

Emergence of rabbit hemorrhagic disease virus 2 in China in 2020

Bo Hu¹, Houjun Wei¹, Zhiyu Fan¹, Yanhua Song¹, Mengmeng Chen¹, Rulong Qiu¹, Weifeng Zhu¹, Weizhong Xu¹, Jiabin Xue¹, and Fang Wang¹

¹Jiangsu Academy of Agricultural Sciences

May 6, 2020

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 hemagglutination tests and phylogenetic analysis of the new RHDV isolate SC2020/04, which was identified as a non-hemagglutinating strain belonging to the RHDV2 (GI.2) genogroup. Considering the serious risk of RHDV2 to the Chinese rabbit industry, the circulation of RHDV2 in the population should be carefully monitored in China.

Emergence of rabbit hemorrhagic disease virus 2 in China in 2020

Running title: RHDV2 in China

Bo Hu[#], Houjun Wei[#], Zhiyu Fan[#], Yanhua Song, Mengmeng Chen, Rulong Qiu, Weifeng Zhu, Weizhong Xu, Jiabin Xue, Fang Wang^{*}

Institute of Veterinary Medicine, Jiangsu Academy of Agricultural Sciences, Key Laboratory of Veterinary Biologicals Engineering and Technology, Ministry of Agriculture, National Center for Engineering Research of Veterinary Bio-products, Nanjing 210014, China

^{*}Corresponding author. Tel: +86 25 84390337; E-mail address: rwangfang@126.com

[#]These authors contributed equally to this work.

Summary: 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 hemagglutination tests and phylogenetic analysis of the new RHDV isolate SC2020/04, which was identified as a non-hemagglutinating strain belonging to the RHDV2 (GI.2) genogroup. 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, RHDV2, *Lagovirus*, rabbits, 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). Rabbit hemorrhagic disease

virus (RHDV) of the family *Caliciviridae*, genus *Lagovirus*, causes high morbidity and mortality in rabbits. Over 90% of RHDV-infected adult rabbits die 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 in France in 2010 (Le Gall-Recule et al., 2013), subsequently spreading to other countries in Europe, Australia, and Africa (Abrantes et al., 2013; Lopes et al., 2019; Mahar et al., 2018; Puggioni et al., 2013).

Based on phylogenetic analyses of RHDV VP60 sequences, RHDV is divided into classical RHDV (GI.1) and RHDV2 (GI.2). Two RHDV genotype strains are considered 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.

MATERIAL AND METHODS

Hemagglutination test

Liver samples collected from three infected rabbits were frozen and stored at -70°C . Liver samples were homogenized (20% in phosphate-buffered saline [PBS]), frozen at -70°C , and thawed twice. The hemagglutination test (Hu et al., 2016) was carried out in U-shaped microtiter plates containing 50 μL of PBS (pH 6.5). Fifty-microliter suspensions of homogenized liver samples were two-fold serially diluted and placed in U-shaped plates; they were then further incubated with an equal volume of 1% human O erythrocytes at 4°C , 25°C , or 37°C . The hemagglutination was visually determined 30 min later. RHDV isolate WF/China/2007 (GenBank accession number: FJ794180) was used as a positive control.

Reverse transcription-polymerase chain reaction (RT-PCR)

The full-length of the *vp60* gene sequence was 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 (sense, 5'-AAGAGAGTCGTCTCGGTAGTA-3' and antisense 5'-GCGCCTGCAAGTCCCAATCC-3') was used as described previously (Duarte et al., 2014). The *vp60* gene was then cloned into a pMD-19T vector (Takara Bio). Positive clones were sequenced and analyzed further.

Phylogenetic analysis

Phylogenetic analysis of *vp60* gene sequences was performed using MEGA 7 (Kumar Stecher, & Tamura, 2016) with the maximum-likelihood approach based on the Kimura 2-parameter model. Reliability of the nodes was assessed with a bootstrap resampling procedure consisting of 1000 replicates.

RESULTS AND DISCUSSION

The clinical symptoms and pathological changes in the dead rabbits 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.

Compared with the positive control, the new isolate failed to hemagglutinate at 4°C , 25°C , and 37°C (hemagglutination titers [?] 1:20). Given that the hemagglutination test remains the routine diagnostic method for RHDV in China, this non-hemagglutinating characteristic warrants further attention in the detection of clinical samples.

The new isolate exhibits the highest nucleotide sequence identity with the NL2016 strain from the Netherlands (98.3%; GenBank accession number: 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 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). This 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 and RHDV2 in China. Indeed, RHDV2 (GI.2) was reported to replace RHDV (GI.1) in some countries, including Sweden and Australia (Mahar et al., 2018; Neimanis et al., 2018). In addition, recombinant events between GI.2 and other genotypes have been reported in Europe (Almeida et al., 2015; Lopes et al., 2015). Considering the distinct serotype from RHDV (GI.1) and the high risk of RHDV2 (GI.2) to the Chinese rabbit industry, ongoing surveillance is important to better understand the epidemic of RHDV in China.

Acknowledgment: This work was supported by the funds earmarked for the China Agriculture Research System (No. CARS-43-C-1) and National Key R&D Program of China (No. 2018YFD0502203). We would like to thank Editage for English language editing.

Conflict of interests: The authors declare no conflict of interest regarding the publication of this manuscript. All authors have read and approved the final manuscript.

Ethical approval: The authors confirm that the ethical policies of the journal have been adhered to. The collection of the liver samples was performed in strict accordance with the guidelines of Jiangsu Province Animal Regulations (Government Decree No. 45).

Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

- Abrantes, J., Lopes, A. M., Dalton, K. P., Melo, P., Correia, J. J., Ramada, M.,... Esteves, P. J. (2013). New variant of rabbit hemorrhagic disease virus, Portugal, 2012-2013. *Emerging Infectious Diseases*, *19*(11), 1900–1902. doi:10.3201/eid1911.130908
- Almeida, T., Lopes, A. M., Magalhaes, M. J., Neves, F., Pinheiro, A., Goncalves, D.,... Abrantes, J. (2015). Tracking the evolution of the G1/RHDVb recombinant strains introduced from the Iberian Peninsula to the Azores islands, Portugal. *Infection Genetics and Evolution*, *34*, 307–313. doi:10.1016/j.meegid.2015.07.010
- Duarte, M. D., Henriques, A. M., Barros, S., Luis, T., Fagulha, T., Ramos, F.,... Fevereiro, M. (2014). New insight into the epidemiology of rabbit hemorrhagic disease viruses in Portugal: retrospective study reveals the circulation of genogroup 5 (G5) in Azores and discloses the circulation of G1 and G6 strains in mainland until 2008. *Infection Genetics and Evolution*, *27*, 149–155. doi:10.1016/j.meegid.2014.07.017
- Hu, B., Fan, Z., Wang, F., Song, Y., Wei, H., Liu, X.,... Xue, J. (2016). A new variant of rabbit hemorrhagic disease virus G2-like strain isolated in China. *Virus Research*, *215*, 20–24. doi:10.1016/j.virusres.2016.01.018
- Hu, B., Wang, F., Fan, Z., Song, Y., Abrantes, J., Zuo, Y.,... Esteves, P. J. (2017). Recombination between G2 and G6 strains of rabbit hemorrhagic disease virus (RHDV) in China. *Archives of Virology*, *162*(1), 269–272. doi:10.1007/s00705-016-3082-6
- Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, *33*(7), 1870–1874. doi:10.1093/molbev/msw054
- Le Gall-Recule, G., Lavazza, A., Marchandea, S., Bertagnoli, S., Zwingelstein, F., Cavadini, P.,... Capucci, L. (2013). Emergence of a new lagovirus related to Rabbit Haemorrhagic Disease Virus. *Veterinary Research*, *44*, 81. doi:10.1186/1297-9716-44-81
- Lopes, A. M., Dalton, K. P., Magalhaes, M. J., Parra, F., Esteves, P. J., Holmes, E. C.,... Abrantes, J. (2015). Full genomic analysis of new variant rabbit hemorrhagic disease virus revealed multiple recombination events. *Journal of General Virology*, *96*(Pt 6), 1309–1319. doi:10.1099/vir.0.000070
- Lopes, A. M., Rouco, C., Esteves, P. J., & Abrantes, J. (2019). GI.1b/GI.1b/GI.2 recombinant rabbit hemorrhagic disease virus 2 (Lagovirus europaeus/GI.2) in Morocco, Africa. *Archives of Virology*, *164*(1), 279–283. doi:10.1007/s00705-018-4052-y

Mahar, J. E., Hall, R. N., Peacock, D., Kovaliski, J., Piper, M., Mourant, R.,... Strive, T. (2018). Rabbit Hemorrhagic Disease Virus 2 (RHDV2; GI.2) is replacing endemic strains of RHDV in the Australian landscape within 18 months of its arrival. *Journal of Virology*, *92*(2), e01374-17 doi:10.1128/JVI.01374-17

Neimanis, A. S., Ahola, H., Zohari, S., Larsson, P. U., Brojer, C., Capucci, L.,... Gavier-Widen, D. (2018). Arrival of rabbit haemorrhagic disease virus 2 to northern Europe: Emergence and outbreaks in wild and domestic rabbits (*Oryctolagus cuniculus*) in Sweden. *Transboundary and Emerging Diseases*, *65*(1), 213–220. doi:10.1111/tbed.12650

Park, J. H., Lee, Y. S., & Itakura, C. (1995). Pathogenesis of acute necrotic hepatitis in rabbit hemorrhagic disease. *Lab Animal Science*, *45*(4), 445–449

Puggioni, G., Cavadini, P., Maestrale, C., Scivoli, R., Botti, G., Ligios, C.,... Capucci, L. (2013). The new French 2010 Rabbit Hemorrhagic Disease Virus causes an RHD-like disease in the Sardinian Cape hare (*Lepus capensis mediterraneus*). *Veterinary Research*, *44*, 96. doi:10.1186/1297-9716-44-96

Wu, L. P., Seema, B., & Huang, D. (2016). The contribution of Chinese rabbit industry and its sustainable development. *Proceedings of the 11th World Rabbit Congress, Qingdao (China)*, 1017–1020.

FIGURE LEGEND

FIGURE 1 . Maximum-likelihood phylogenetic trees for the complete nucleotide sequences of RHDV *vp60* genes. Bootstrap probability values above 50% with 1000 replicates are indicated at the nodes. The branch lengths are proportional to the genetic distance. European brown hare syndrome virus (EBHSV) strain BS89 was used as the outgroup to root the tree.

