Archives of Virology (2020) 165:2367–2372
https://doi.org/10.1007/s00705-020-04712-5

BRIEF REPORT

Molecular characterization of an emerging reassortant mammalian orthoreovirus in China

Dandan Ye[1] · Zhaoyang Ji[1] · Hongyan Shi[1] · Jianfei Chen[1] · Da Shi[1] · Liyan Cao[1] · Jianbo Liu[1] · Mingwei Li[1] · Hui Dong[1] · Zhaoyang Jing[1] · Xiaobo Wang[1] · Qiuge Liu[1] · Qianjin Fan[1] · Guangyi Cong[1] · Jiyu Zhang[1] · Yuru Han[1] · Jiyong Zhou[2] · Jinyan Gu[2] · Xin Zhang[1] · Li Feng[1]

Received: 1 April 2020 / Accepted: 22 May 2020 / Published online: 5 August 2020

Abstract

Mammalian orthoreoviruses (MRVs) infect almost all mammals, and there are some reports on MRVs in China. In this study, a novel strain was identified, which was designated as HLJYC2017. The results of genetic analysis showed that MRV HLJYC2017 is a reassortant strain. According to biological information analysis, different serotypes of MRV contain specific amino acid insertions and deletions in the σ1 protein. Neutralizing antibody epitope analysis revealed partial cross-protection among MRV1, MRV2, and MRV3 isolates from China. L3 gene recombination in MRV was identified for the first time in this study. The results of this study provide valuable information on MRV reassortment and evolution.

Mammalian orthoreoviruses (MRVs) belong to the genus *Orthoreovirus* in the family *Reoviridae*. MRVs are non-enveloped, double-stranded (ds) RNA viruses that cause symptomatic or asymptomatic infection in mammals [1]. They are classified into four serotypes: MRV serotype 1 (MRV1) (Lang, T1L), MRV serotype 2 (MRV2) (Jones, T2J), MRV serotype 3 (MRV3) (Dearing, T3D), and MRV serotype 4 (MRV4) (Ndelle, T4N) [2]. The genome of MRVs is approximately 23,500 bp in total and contains 10 segments: three large (L) segments (L1-L3), three medium (M) segments (M1-M3), and four small (S) segments (S1-S4) [3]. Segments L1-L3 encode the λ3, λ2, and λ1 protein, respectively; segments M1-M3 encode the μ2, μ1, and μNS protein, respectively; and segments S1-S4 encode the σ1, σ2, σNS, and σ3 protein, respectively. Neutralization and hemagglutinin activities are restricted to the σ1 protein, which is located on the outer capsid of the virion [4]. The σ1 protein is responsible for attachment of the virus to the cell receptor and is used for serotype determination of MRVs [5].

MRVs are considered to be nonpathogenic, causing mild respiratory and enteric symptoms only occasionally [6]. However, some reports have shown that they can cause severe illness in animals and children. MRV was detected in a child with acute gastroenteritis, and this virus showed the highest similarity to an MRV recently reported in European bats [7]. Furthermore, an increasing number of reports have indicated that MRV is the cause of diarrhea in newborn piglets. In 2015, a novel pathogenic MRV was found in diarrheic pigs in the United States [8], and in 2017, a novel reassortant MRV3 was identified in diarrheic pigs in eastern China [2]. To study the biological characteristics of MRV in China, further investigation of MRV diversity is necessary.

In 2017, 19 fecal samples from suckling piglets with diarrhea were collected from pig farms in Heilongjiang Province in China. The positivity rate of MRV in Heilongjiang Province was found to be 15.79% (3/19) by RT-PCR using

Handling Editor: William G. Dundon.

Dandan Ye and Zhaoyang Ji contributed equally to this paper.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00705-020-04712-5) contains supplementary material, which is available to authorized users.

✉ Jinyan Gu
gjy@njau.edu.cn

✉ Xin Zhang
zhangxin01@caas.cn

✉ Li Feng
fengli@caas.cn; fengli_h@163.com

[1] Division of Swine Infectious Diseases, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, No. 427 Maduan Street, Nangang District, Harbin 150001, China

[2] Key Laboratory of Animal Virology of Ministry of Agriculture, Department of Veterinary Medicine, Zhejiang University, Hangzhou, China

previously described primers [9]. MRV-positive fecal samples were inoculated onto MDBK cells. After five passages, a cytopathic effect (CPE) was found with one MRV-positive fecal sample, as evidenced by enlargement of the cells, cell rupture, and granular changes. Electron microscopic observation revealed the presence of virus particles with a diameter of approximately 70 nm (Supplementary Fig. S1A). The virus strain was named HLJYC2017.

The whole genome of MRV HLJYC2017 was amplified and sequenced (Supplementary Table S1, Fig. S1B). The sequence of each segment of HLJYC2017 was deposited in the GenBank database (Supplementary Table S2). The nucleotide sequences of MRV HLJYC2017 S1, S2, S3 and S4 are 1413 nt, 1257 nt, 1101 nt, and 1098 nt in length, respectively, encoding predicted σ1, σ2, σNS, and σ3 proteins consisting of 470 aa, 418 aa, 366 aa, and 365 aa, respectively. The nucleotide sequences of the MRV HLJYC2017 M1, M2, and M3 genes are 2211 nt, 2127, and 2166 nt in length, respectively, encoding predicted μ2, μ1, and μNS proteins of 736 aa, 708 aa, and 721 aa, respectively. The nucleotide sequences of the MRV HLJYC2017 L1, L2, and L3 genes are 3804 nt, 3870 nt, and 3828 nt in length, respectively, encoding predicted λ3, λ2, and λ1 proteins of 1267 aa, 1289 aa, and 1275 aa, respectively.

Phylogenetic analysis based on complete nucleotide sequences of the MRV HLJYC2017 S1 gene and the S1 genes of other MRVs with sequences available in the GenBank database (Supplementary Table S3) indicated a close relationship with the MRV isolate T1/T28/KM/2013 (Supplementary Table S2, Fig. 1) (86.9% nt and 92.8% aa sequence identity) (host: *Tupaia* sp.). The serotype was classified as MRV1. Phylogenetic analysis based on complete nucleotide sequences of the MRV HLJYC2017 S2, S3, S4, M1, M3, L1 and L2 genes showed that this strain is closely related to the MRV isolate WIV3 (Supplementary Table S2, Supplementary Fig. S2) isolated in Wuhan, China (host: *Hipposideros* sp.). Phylogenetic analysis based on the complete MRV HLJYC2017 M2 gene showed that it is closely related to the isolate GD-1 (Supplementary Table S2) isolated in China (host: pig), and phylogenetic analysis based on the complete MRV HLJYC2017 L3 gene indicated that it is closely related to the MRV2 isolate OV204 (Supplementary Table S2) isolated at the University of Florida in the USA (host: *Odocoileus virginianus*). These results indicate that MRV HLJYC2017 is a reassortant strain.

Reassortment of genome segments might play an important role in the emergence of new reovirus variants [1, 10–14], and reassortment events between MRV strains have been reported previously [2, 8, 15–20]. In this study, of the 10 segments of the MRV HLJYC2017 strain, seven (S2, S3, S4, M1, M3, L1, and L2) possess sequences that are nearly identical to those of WIV3 (host: *Hipposideros*), indicating that MRV HLJYC2017 might be of bat origin. The S1 gene of the MRV HLJYC2017 strain appears to have murine origins, because it exhibits the closest relationship to the S1 gene of MRV isolate T1/T28/KM/2013 (host: *Tupaia*), and its M2 gene appears to have a porcine origin, because M2 has the closest relationships to the MRV isolate GD-1 (host: pig). Furthermore, the L3 gene of the MRV HLJYC2017 strain appears to be of deer origin, because it is most closely related to that of the MRV isolate OV204 (host: *Odocoileus virginianus*). These results indicate that MRV HLJYC2017 might be a reassortant strain with segments derived from bat, murine, porcine, and deer viruses (Fig. 2). These results suggest that MRV infection on farms in China is becoming increasingly complex.

The trimeric σ1 protein is divided into two domains: an N-terminal tail and a C-terminal head [21]. Through aa sequence comparisons, we found two large differences in the N-terminal tails of MRV1, MRV2 and MRV3 (Supplementary Fig. S3A). The first difference is that, compared with MRV1 and MRV2 strains, all MRV3 strains have an insertion of 4 aa (GLES) located between 26K and 27E (aa numbering based on MRV1). The second difference is that there is a large gap in the S1 protein between 155D and 171R of MRV1 compared to MRV2 and MRV3 (Supplementary Fig. S3A). There is also a 7-aa deletion (156SLINSGQ162, between 155D and 171R) in the S1 protein of MRV2 compared with that of MRV1 strains, and there is a 14-aa deletion (157LINSGQSSIGELSA170, between 156S and 171R) in the S1 protein of MRV3 compared with that of MRV1 strains (Supplementary Fig. S3A).

Serotype-dependent differences in MRV are related to the S1 gene fragment encoding the σ1 protein, which is responsible for viral attachment to cellular receptors [22]. Further analysis of the influence of these aa changes on the spatial structure of the N-terminal tail of the S1 protein was performed. The S1 protein sequences of MRV1 strain HLJYC20172017 and MRV3 strain MPC/04 [23] were used to search the SWISS-MODEL template library using PyMOL software [18]. The solution structure of the outer capsid protein sigma-1 (VP4) (SMTL ID: 6gao.1 and 6gap.1) [24] was selected for model construction, which showed that the spatial structure of the S1 protein N-terminal tail of HLJYC20172017 is different from that of the MRV3 MPC/04 strain. Specifically, a longer α-helix forms in the N-terminal tail of the MRV1 HLJYC20172017 strain than in that of the MRV3 MPC/04 strain (Supplementary Fig. S3B).

Structures of two neutralizing epitopes (5C6 and 9BG5) have been identified on the σ1 protein [21]. The two neutralizing epitope regions of the σ1 protein of HLJYC2017 were compared with those of MRV strains (encoding the full-length σ1 protein) from China obtained from the GenBank database. In the 5C6 neutralizing epitope, 13 aa positions (415A, 417Q, 424R, 426D, 429R, 430I, 446Y, 447G,

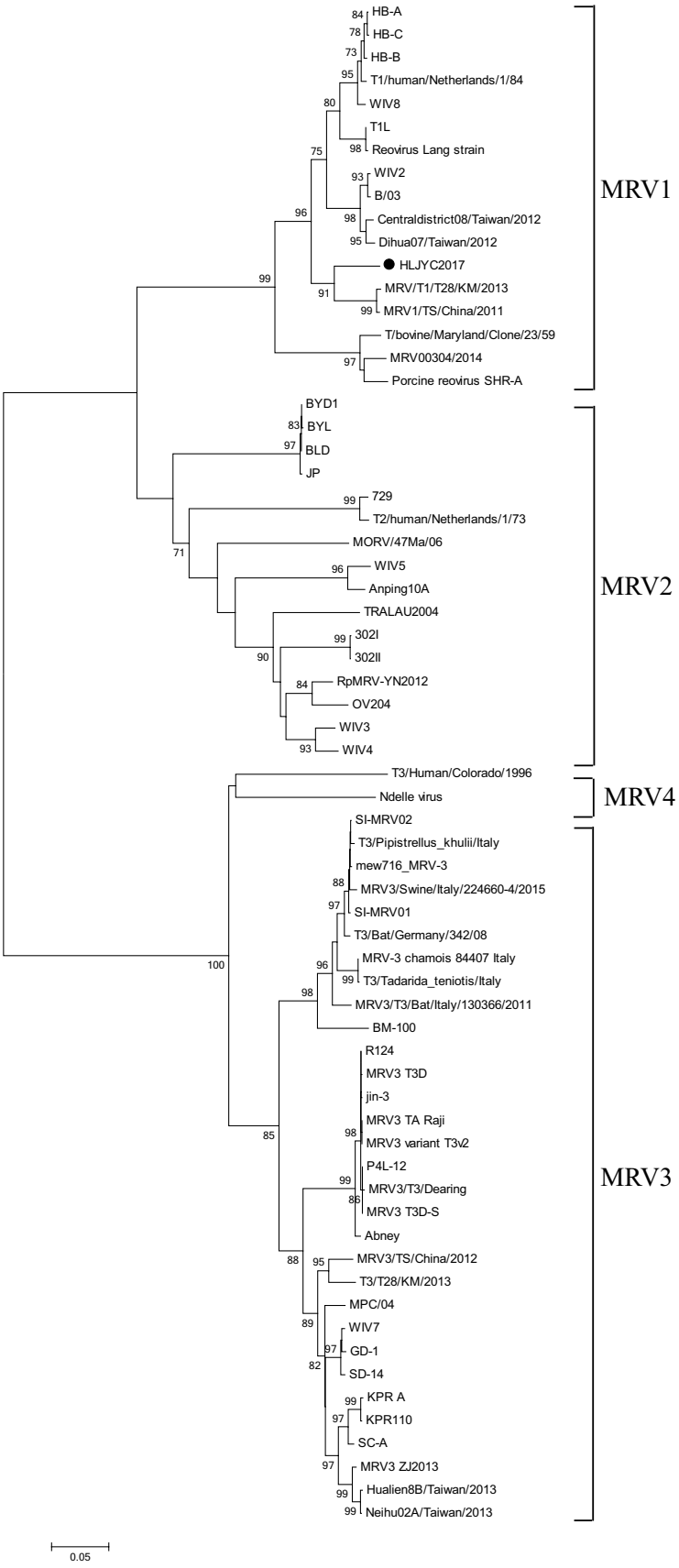

Fig. 1 Phylogenetic analysis of MRV HLJYC2017 based on the S1 sequence. Phylogenetic trees were constructed using MEGA 5.2 software, using the Construct/Test neighbor-joining method (bootstrap method with 1000 replications). Bootstrap values > 70% are shown

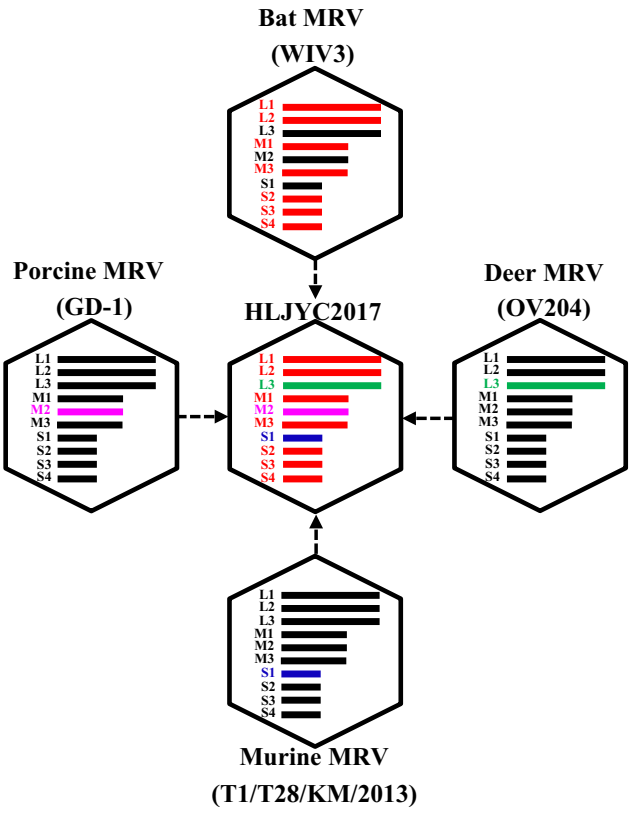

Fig. 2 The 10 segments of HLJYC2017 that are closely related to those of bat, murine, porcine and deer MRVs. HLJYC2017 is likely to have been formed by a reassortment process, with L1, L2, M1, M3, S2, S3, and S4 from a bat MRV; L3 from a deer MRV; M2 from a porcine MRV; and S1 from a murine MRV

448G, 449T, 450Y, 458W and 459A) are conserved between MRV1 and MRV2 strains (Supplementary Fig. S4A), and 5 aa positions (415A, 422G, 447G, 448G and 458W) are conserved between MRV1 and MRV3 strains (Supplementary Fig. S4A). In the 9BG5 neutralizing epitope, 14 aa positions (344Q, 346P, 354V, 355T, 368S, 381G, 382K, 408P, 410S, 432G, 434S, 435E, 442E, and 446Y) are conserved between MRV1 and MRV2 strains (Supplementary Fig. S4B) and four (351R, 354V, 381G, and 407) are conserved between MRV1 and MRV3 strains (Supplementary Fig. S4B). There is a partial overlap of neutralizing antibody epitopes among MRV1, MRV2 and MRV3, suggesting some cross-protection among them.

Recombination is a common process that produces diversity in most RNA viruses and creates new opportunities for them to overcome selective pressures and adapt to new environments and hosts [25]. Recombination analysis was performed using RDP4 software [26], revealing recombination in the L3 gene sequence, as shown in Fig. 3. A breakpoint for a potential recombination zone was detected in the L3 gene (nt 1619–1663). The recombinant strain is the MRV BatMRV1-IT2011 strain (KT900697). The major parental strain for the recombination site located in the L3 gene is HLJYC2017, and the minor parental strain is the MRV strain FS-03 (KM820756).

Analysis of viral recombination has attracted the interest of epidemiologists, molecular biologists, and evolutionary biologists. Under field conditions, mixed infections are required to give rise to recombination events [27, 28]. Some MRV strains have been found in China, such as MRV-ZJ2013 [2], MRV variant B/03 [29], MRV3 SD-14 [30, 31], BtMRV WIV2-5, WIV7 and WIV8 [31], MRV3 MPC/04 [23], RpMRV-YN2012 [32], MRV2tou05 [33], MRV-HLJ/2007 [34], and BYD1 [35], providing increased opportunities for genome reorganization. In addition, the transmission of MRV from one host to another is not limited to close contact but may occur due to indirect contamination [15]. As infectious particles of MRV are found in environmental samples, it is likely that infection can be transmitted in the environment through contaminated food, water, or other factors [36]. This provides sufficient opportunity for the occurrence of recombination. In avian orthoreoviruses, several reports have identified recombinant sequences in L1

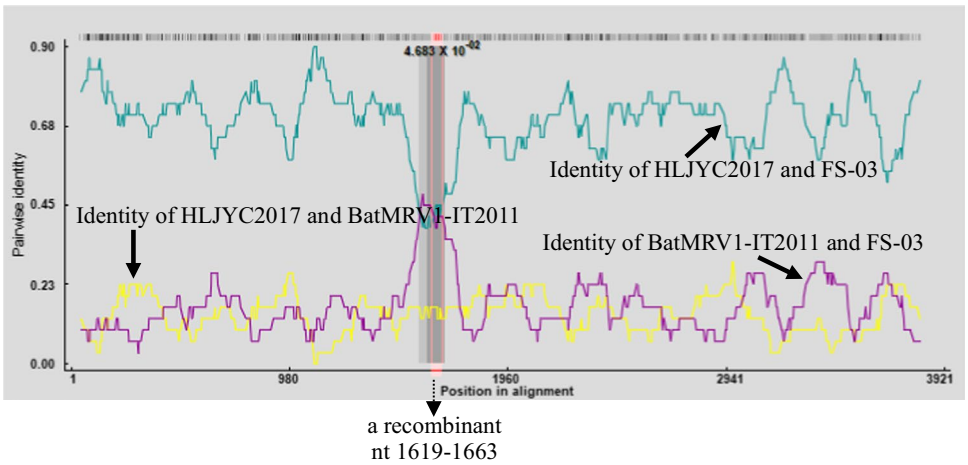

Fig. 3 Recombination analysis of MRV HLJYC2017 and other MRV strains

from Muscovy duck reovirus [11]; L1, L2, M1, M2, M3, and S2 from chicken orthoreovirus [12]; and M1 and S2 from avian orthoreovirus [1] (Supplementary Table S4). In MRVs, recombination in the L1, L2, M1, M2, M3 and S1 genes has also been found [2, 15]. In this study, recombination in the L3 gene of MRV was identified for the first time. Further studies of the genetic evolution of new MRVs in China are necessary.

In summary, a novel reassortant of MRV from China was isolated in this study. The insertion and deletion of specific amino acids in the σ1 protein have occurred between different serotypes of MRV. Neutralizing antibody epitope analysis showed partial cross-protection between MRV1, MRV2, and MRV3 in China. These results provide valuable information on MRV evolution and will facilitate future investigation of the molecular pathogenesis of MRVs.

Acknowledgements This study was supported by the National Key Research and Development Program of China (2017YFD0500100 and 2017YFD0501603), the National Natural Science Foundation of China (31602072) and the Heilongjiang Science Foundation Project (C2018066 and C2017079).

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest with this research.

References

1. Noh JY, Lee DH, Lim TH, Lee JH, Day JM, Song CS (2018) Isolation and genomic characterization of a novel avian orthoreovirus strain in Korea, 2014. Arch Virol 163(5):1307–1316. https://doi.org/10.1007/s00705-017-3667-8
2. Qin P, Li H, Wang JW, Wang B, Xie RH, Xu H, Zhao LY, Li L, Pan YF, Song YH, Huang YW (2017) Genetic and pathogenic characterization of a novel reassortant mammalian orthoreovirus 3 (MRV3) from a diarrheic piglet and seroepidemiological survey of MRV3 in diarrheic pigs from east China. Vet Microbiol 208:126–136. https://doi.org/10.1016/j.vetmic.2017.07.021
3. Nibert ML, Dermody TS, Fields BN (1990) Structure of the reovirus cell-attachment protein: a model for the domain organization of sigma 1. J Virol 64(6):2976–2989
4. Weiner HL, Fields BN (1977) Neutralization of reovirus: the gene responsible for the neutralization antigen. J Exp Med 146(5):1305–1310. https://doi.org/10.1084/jem.146.5.1305
5. Lee PW, Hayes EC, Joklik WK (1981) Protein sigma 1 is the reovirus cell attachment protein. Virology 108(1):156–163. https://doi.org/10.1016/0042-6822(81)90535-3
6. Lelli D, Beato MS, Cavicchio L, Lavazza A, Chiapponi C, Leopardi S, Baioni L, De Benedictis P, Moreno A (2016) First identification of mammalian orthoreovirus type 3 in diarrheic pigs in Europe. Virol J 13:139. https://doi.org/10.1186/s12985-016-0593-4
7. Steyer A, Gutierrez-Aguire I, Kolenc M, Koren S, Kutnjak D, Pokorn M, Poljsak-Prijatelj M, Racki N, Ravnikar M, Sagadin M, Fratnik Steyer A, Toplak N (2013) High similarity of novel orthoreovirus detected in a child hospitalized with acute gastroenteritis to mammalian orthoreoviruses found in bats in Europe. J Clin Microbiol 51(11):3818–3825. https://doi.org/10.1128/JCM.01531-13
8. Thimmasandra NA, Sooryanarain H, Deventhiran J, Cao D, Ammayappan VB, Kambiranda D, LeRoith T, Heffron CL, Lindstrom N, Hall K, Jobst P, Sexton C, Meng XJ, Elankumaran S (2015) A novel pathogenic Mammalian orthoreovirus from diarrheic pigs and Swine blood meal in the United States. MBio 6(3):00593–1515. https://doi.org/10.1128/mBio.00593-15
9. Leary TP, Erker JC, Chalmers ML, Cruz AT, Wetzel JD, Desai SM, Mushahwar IK, Dermody TS (2002) Detection of mammalian reovirus RNA by using reverse transcription-PCR: sequence diversity within the lambda3-encoding L1 gene. J Clin Microbiol 40(4):1368–1375
10. Wang H, Gao B, Chen H, Diao Y, Tang Y (2019) Isolation and characterization of a variant duck orthoreovirus causing spleen necrosis in Peking ducks. China Transbound Emerg Dis 66(5):2033–2044. https://doi.org/10.1111/tbed.13252
11. Zhang XL, Shao JW, Li XW, Mei MM, Guo JY, Li WF, Huang WJ, Chi SH, Yuan S, Li ZL, Huang SJ (2019) Molecular characterization of two novel reoviruses isolated from Muscovy ducklings in Guangdong, China. BMC Vet Res 15(1):143. https://doi.org/10.1186/s12917-019-1877-x
12. Farkas SL, Marton S, Dandar E, Kugler R, Gal B, Jakab F, Balint A, Kecskemeti S, Banyai K (2016) Lineage diversification, homo- and heterologous reassortment and recombination shape the evolution of chicken orthoreoviruses. Sci Rep 6:36960. https://doi.org/10.1038/srep36960
13. Yang J, Tian J, Chen L, Tang Y, Diao Y (2018) Isolation and genomic characterization of a novel chicken-origin orthoreovirus causing goslings hepatitis. Vet Microbiol 227:69–77. https://doi.org/10.1016/j.vetmic.2018.10.017
14. Farkas SL, Varga-Kugler R, Marton S, Lengyel G, Palya V, Banyai K (2018) Genomic sequence and phylogenetic analyses of two novel orthoreovirus strains isolated from Pekin ducks in 2014 in Germany. Virus Res 257:57–62. https://doi.org/10.1016/j.virusres.2018.09.001
15. Lelli D, Moreno A, Steyer A, Nagli T, Chiapponi C, Prosperi A, Faccin F, Sozzi E, Lavazza A (2015) Detection and characterization of a novel reassortant mammalian orthoreovirus in bats in Europe. Viruses 7(11):5844–5854. https://doi.org/10.3390/v7112908
16. Feher E, Kemenesi G, Oldal M, Kurucz K, Kugler R, Farkas SL, Marton S, Horvath G, Banyai K, Jakab F (2017) Isolation and complete genome characterization of novel reassortant orthoreovirus from common vole (Microtus arvalis). Virus Genes 53(2):307–311. https://doi.org/10.1007/s11262-016-1411-1
17. Mikuletic T, Steyer A, Kotar T, Zorec TM, Poljak M (2019) A novel reassortant mammalian orthoreovirus with a divergent S1 genome segment identified in a traveller with diarrhea. Infect Genet Evol 73:378–383. https://doi.org/10.1016/j.meegid.2019.06.002
18. Naglic T, Rihtaric D, Hostnik P, Toplak N, Koren S, Kuhar U, Jamnikar-Ciglenecki U, Kutnjak D, Steyer A (2018) Identification of novel reassortant mammalian orthoreoviruses from bats in Slovenia. BMC Vet Res 14(1):264. https://doi.org/10.1186/s12917-018-1585-y
19. Besozzi M, Lauzi S, Lelli D, Lavazza A, Chiapponi C, Pisoni G, Vigano R, Lanfranchi P, Luzzago C (2019) Host range of mammalian orthoreovirus type 3 widening to alpine chamois. Vet Microbiol 230:72–77. https://doi.org/10.1016/j.vetmic.2019.01.012
20. Niu X, Tian J, Yang J, Jiang X, Wang H, Tang Y, Diao Y (2018) Complete genome sequence of a novel avian orthoreovirus isolated from gosling. China Arch Virol 163(12):3463–3466. https://doi.org/10.1007/s00705-018-4035-z

21. Dietrich MH, Ogden KM, Katen SP, Reiss K, Sutherland DM, Carnahan RH, Goff M, Cooper T, Dermody TS, Stehle T (2017) Structural Insights into Reovirus sigma1 Interactions with Two Neutralizing Antibodies. J Virol 91 (4). doi:10.1128/JVI.01621-16
22. Yeung MC, Gill MJ, Alibhai SS, Shahrabadi MS, Lee PW (1987) Purification and characterization of the reovirus cell attachment protein sigma 1. Virology 156(2):377–385. https://doi.org/10.1016/0042-6822(87)90417-x
23. Li Z, Shao Y, Liu C, Liu D, Guo D, Qiu Z, Tian J, Zhang X, Liu S, Qu L (2015) Isolation and pathogenicity of the mammalian orthoreovirus MPC/04 from masked civet cats. Infect Genet Evol 36:55–61. https://doi.org/10.1016/j.meegid.2015.08.037
24. Dietrich MH, Ogden KM, Long JM, Ebenhoch R, Thor A, Dermody TS, Stehle T (2018) Structural and functional features of the reovirus sigma1 tail. J Virol 92 (14). doi:10.1128/JVI.00336-18
25. Perez-Losada M, Arenas M, Galan JC, Palero F, Gonzalez-Candelas F (2015) Recombination in viruses: mechanisms, methods of study, and evolutionary consequences. Infect Genet Evol 30:296–307. https://doi.org/10.1016/j.meegid.2014.12.022
26. Martin DP, Murrell B, Golden M, Khoosal A, Muhire B (2015) RDP4: detection and analysis of recombination patterns in virus genomes. Virus Evol 1 (1):vev003. doi:10.1093/ve/vev003
27. Woods RD, Wesley RD (1992) Seroconversion of pigs in contact with dogs exposed to canine coronavirus. Can J Vet Res 56(1):78–80
28. Jing Z, Zhang X, Shi H, Chen J, Shi D, Dong H, Feng L (2018) A G3P[13] porcine group A rotavirus emerging in China is a reassortant and a natural recombinant in the VP4 gene. Transbound Emerg Dis 65(2):e317–e328. https://doi.org/10.1111/tbed.12756
29. Li Z, Liu D, Ran X, Liu C, Guo D, Hu X, Tian J, Zhang X, Shao Y, Liu S, Qu L (2016) Characterization and pathogenicity of a novel mammalian orthoreovirus from wild short-nosed fruit bats. Infect Genet Evol 43:347–353. https://doi.org/10.1016/j.meegid.2016.05.039
30. Zhang YW, Liu Y, Lian H, Zhang F, Zhang SF, Hu RL (2016) A natural reassortant and mutant serotype 3 reovirus from mink in China. Arch Virol 161(2):495–498. https://doi.org/10.1007/s00705-015-2670-1
31. Yang XL, Tan B, Wang B, Li W, Wang N, Luo CM, Wang MN, Zhang W, Li B, Peng C, Ge XY, Zhang LB, Shi ZL (2015) Isolation and identification of bat viruses closely related to human, porcine and mink orthoreoviruses. J Gen Virol 96(12):3525–3531. https://doi.org/10.1099/jgv.0.000314
32. Wang L, Fu S, Cao L, Lei W, Cao Y, Song J, Tang Q, Zhang H, Feng Y, Yang W, Liang G (2015) Isolation and identification of a natural reassortant mammalian orthoreovirus from least horseshoe bat in China. PLoS ONE 10(3):e0118598. https://doi.org/10.1371/journal.pone.0118598
33. Lian H, Liu Y, Zhang S, Zhang F, Hu R (2013) Novel orthoreovirus from mink, China. Emerg Infect Dis 19(12):1985–1988. https://doi.org/10.3201/eid1912.130043
34. Zhang C, Liu L, Wang P, Liu S, Lin W, Hu F, Wu W, Chen W, Cui S (2011) A potentially novel reovirus isolated from swine in northeastern China in 2007. Virus Genes 43(3):342–349. https://doi.org/10.1007/s11262-011-0642-4
35. Song L, Zhou Y, He J, Zhu H, Huang R, Mao P, Duan Q (2008) Comparative sequence analyses of a new mammalian reovirus genome and the mammalian reovirus S1 genes from six new serotype 2 human isolates. Virus Genes 37(3):392–399. https://doi.org/10.1007/s11262-008-0283-4
36. Spinner ML, Di Giovanni GD (2001) Detection and identification of mammalian reoviruses in surface water by combined cell culture and reverse transcription-PCR. Appl Environ Microb 67(7):3016–3020. https://doi.org/10.1128/AEM.67.7.3016-3020.2001