

Complete Genome Sequence of Avian Bornavirus Genotype 1 from a Macaw with Proventricular Dilatation Disease

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Avian bornaviruses (ABV) were first detected and described in 2008. They are the etiologic agents of proventricular dilatation disease (PDD), a frequently fatal neurologic disease of captive parrots. Seven ABV genogroups have been identified worldwide from a variety of sources, and that number may increase as surveillance for novel bornaviruses continues. Here, we report the first complete sequence of a genogroup 1 avian bornavirus (ABV1).

Bornaviruses (order *Mononegavirales*, family *Bornaviridae*) are nonsegmented negative-strand RNA (NNSR) viruses whose prototype member is Borna disease virus (BDV) (1). BDV causes a neurologic disease of sheep and horses and a spectrum of neurologic disorders in small-animal models (4). In 2008, five bornavirus genogroups were identified from brain samples of psittacine birds (macaws, parrots, and conures, among others) with proventricular dilatation disease (PDD) (2, 3). These studies demonstrated the genetic and ecological diversity of bornaviruses. In 2011, we reported on a Canada goose bornavirus isolate (5) that was distinct from other avian bornaviruses (ABV), a finding that highlighted the need to generate complete genome sequences of diverse avian bornaviruses for further studies of their ecology, evolution, and pathogenesis.

The genogroup 1 ABV (ABV1) isolate NM-M25 was recovered from the brain of a macaw (*Diopsittaca nobilis*) with a diagnosis of PDD (9). Virus was cultured in duck embryo fibroblasts, and sequences from the cultured virus were compared to viral sequences obtained directly from the infected brain sample. Overlapping PCR fragments were amplified using 8 primer sets, and the fragments were sequenced directly. Rapid amplification of cDNA ends (RACE) was used to determine the 5' and 3' genome ends. These products were cloned into TOPO TA vector (Invitrogen), and individual plasmids were sequenced. DNA sequencing reactions were performed with the ABI BigDye Terminator cycle sequencing kit (Applied Biosystems), and sequences were generated with an ABI Prism 3100 genetic analyzer (Applied Biosystems). Sequences were assembled with Geneious Pro 4.6.2 software. Genome sequences were aligned using ClustalW in the MEGA5 software package (8).

The ABV genotype 1 isolate NM-M25 shares, overall, 81 to 82% nucleotide sequence identity with ABV2 (GenBank accession numbers [FJ620690](#) and [HM998710](#)) and ABV4 (GenBank accession numbers [FJ002331](#) and [JN035149](#)) and 64% pairwise identity with BDV (GenBank accession number [NC001607.1](#)). As for individual genes, M is the most highly conserved at the nucleotide level, sharing 89% and 86% nucleotide (nt) identity with ABV2 and ABV4, respectively. M sequences are 74% identical to those of BDV. In comparison, M25 N sequences are 81 to 82% identical to those of ABV2 and ABV4 and only 66% identical to those of BDV. The region between the bornavirus N and X genes (N/X intergenic region) is also diverse among bornaviruses (3, 7). At 27 nt, the ABV1 N/X intergenic region is the same length as in other psitta-

cine bornaviruses, differing from that of ABV2s by 1 nt, from that of ABV4s by 2 nt, and from that of ABV3 by 3 nt. However, the ABV1 N/X intergenic region contains a short (7-amino-acid [aa]) upstream open reading frame (uORF) analogous to those found in BDV (6) and Canada goose bornaviruses (5) that is not present in ABV2, ABV4, or ABV3.

Nucleotide sequence accession number. We have deposited the sequence of the genotype 1 ABV (ABV1) isolate NM-M25 into GenBank under accession no. [GU249595.2](#).

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