EMERGENCE OF RABBIT HEMORRHAGIC DISEASE VIRUS *RHDV-2* IN CHINA

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ABSTRACT

Rabbit hemorrhagic disease (RHD) is an acute fatal disease caused by the *Lagovirus* rabbit hemorrhagic disease virus (RHDV), which was first reported in 1984 in China. Strains of two different genotypes (GI.1a and GI.1c) have been detected in China to date. In 2010, a new RHDV variant with a unique genetic and antigenic profile was identified in France, designated RHDV2, which rapidly spread throughout continental Europe and nearby islands. Here, we report the first outbreak of RHD induced by RHDV2 (GI.2) in rabbit farms in the Sichuan province of China. We conducted phylogenetic analysis of the new RHDV isolate SC2020/04, which was identified as a non-recombinant strain belonging to the RHDV2 (GI.2) genogroup. The current GI.1 licensed vaccine used in China could not provide effective protection against the new isolate. Considering the serious risk of RHDV2 to the Chinese rabbit industry, the circulation of RHDV2 in the population should be carefully monitored in China.

Key words: rabbit hemorrhagic disease virus 2 (RHDV2), *Lagovirus*, phylogenetic analysis, China

INTRODUCTION

China is highly ranked in the global rabbit industry, accounting for 43% of the worldwide slaughtered rabbits with 44% of the global share of rabbit meat output (Wu, Seema, & Huang, 2016). Members of the family *Caliciviridae* and genus *Lagovirus*, Rabbit hemorrhagic disease virus (RHDV) causes high morbidity and mortality in rabbits, resulting in over 90% of RHDV-infected adult rabbits deaths owing to fulminant hepatic failure within 3 days of infection (Park, Lee, & Itakura, 1995). RHDV was first reported in China in 1984. However, a new RHDV-related virus designated RHDV2 was detected, for the first time, in France in 2010 (Le Gall-Recule et al., 2013), and subsequently spread worldwide.
Based on phylogenetic analyses of RHDV VP60 sequences, RHDV was divided into classical RHDV G1−G5 and G6 or RHDVa and the new strain named RHDV2 (Le Gall-Recule et al., 2003 and 2013). In 2017, a new RHDV nomenclature was proposed that changed G1, G2, G3−G5, and G6 to GI.1b, GI.1c, GI.1d, and GI.1a, respectively, and RHDV2 was called GI.2 (Le Pendu et al., 2017). To date, only two RHDV genotypes were known to be present in China, G2 (GI.1a) and G6 (GI.1c) (Hu et al., 2017). Here, we report a new RHDV isolate collected from three infected rabbits at farms in the Sichuan province of China in April 2020, representing the first report of GI.2 in China.

MATERIAL AND METHODS

Virus samples

The new RHDV isolate SC2020/04 was collected from rabbit farms experiencing RHDV outbreaks in Sichuan province of China. Liver samples were homogenized (20% in phosphate-buffered saline [PBS]), frozen at -70°C, and thawed twice. The new isolate was compared with the RHDV isolate WF/China/2007 (FJ794180; GI.1a) maintained in our laboratory.

Reverse transcription-polymerase chain reaction (RT-PCR)

The *vp60* gene and full-length genomic sequence were amplified by RT-PCR using the Reverse Transcriptase XL (AMV) kit (Takara Bio, Dalian, China) and the Ex *Taq* kit (Takara Bio). A specific

primer pair was used for *vp60* gene amplify and eight pairs of primers were used for genomic sequence amplify (Duarte et al., 2014; Lopes et al., 2015). The gene fragments were then cloned into a pMD-19T vector (Takara Bio). Positive clones were sequenced and analyzed further.

Phylogenetic analysis

Phylogenetic analysis of *vp60* and full-length genomic sequences were performed using MEGA 7 with the neighbor-joining approach based on the Kimura 2-parameter model. Reliability of the nodes was assessed with a bootstrap resampling procedure consisting of 1000 replicates.

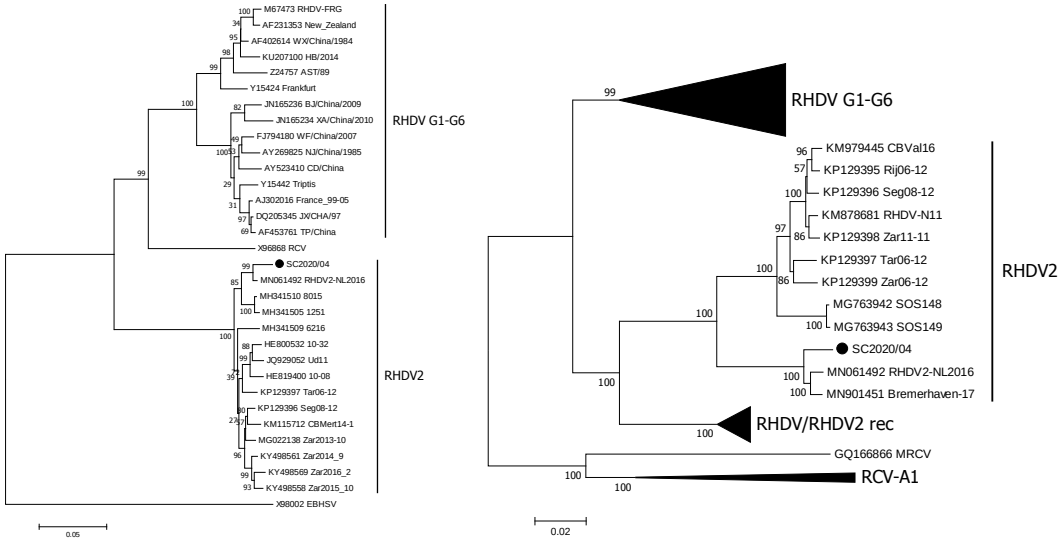
Challenge experiment

Ten 8-week-old New Zealand White rabbits purchased from Qingdao Kangda Rabbit Industry Development Co. Ltd (Qingdao, China) were randomly divided into 2 groups. One group was subcutaneously immunized with commercial inactivated vaccine (WF/China/2007) and the other five rabbits were injected with PBS (pH 6.5) as an unvaccinated control. The challenge experiment was performed on 14 days post immunization by infecting the rabbits with 1 mL of the liver homogenates of SC2020/04.

RESULTS AND DISCUSSION

The clinical symptoms and pathological changes of the infection rabbits in farms were similar to those of rabbit hemorrhagic disease. The mortality rate was more than 70% (approximately 1300 rabbits died), although weaning rabbits had been immunized with a commercial inactivated RHD vaccine. Importantly, most of the unweaned rabbits died of the disease, indicating that RHDV2 might be the causal pathogen, because RHDV2 is able to fatally affect a high proportion of young rabbits.

The new isolate exhibits the highest nucleotide sequence identity with the NL2016 strain from the Netherlands (98.3%; MN061492), which corresponds to RHDV2. Phylogenetic analysis was employed to determine the evolution of the new isolate. As shown in Figure 1, the new isolate, identified as a non-recombinant strain, is in the same branch of the other RHDV2 strains. These results support the conclusion that the isolate collected from the Sichuan province of China in 2020 belongs to the RHDV2 (GI.2) genogroup, which was designated strain SC2020/04 (GenBank accession number MT383749 for *vp60* gene and MT586027 for genomic sequence). It represents the first outbreak of RHDV2-induced RHD in rabbit farms in China. We previously classified all RHDV isolates in China collected before 2017 in GI.1 (Hu et al., 2017); therefore, the present finding indicates the potential for co-circulation of RHDV (GI.1)and RHDV2 (GI.2) in China. Indeed, RHDV2 (GI.2) was reported to replace RHDV (GI.1) in some countries, including Portugal, Sweden, and Australia (Lopes et al., 2014; Mahar et al., 2018; Neimanis et al., 2018). In addition, recombinant events between GI.2 and other genotypes have also been reported in recent years (Almeida et al., 2015; Silverio et al., 2018; Lopes et al., 2015).



A) B)

Figure 1. Neighbor-joining phylogenetic trees for the complete nucleotide sequences of RHDV *vp60*

genes (A) and full genomic sequences (B). Bootstrap probability values above 50% with 1000 replicates are indicated at the nodes. The branch lengths are proportional to the genetic distance.

Considering the distinct serotype from RHDV (GI.1), we further checked the morbidity and mortality in rabbits immunized commercial inactivated vaccine (WF/China/2007) against SC2020/04. The result suggests that the current GI.1 licensed vaccine used in China against RHDV2 was limited since only 40% of vaccinated rabbits survived during the infection. These indicated rabbits in the farms at high risk of exposure to RHDV2 (GI.2). More importantly, ongoing surveillance and vaccine formulation update are most imminent requirements for control of the disease induced by RHDV2 in China.

CONCLUSIONS

In summary, analysis of the new RHDV isolate SC2020/04 suggests the first outbreak of RHDV2 (GI.2) in rabbit farms in China. Furthermore, current licensed vaccine used in China could not provide effective protection against the new isolate. Considering the co-circulation of RHDV (GI.1) and RHDV2 (GI.2) in China, combined vaccine with both antigenic types might be useful for controlling rabbit hemorrhagic disease in China.

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