

A natural reassortant and mutant serotype 3 reovirus from mink in China

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Abstract Mammalian orthoreoviruses (MRVs) are widespread and infect virtually all mammals. We report here the first case of a natural mutant and reassortant serotype 3 reovirus from mink in China, known as MRV3 SD-14. Whole-genome sequence analysis showed that the MRV3 SD-14 may have resulted from a reassortment involving MRVs that infected swine, humans and mink. Interestingly, the S1 segment, which encodes the viral attachment protein $\sigma 1$, which influences viral virulence and cell tropism in the host, had a stop codon mutation at amino acid 246. Surveillance of the virulence and evolution of MRVs in humans and other animals deserves more attention.

Mammalian orthoreoviruses (MRVs) belong to the family *Reoviridae* and genus *Orthoreovirus* [1]. MRVs are spherical, nonenveloped particles approximately 85 nm in diameter as assessed by cryoelectron microscopy (cryo-EM) image reconstruction [2]. Virions are formed by two concentric capsid protein shells that surround and protect a

genome of 10-segmented double-stranded RNA (dsRNA) that are divided into three groups, large (L1–L3), medium (M1–M3) and small (S1–S4), depending on their size and mobility in polyacrylamide gel electrophoresis [3]. Four major reovirus serotypes have been recognized so far (type 1 Lang, type 2 Jones, type 3 Dearing, type 4 Ndele), which are differentiated by the capacity of antisera to neutralize viral infectivity and inhibit hemagglutination [4, 5].

MRVs are zoonotic and infect a wide variety of species including humans, mink, swine, cattle, bats, and dogs [5–8]. In addition, MRVs are relatively stable in the environment and retain their infectivity for several years at temperatures below 4 °C [9]. Although MRV infection is rarely symptomatic in humans and other animals, previous reports have described the isolation of some reoviruses from cerebrospinal fluid (CSF) of individuals with meningitis [10, 11]. MRVs can spread from the site of entry to the central nervous system (CNS) [5]. Type 3 reoviruses cause lethal encephalitis after spreading neurally to the CNS and infecting neurons [5]. This is determined by the viral attachment protein $\sigma 1$ and nonstructural protein $\sigma 1s$, which are encoded by the S1 gene.

In this study, a dead mink with an unidentified disease with clinical signs including diarrhea and emaciation was autopsied (collected in 2014 in Shandong, China). Mink enteritis virus, canine distemper virus, Aleutian mink disease virus, and mink coronavirus were excluded by PCR or RT-PCR. To identify the cause of the disease, the intestinal contents were suspended in phosphate-buffered saline (PBS) and centrifuged at $12000 \times g$ for 10 min at 4 °C. Then supernatants were filtered through a 0.22- μm filter (Pall Life Sciences) and inoculated onto an 80 % confluent monolayer of Vero and BHK cells at 37 °C with 5 % CO_2 . The cells were passaged every 3 days, and a clear cytopathic effect (CPE) was observed in the third passage, with

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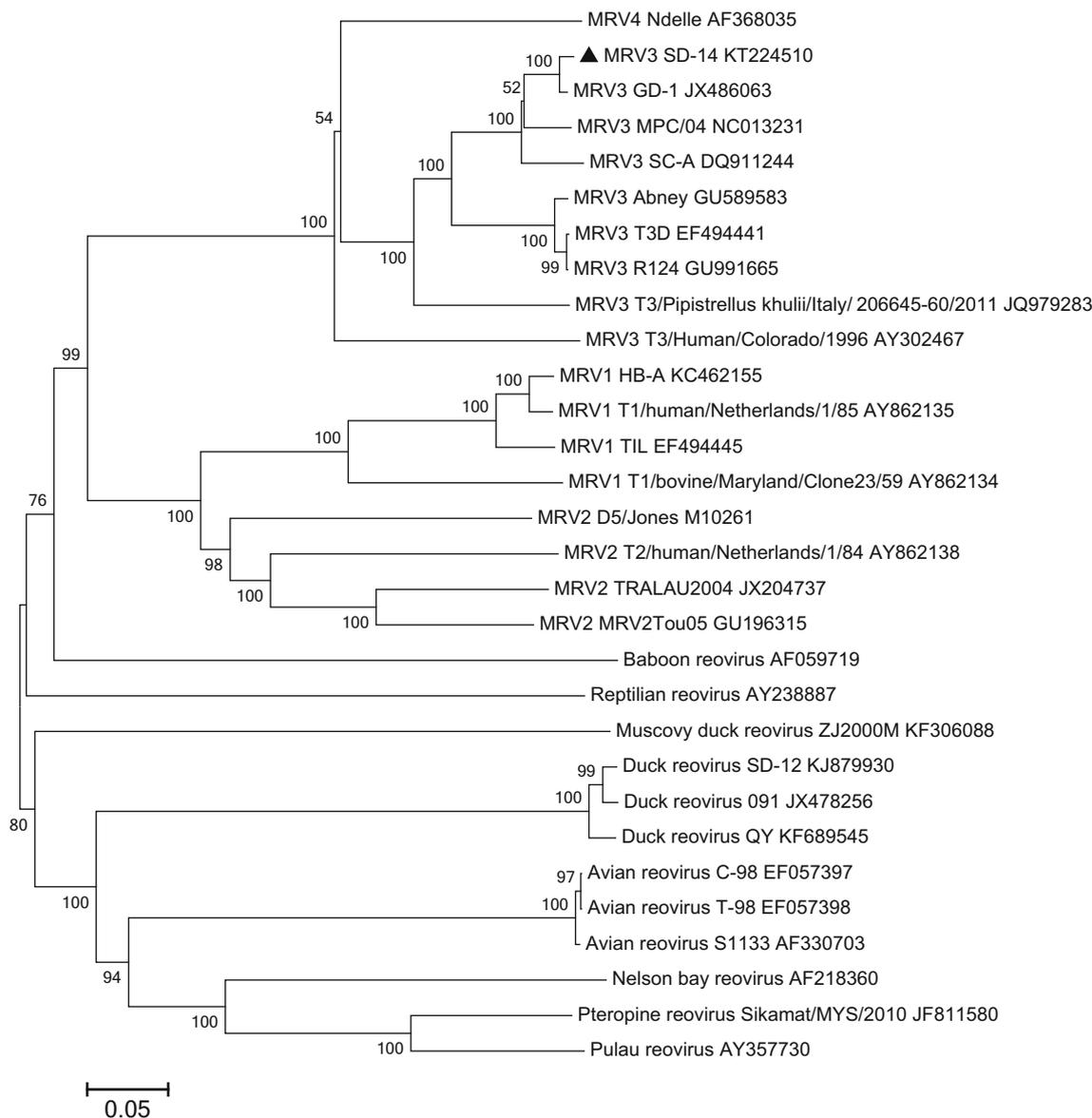


Fig. 1 Phylogenetic analysis of the S1 segment for the MRV3 SD-14 strain. the neighbor-joining method was used for the construction of the phylogenetic tree, with bootstrap values for 1,000 replicates

shown at the branches. The scale bar represents the p -distance. ▲, the sequence of MRV3 SD-14 from this study

rounded and detached cells. Subsequently, the cells were harvested after three freeze-thaw cycles and used as viral stocks. The supernatants of medium containing viral particles were observed by negative staining electron microscopy. The observed virus particles were nonenveloped and isometric with a double-layered capsid, and they were proximately 75 nm in diameter (supplementary material). These characteristic are consistent with those of mammalian orthoreoviruses. PCR products of 400 bp were amplified using a pair of primers for the large segment (L1) and sequenced. The results of BLAST analysis ([\[www.ncbi.nlm.nih.gov\]\(http://www.ncbi.nlm.nih.gov\)\) showed that the sequence corresponded to the L1 segment of mammalian orthoreovirus.](http://</p>
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The complete genome sequence of 10 segments of the virus was determined using specific primers and sequenced by Comate Bioscience Co., Ltd., Changchun, China. The data were submitted to the GenBank database (accession numbers KT224504-KT224513).

A phylogenetic tree was constructed using MEGA (version 6.06) based on the S1 segment, and phylogenetic relationships were assessed by the neighbor-joining method [3, 12, 13]. The analysis showed that MRV3 SD-14

Table 1 Percent nucleotide sequence identity of segments of novel mink orthoreovirus MRV3 SD-14 from China to those of other reoviruses

MRV3 SD-14	MRV prototype strains, %				Human reovirus strains, %		Swine reovirus strains, %		Mink reovirus strain, %
	T1L	T2J	T3D	T4N	Abney	MRV2 tou05	GD-1	SC-A	HB-A
L1	89.26	75.51	89.30	89.80	88.81	97.40	96.13	96.52	96.60
L2	86.36	72.77	77.32	NA	77.32	96.31	97.39	96.42	95.30
L3	84.57	77.80	84.57	NA	84.67	96.92	95.10	95.64	97.33
M1	94.62	70.62	93.92	NA	93.06	89.90	92.75	95.53	95.49
M2	85.07	76.67	89.42	88.74	89.49	95.99	98.37	95.23	96.23
M3	85.36	71.01	85.23	NA	85.36	97.09	84.20	96.07	96.65
S1	40.97	44.99	85.66	70.06	85.73	44.61	98.66	93.71	43.23
S2	96.17	77.54	85.80	85.95	95.64	85.26	85.05	86.63	98.95
S3	90.57	74.37	85.64	NA	85.14	97.20	89.07	89.82	96.33
S4	88.04	80.43	87.46	90.12	88.46	94.21	77.76	97.58	96.91

T1L, type1 Lang; T2J, type 2 Jones; T3D, type3 Dearing; T4N, type 4 Ndelle; L, large segment; M, medium segment; S, small segment; NA, not available; **Boldface** indicates high sequence identity

belonged to serotype 3 (Fig. 1). The S1 genes shared 85.66 % nucleotide sequence identity between the prototype T3D and MRV3 SD-14, which also provided sequence confirmation that this new isolate was a serotype 3 strain.

The nucleotide sequence of each segment was compared with those of other orthoreoviruses, including the prototype MRVs, using DNAMAN (Lynnon Biosoft, version 6.0, Table 1). The results showed that four segments (L1, L3, M3, S3) of MRV3 SD-14 were closely related to a human reovirus (MRV2Tou05) isolated from a child with acute necrotizing encephalopathy in France, and the sequences coincidence rates were 97.40 %, 96.92 %, 97.09 % and 97.20 %, respectively [14]. The S2 segment of MRV3 SD-14 was most similar to that of type 1 Lang (96.17 % identity) which can infect humans. The other five segments shared high sequence similarity with swine-origin MRVs strains GD-1 and SC-A, which were isolated in Guangdong and Sichuan Province, China (sequence coincidence rates were from 95.53 % to 98.66 %). MRV3 SD-14 showed high similarity to MRV1 HB-A, which was also isolated from mink in 2011 in Hebei, China, except for the S1 segment [8]. Each segment was also sequenced and analyzed phylogenetically using MEGA (version 6.06) (supplementary material). We conclude that MRV3 SD-14 is a reassortant derived from human, swine and/or mink strains.

The whole genome sequence of MRV3 SD-14 was 23,558 nt in length. The open reading frames (ORFs) and deduced protein sequences of each segment were determined and analyzed using DNASTAR (Lasergene version 7.1). The lengths of L1-L3, M1-M3, and S1-S4 were 3854 nt, 3915 nt, 3901 nt, 2304 nt, 2203 nt, 2241 nt, 1415 nt, 1331 nt, 1198 nt, and 1196 nt, respectively, encoding λ 3 (1267 amino acids, [aa]), λ 2 (1289 aa), λ 1 (1275 aa), μ 2 (736 aa), μ 1 (708 aa), μ NS (721 aa), σ 1 (245 aa), σ 1s (120

aa), σ 2 (418 aa), σ NS (366 aa), and σ 3 (365 aa) [5]. Surprisingly, the S1 segment of the MRV3 SD-14 strain contained a stop codon mutation at amino acid 246 of the σ 1 protein, which mediates viral binding to cellular receptors and influences target-cell selection in the infected host [5]. The stop codon mutation at amino acid 246 of the σ 1 protein was also found in the original animal samples. The normal S1 had two major ORFs, encoding a structural protein (σ 1, ORF1, 455-470 aa) and a nonstructural protein (σ 1s, ORF2). The S1 segment of MRV3 SD-14 strain was analyzed using DNASTAR (Lasergene version 7.1), and the results showed that the deduced amino acid sequences of σ 1 had only residues 1-245. Two previous studies revealed that the amino acids of σ 1 had only residues 1-250 or 1-252, and this did not affect viral growth [15, 16].

To date, there have been few reports on the isolation of orthoreoviruses from minks. Here, we isolated a novel natural reassortant and mutant reovirus, a serotype 3 reovirus named MRV3 SD-14, from mink.

Genetic reassortment and mutation are common in segmented dsRNA viruses and are important for their evolution and virulence. We cannot ignore the risk of novel reassortant and mutant viruses. In Shandong Province, China, there are many swine and mink farms, and indirect infection through contaminated food or water could occur. Thus, monitoring the evolution and virulence of mammalian reovirus in animals and humans is necessary.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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