticated wild duck. Subsequently, researchers isolated and identified a novel duck reovirus (NDRV) from affected ducklings (Chen et al., 2012; Liu et al., 2011), and thirteen isolates are found in National Center for Biotechnology Information using the Blast method, such as 091 strain and TH11 strain (Ma et al., 2012; Zhu et al., 2015). This is called the new reovirus, because compared to avian orthoreovirus (ARV) and Muscovy duck reovirus (MDRV), clinical symptoms and cytopathic effects are different, and the phylogenetic analysis demonstrates that the proteins encoded by S2 and S3 genes are in a different branch of ARV and MDRV, indicating that NDRV are significantly different from ARV and MDRV (Chen et al., 2010).

Although some outbreaks and detection methods of NDRV have been reported (Chen et al., 2012; Yuan et al., 2013), little research focuses on the pathogenicity of NDRV in ducklings, and the role of the host's innate immune responses in this process has not been studied. Here, pathological lesions and the viral distribution in the tissues of the NDRV-infected ducks were analyzed, and a special focus on the innate immune response to this virus was evaluated to systemically explore the pathogenicity of NDRV in Cherry Valley ducks.

2. Materials and methods

2.1. Ethics statement

The study was approved by the Committee on the Animal Ethics of Shandong Agricultural University. Experiments were carried out in accordance with the approved guidelines (No. SDAUA-2015-004).

2.2. Virus, cell, and animal

The NDRV strain used in this experiment was isolated from one clinically infected mallard duck in Linyi City, Shandong province in

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http://dx.doi.org/10.1016/j.vetmic.2016.07.015
0378-1135/

Table 1
Primers used in this study.

Primer name	Sequence (5′-3′)	Product size (bp)	GenBank No.
TRIF-F	GCTTTCAGGATGCTTTGGAG	155	KJ466051.1
TRIF-R	CTTGGGAACAAAAGGGATGA		
IPS-1-F	ACATCCTGAGGAACATGGAC	243	KJ466052.1
IPS-1-R	AGACCTCCTGCAGCTCTTCG		

2012. Twelve-day-old duck embryos were prepared to make duck embryo fibroblasts (DEF). The virus titer was determined using the $TCID_{50}$ method by the infection of DEF (Reed and Muench, 1938). Eighty one-day-old Cherry Valley ducks were purchased from the healthy farm, and raised in isolators. Serum samples were detected to verify that all ducks used in this study were serologically negative for NDRV.

2.3. Experimental procedure

After being raised for three weeks, the ducklings were randomly divided into two groups (40 ducks/group) and housed in separate isolators. The first group was used for an infection experiment in which ducks were intramuscularly inoculated with 0.5 mL virus stocks containing $1.0 \times 10^{4.5}$ $TCID_{50}$. The other group was set as the control group and received an intramuscular injection of 0.4 mL sterile PBS. The related signs of this disease were continuously observed for 14 days. On 1, 3, 5, 7, 9, and 14 days post-inoculation (dpi), five ducks from each group were sacrificed and their tissues (heart, liver, spleen, lung, kidney, and brain) were collected, and part were fixed with 4% paraformaldehyde solution for histopathological examination while others were stored at −80 °C for RNA extraction. The remaining ducks were euthanized at the end of this study through the intravenous administration of sodium pentobarbital (100 mg/kg body weight).

2.4. Viral load in the tissues of the infected ducks

The total RNA of samples was extracted by using the EasyPure RNA Kit according to the instruction (TransGen Biotech, Beijing, China). 1 μg total RNA was reverse-transcribed with HiScriptRII One Step RT-PCR kit (Vazyme, Nanjing, China). The detection method of viral RNA load in the tissues was Real-time quantitative PCR (RT-qPCR) and the primers were designed as previously described (Yuan et al., 2013). RT-qPCR was performed using the Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, CA, USA) with the SYBR Green PCR kit (Vazyme Biotech Co., Ltd., Nanjing, China).

2.5. The innate immune response of the infected ducks

RT-qPCR was used to detect the mRNA expression of the innate immune-related genes in the liver and spleen of the experimental ducks, and their relative level was quantified using the previously reported primers (Jiao et al., 2012; Li et al., 2015). The primers for the duck TRIF and IPS-1 genes were designed by the Primer3 software (http://bioinfo.ut.ee/primer3-0.4.0/) on the basis of the published GenBank sequences (Table 1), and their sensitivity and specificity were tested. The operation method of RT-qPCR was as described above.

2.6. Statistical analysis

The fold changes of the target genes in the experiment versus the control groups were calculated by the $2^{-\Delta\Delta Ct}$ method using glyceraldehyde-3-phosphate dehydrogenase. The fold changes were logarithmically transformed. All data were expressed as means ± standard deviation (n = 5) and were analyzed with a Student's *t*-test through GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA). $P < 0.05$ or $P < 0.01$ was considered significant.

3. Results

3.1. Clinical symptoms and gross lesions

Three-week-old Cherry Valley ducks were intramuscularly inoculated with NDRV to study the pathogenicity of this disease. The experimental ducks only showed a transient lassitude and anorexia, beyond that, there were no obvious clinical signs of NDRV or death during the tested period. However, livers were enlarged, hemorrhagic and necrotic spots were observed in some infected ducks as early as day three (Fig. 1A). Swollen spleens and small necrotic foci were also found at 3 and 5 dpi (Fig. 1B). Additionally, no other gross lesions were observed in both groups after 7 dpi.

3.2. Histopathologic analysis

Histopathological analysis showed that there were no microscopic lesions in the control group throughout the experimental infection. Although the gross lesions of infected ducks were not serious, microscopic lesions were observed in a variety of tissues. Analysis indicated necrosis of a large number of cells and inflammatory cell infiltration in the liver (Fig. 2A). Necrotic foci were obvious in the spleen (Fig. 2B). Coincidently, in the bursa of

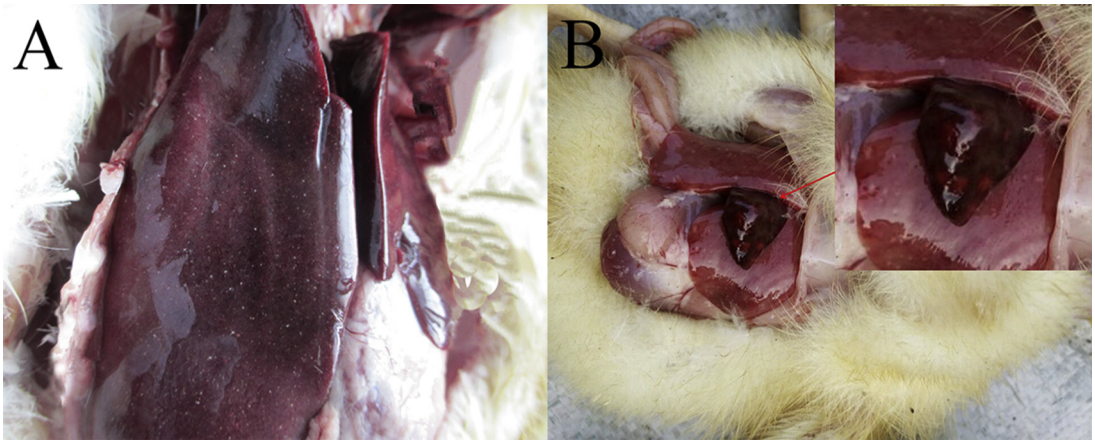

Fig. 1. Gross lesions of the NDRV-infected ducks. (A) Swollen and necrotic spots in the liver; (B) Splenomegaly with necrotic foci.