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LETTER TO THE EDITOR

L2 segment-based phylogenetic relationships among duck reoviruses from China

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In the late 1990s, we reported a highly fatal virus disease in Muscovy ducklings (Cairina Moschata), characterized by white necrotic foci in the enlarged liver and spleen. The disease was caused by muscovy duck reovirus (MDRV). It is apparent that muscovy ducks are particularly susceptible to MDRV infection and that the infection is less obvious in other duck species (*1*).

Towards the end of the first decade of the 21st century, many cases of a new disease caused by a related reovirus appeared in ducks in Southeast China. The disease is characterized by multiple organ hemorrhage and necrosis in the liver and spleen of the sick ducklings, however, the morbidity and mortality is usually low unless accompanied by secondary bacterial or viral infections or aggravated by environmental stress factors. In 2009, we described the first isolation of novel duck reovirus (NDRV) from affected domestic ducklings showing the above symptoms in Fujian Province. In contrast to MDRV, NDRV can infect a variety of domestic ducks, including Muscovy ducks, Mule ducks and Pekin ducks. Results of the neutralization assay have shown that MDRV and NDRV isolates are different, with less cross neutralization than expected (*2*).

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Abbreviations: ARV = avian reovirus; DRV = duck reovirus; MDRV = muscovy duck reovirus; NDRV = novel duck reovirus

Like avian reovirus (ARV) (chicken origin), duck reovirus (DRV) is a member of the genus *Orthoreovirus*, the family *Reoviridae*. Each DRV particle contains ten dsRNA genome segments, named according to their migration in gel electrophoresis: large (L1, L2 and L3), medium (M1, M2 and M3) and small (S1, S2, S3, and S4), encoding λ-, μ- and σ-class proteins, respectively (*3, 4*). MDRV and NDRV isolates have been classified into several groups and lineages based on sequence variability of the antigenically related proteins. Recent studies have begun to focus on the evolutionary relationship of the M- and S-class genome segments of DRV field-isolates (*5–8*). It is interesting to note that MDRV σC protein is encoded by S4 and not by S1, as usually described for NDRV. Similar to the chicken ARV S1 gene, NDRV S1 is tricistronic, but MDRV S4 is a bicistronic genomic segment containing two overlapping ORFs (*9, 10*).

There is little information, however, about the L2 genome segment of MDRV isolated in China within isolated cases. The segment is predicted to encode the RNA-dependent RNA polymerase (also called λB protein), which is required for viral propagation (*11*). In order to further understand the phylogenetic relationship between MDRV and NDRV strains, we determined the nucleotide sequence of the polymerase domains of MDRV-MW9710 strain L2 genome segment. The viral RNA was extracted from purified virions of MDRV-MW9710 strain using TRIzol LS reagent (Invitrogen, Carlsbad, CA) following the manufacturer's recommendations. The PCR primers L2F (GTCTCAGCAGCTCACACCT) and L2R

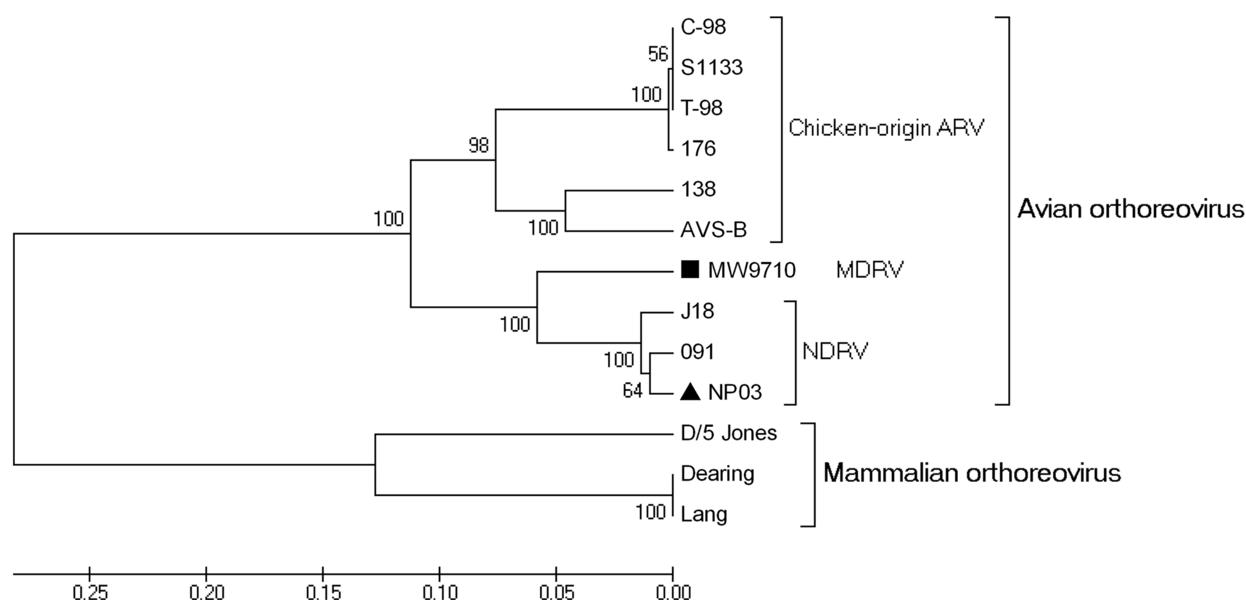

Fig. 1
Phylogenetic relationship between MDRV MW9710, NDRV, chicken ARV, and mammalian reovirus
The neighbor-joining tree is based on the sequence of 564 nts in the λB gene. Numbers at nodes represent the percentage of 1,000 bootstrap replicates (values <50 are not shown). Scale bar indicates a branch length corresponding to 100 character-state changes. Acc. No.: JN641888, DQ534201, JN641889, EU707936, EU707935, FR694192, KC311940, JX478261, JX478251, KC312701, M31057, NC_004282, M24734. (MDRV strain MW9710 was marked with solid square, NDRV strain NP03 was marked with solid triangle. The two DRV strains were isolated from affected ducklings in Fujian Province, China).

(GATGGAAATGGAACGACAAAACT) were designed using the Oligo 6 primer design software (NBI, Plymouth, MN) based on conserved regions identified from an alignment of the published complete L2 nucleotide sequences for NDRV and chicken ARV strains. The RT-PCR was performed according to the method described previously by Xu et al (*11*). The PCR products were analyzed by gel electrophoresis, cut out from the gel, cloned into pMD18-T vectors, and transformed into *Escherichia coli* strain JM109. Bacterial colonies were screened for the correct recombinant vector by PCR, using the PCR primers (L2F and L2R) to amplify the insert DNA. DNA sequencing of the inserted gene was performed with the ABI BigDye Terminator v3.1 Cycle Sequencing kit, and completed reactions were run on an ABI 377 Automated DNA Sequencer (Perkin-Elmer Applied Biosystems). Each sequence given represents the consensus of at least five different plasmid clones. Sequence assembly was carried out using the SeqMan program of the Lasergene software package. The partial L2 gene nucleotide sequence of MDRV MW9710 strain was directly determined (564 nts, Acc. No. KC311940) and was aligned with those of other 12 published orthoreovirus sequences by means of the DNAStar software. Phylogenetic tree was generated using the neighbor-joining method in the MEGA 4.1 program with 1000 bootstrap replicates. The resulting phylogenetic tree (Fig. 1) showed that DRV strains are more distant from the chicken ARV strains; they form a separate cluster consisting of a MDRV and a NDRV subcluster, indicating that DRV viruses continuously evolve and occur as different strains.

Using the nucleotide sequences of the L2 gene polymerase domains, we provide additional phylogenetically informative data of DRV strains circulating in China. Therefore, this method will be useful for classification of currently prevalent MDRV and NDRV strains isolated from domestic ducks. Finally, further studies are necessary to investigate the ecology and epidemiology of the two MDRV/NDRV isolates of distinct genotypes from China.

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