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Complete Genome Sequencing of a Velogenic Viscerotropic Avian Paramyxovirus 1 Isolated from Pheasants (Pucrasia macrolopha) in Lahore, Pakistan

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GENOME ANNOUNCEMENT

Complete Genome Sequencing of a Velogenic Viscerotropic Avian Paramyxovirus 1 Isolated from Pheasants (*Pucrasia macrolopha*) in Lahore, Pakistan

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We report the complete genome sequence of avian paramyxovirus 1 (APMV-1) isolated from an acute and highly contagious outbreak in pheasants (*Pucrasia macrolopha*) in Lahore, Pakistan. Biological and serological characterization showed a velogenic strain of APMV-1, which was further confirmed by the sequence analysis of the cleavage site in the fusion protein. Complete genome sequencing and phylogenetic analysis indicated that this isolate belonged to genotype VII, specifically to subgenotype VIIa, and clustered closely with isolates characterized from Indonesia. Notably, the isolate showed significant differences from previously characterized APMV-1 from Pakistani commercial and rural chicken.

Newcastle disease (ND), caused by avian paramyxovirus serotype 1 (APMV-1), is a highly contagious viral disease that affects both domestic and wild bird species worldwide (1, 2). The enveloped virus has a negative-sense, nonsegmented single-stranded RNA (ssRNA) genome of either 15,186, 15,192, or 15,198 nucleotides in length and follows the so-called "rule of six" (4). From the 5′ to the 3′ terminus, the ND virus (NDV) genome encodes six structural proteins that include nucleocapsid (NP), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin-neuraminidase (HN), and large RNA-dependent RNA polymerase (L) (2). Based upon genome size, the NDVs are grouped into class I and class II. Class II viruses are further divided into 11 genotypes (I to XI), and genotypes VI and VII are subdivided, due to high genetic divergence, into eight (a to h) and five (a to e) subgenotypes, respectively (1). Based upon pathogenicity in chicken, the NDVs are classified into velogenic, mesogenic, and lentogenic (1, 2), types which correspond to the amino acid sequence motif present in the protease cleavage site of the precursor fusion protein (F_0) and the subsequent abilities of the cellular proteases to cleave this F_0 protein (5, 8).

Despite extensive live vaccination in poultry with lentogenic strains (i.e., Lasota), outbreaks are continuously occurring in Pakistan. A number of outbreaks have recently been reported (3, 6–8); nevertheless, the nature of circulating NDV in wild birds has largely remained elusive. Concerns have been raised regarding the efficacy of ongoing vaccination programs that have urged the necessity for comprehensive epidemiological surveillance to improve the knowledge related to the diagnosis and control of this disease.

An outbreak of suspected APMV-1 occurred on a wildlife farm in Lahore, resulting in deaths of approximately 67 pheasants (*Pucrasia macrolopha*) within 2 weeks of the onset of clinical signs. The apparent case fatality rate was 80%, with a loss of 60% of the susceptible birds. Conventional diagnostic methods such as virus isolation, enzyme-linked immunosorbent assay (ELISA) (percent inhibition [PI], >95%), and hemagglutination inhibition (HI) (1:1,024) test identified NDV, which was further confirmed by molecular diagnostic tests such as real-time PCR and sequencing. The F protein cleavage site of the reported strain Pheasant/MM20/Pakistan/2011 (referred to as APMV1/MM20/11) was determined to be ^{112}RRQKRF^{117}, which corresponds to the velogenic strains of NDV. Comparing the identities of the F protein amino acid and the whole genome indicated divergences of 18.4% and 19.2% between APMV1/MM20/11 and the Lasota strain, respectively. The phylogenetic analysis of the F gene of the isolate, performed along with analysis of 150 F genes representing all genotypes, including those characterized previously from commercial and rural Pakistan poultry using the neighbor-joining method in MEGA5 (7, 8), clustered APMV1/MM20/11 with isolates of genotype VII. Since VII is further divided into subgenotypes, analysis with representatives of all reported subgenotype VII isolates grouped APMV1/MM20/11 into VIIa along with isolates reported earlier from Indonesia. Notably, the closely related isolate from Indonesia (cockatoo/Indonesia/14698/90, AY562985), was also isolated from a wild captive bird, the cockatoo. Further analysis of the APMV1/MM20/11 isolate indicated significant divergence from previously reported NDV strains in domestic poultry in Pakistan.

Nucleotide sequence accession number. The complete genome sequence of Pheasant/Pakistan/MM20/11 has been deposited in GenBank under the accession number JX854452.

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