

Complete Genome Sequence of a Velogenic Neurotropic Avian Paramyxovirus 1 Isolated from Peacocks (*Pavo cristatus*) in a Wildlife Park in Pakistan

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Avian paramyxovirus serotype 1 (APMV-1) was isolated from an acute and highly contagious outbreak in peacocks (*Pavo cristatus*) in a wildlife park in Pakistan. A velogenic neurotropic form of APMV-1 caused a 100% case fatality rate and killed 190 peacocks within a week. Biological and serological characterizations showed features of a velogenic strain of APMV-1, and these results were further confirmed by sequence analysis of the cleavage site in the fusion protein. The complete genome of one of the isolates was sequenced, and phylogenetic analysis was conducted. The analysis showed that this isolate belonged to genotype VII, specifically, to subgenotype VIIa, and clustered closely with isolates characterized from Indonesia in the 1990s. Interestingly, the isolate showed significant differences from previously characterized APMV-1 isolates from commercial and rural chickens in Pakistan. The work presented here is the first complete genome sequence of any APMV-1 isolate from wild birds in the region and therefore highlights the need for increased awareness and surveillance in such bird species.

Newcastle disease virus (NDV), also designated avian paramyxovirus serotype 1 (APMV-1), causes a highly contagious disease in domestic and wild bird populations and results in significant economic losses in the poultry industry around the globe (2). Based on partial sequence analysis of the fusion protein (F) gene, NDV strains can be classified into one of two classes, I or II. Strains of class I are avirulent in chickens, whereas class II strains can be lentogenic, mesogenic, or velogenic. Class II viruses are subdivided into 11 genotypes (I to XI) (1, 3).

APMV-1 infection is not limited to domestic chickens; it has been reported in at least 250 species of birds. APMV-1 viruses circulating within Pakistani poultry flocks have been thoroughly characterized in several recently published studies (4, 5–7). However, most of these studies focused on chicken populations, in either industrial or village farming production facilities. However, strains isolated from peacocks have been poorly characterized, and there have been few reports of isolates found in these birds (1). These facts have raised concerns regarding the efficacy of implemented vaccination programs and indicate the necessity for stringent epidemiological surveillance strategies to help improve knowledge about the disease in wild bird species.

A severe outbreak, suspected to have been caused by APMV-1, occurred in a wildlife park in Lahore, Pakistan, and took the lives of approximately 190 peacocks (*Pavo cristatus*) within a week, with a 100% apparent case fatality rate and loss of 50% of the susceptible birds. Virus isolation and subsequent serological diagnostic tests, such as hemagglutination inhibition (HI; titer of 1:1,024) and an enzyme-linked immunosorbent assay (ELISA; percentage inhibition of >90%), and molecular diagnostic tests, such as real-time PCR, confirmed the presence of velogenic NDV. Virus isolation was only possible from brain tissues of dead birds and was consistent with the nature of the clinical disease, in which severe neurological signs are observed in most sick peacocks.

The sequence at the cleavage site in the F protein is a predictor

of virulence. The sequence of the cleavage site of the strain peacock/Pakistan/MM19/2012 (APMV1/MM19/12) was determined to be ¹¹²RRQKRF¹¹⁷, which corresponds to the cleavage site of velogenic strains of APMV-1. The complete genome sequence of the APMV1/MM19/12 strain was determined and phylogenetically analyzed by using neighbor-joining and Bayesian inference methods within the MEGA5 and MrBayes programs, respectively (6, 7). From the topology of the phylogenetic trees, it was apparent that APMV1/MM19/12 clustered with isolates of genotype VII. Further analysis indicated that the present isolate clustered with Indonesian APMV-1 within subgenotype VIIa. Interestingly, the strain that appeared most closely related to APMV1/MM19/12 was cockatoo/Indonesia/14698/90 (AY562985), which was also isolated from a wild captive bird, a cockatoo. A comparison of F proteins and the complete genomes of APMV1/MM19/12 and the LaSota vaccine strain (JF950510) showed only 86.8% and 83.6% identity. Our further evolutionary and bioinformatics analysis showed that APMV1/MM19/12 was significantly divergent from the previously characterized APMV-1 isolate from commercial and rural poultry of Pakistan (5–7).

Nucleotide sequence accession number. The complete genome sequence of peacock/Pakistan/MM19/2012 has been deposited in GenBank under the accession number [JX532092](https://www.ncbi.nlm.nih.gov/nuccore/JX532092).

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