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Complete Genome Sequence of a Vaccinal Newcastle Disease Virus Strain Isolated from an Owl (*Rhinoptynx clamator*)

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A Newcastle disease virus (NDV) was isolated in chicken embryonated eggs after detection by real-time reverse transcription-PCR (RRT-PCR) from a captive owl swab. The complete genome sequence of APMV-1/*Rhinoptynx clamator*/Brazil/22516/2009 (APMV-1, avian paramyxovirus type 1) was obtained using Illumina sequencing. Phylogenetic analysis of the complete genome classified the isolate within NDV class II genotype II.

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In Brazil, Newcastle disease virus (NDV), an avian paramyxovirus type 1 (APMV-1), is an economically important poultry disease that can be maintained in wild birds (1–3).

During an ongoing wild bird surveillance project in the Southeast of Brazil, São Paulo state, an NDV was isolated after three passages in embryonated chicken eggs, as previously described (4), from a real-time RT-PCR-positive (as previously described [5]) oropharyngeal swab taken from a clinically healthy striped owl (*Rhinoptynx clamator*).

The virus isolate (allantoic fluid) was centrifuged at 8,000 rpm for 2 min at 4°C and filtered with 0.45-μm disk filters (Millipore), followed by a nuclease treatment (50 U of Ambion Turbo DNase in 150 μl with incubation at 37°C for 1 h) and RNA purification using TRIzol LS (Invitrogen) with purification of the aqueous phase containing the RNA using the QIAamp viral RNA minikit (Qiagen). The RNA concentration was quantified using a DS-11 spectrophotometer (DeNovix) and a Qubit fluorometer (Invitrogen). cDNA was synthesized using the SuperScript IV first-strand synthesis system (Invitrogen) and random hexamer primers, followed by double-strand cDNA synthesis using T4 DNA polymerase and T4 DNA ligase (Thermo Scientific), as previously described (6). Nextera XT libraries (Illumina) were prepared using 1 ng of double-stranded cDNA (6), quantified with a Kapa library quantitation kit Illumina Platforms (Kapa Biosystems) diluted to 1 nM, and sequenced on the NextSeq system (Illumina, Inc., San Diego, CA, USA) using a NextSeq 500 mid output kit (1 × 150 cycles) (Illumina Inc.). The quality of the sequences was checked with FastQC version 0.10.1 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Stretches containing unidentified nucleotides ("N") were trimmed using Cutadapt version 1.3 (7) prior to quality trimming using Sickle version 1.210 (*Q* score, <30; length, <50 bp) (8). Host reads were removed by mapping to the *Gallus gallus* genome (accession no. GCF_000002315.4) (bwa version 0.7.12 [9]). Fifty thousand randomly selected unmapped reads were used for a *de novo* assembly (Newbler version 2.9; Roche), resulting in a 15,110-bp NDV contig. All *G. gallus* unmapped reads (2,053,959 reads) were subsequently mapped (Newbler version 2.9; Roche) to a hybrid reference consisting of the 15,110-bp *de novo* contig supplemented with the missing 5′ noncoding region (76 nucleotides) from its closest BLASTn hit, accession no. KM056358 (4).

The complete genome sequence of NDV isolate APMV-1/*Rhinoptynx clamator*/Brazil/22516/2009 was thus assembled (average coverage, 1,971×) as a 15,186-bp contiguous sequence, confirming to the rule of six described for paramyxoviruses (10). The protein-coding genes were predicted relative to reference sequence KM056358 by GATU (11)

Phylogenetic analysis of the complete genome classified the isolate as members of NDV class II, genotype II (data not shown). Pathogens can be transmitted from domestic animals to free-ranging hosts and vice versa (12, 13). Indeed, the continuous expansion of the poultry industry coupled with the mass employment of live-virus vaccines (14) may result in a spillover of vaccinal strains to wildlife reservoirs (15). The impact of these vaccinal strains in wildlife reservoirs remains unknown.

Accession number(s). The complete genome sequence of NDV isolate APMV-1/*Rhinoptynx clamator*/Brazil/22516/2009 has been deposited in GenBank under accession number KX822746.

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REFERENCES

1. **Orsi MA**. 2010. Caracterização biológica, molecular, imunológica e estabilidade térmica das estirpes vacinais e de isolados da doença de Newcastle de aves de produção industrial e migratórias no Brasil. Ph.D. thesis. UNICAMP, Campinas, Brazil.
2. **Thomazelli L, de Araujo J, Ferreira CS, Hurtado R, Oliveira D, Ometto T, Golono M, Sanfilippo L, Demetrio C, Figueiredo M, Durigon E**. 2012. Molecular surveillance of the Newcastle disease virus in domestic and wild Birds on the North Eastern Coast and Amazon biome of Brazil. Rev Bras Cienc Avic **14:**01–07. http://dx.doi.org/10.1590/S1516-635X2012000100001.
3. **Carrasco Ade O, Rodrigues JN, Seki MC, de Moraes FE, Silva JR, Durigon EL, Pinto AA**. 2013. Use of reverse transcriptase polymerase chain reaction (RT-PCR) in molecular screening of Newcastle disease virus in poultry and free-living bird populations. Trop Anim Health Prod **45:**569–576. http://dx.doi.org/10.1007/s11250-012-0261-7.
4. **OIE**. 2012. Newcastle disease (infection with Newcastle disease virus), p 555–574. Manual of diagnostic tests and vaccines for terrestrial animals: mammals, birds and bees, 7th ed, vol I. Biological Standards Commission, World Organisation for Animal Health, Paris, France.
5. **Wise MG, Suarez DL, Seal BS, Pedersen JC, Senne DA, King DJ, Kapczynski DR, Spackman E**. 2004. Development of a real-time reverse-transcription PCR for detection of Newcastle disease virus RNA in clinical samples. J Clin Microbiol **42:**329–338. http://dx.doi.org/10.1128/JCM.42.1.329-338.2004.
6. **Ullmann LS, de Camargo Tozato C, Malossi CD, da Cruz TF, Cavalcante RV, Kurissio JK, Cagnini DQ, Rodrigues MV, Biondo AW, Araujo JP, Jr.** 2015. Comparative clinical sample preparation of DNA and RNA viral nucleic acids for a commercial deep sequencing system (Illumina MiSeq). J Virol Methods **220:**60–63. http://dx.doi.org/10.1016/j.jviromet.2015.04.009.
7. **Martin M**. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J **17:**10–12. http://dx.doi.org/10.14806/ej.17.1.200.
8. **Joshi NA, Fass JN**. 2011. Sickle: a sliding-window, adaptive, quality-based trimming tool for FastQ files (version 1.33). https://github.com/najoshi/sickle.
9. **Li H, Durbin R**. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics **25:**1754–1760. http://dx.doi.org/10.1093/bioinformatics/btp324.
10. **Kolakofsky D, Roux L, Garcin D, Ruigrok RW**. 2005. Paramyxovirus mRNA editing, the "rule of six" and error catastrophe: a hypothesis. J Gen Virol **86:**1869–1877. http://dx.doi.org/10.1099/vir.0.80986-0.
11. **Tcherepanov V, Ehlers A, Upton C**. 2006. Genome annotation transfer utility (GATU): rapid annotation of viral genomes using a closely related reference genome. BMC Genomics **7:**150. http://dx.doi.org/10.1186/1471-2164-7-150.
12. **Miller M, Olea-Popelka F**. 2013. One health in the shrinking world: experiences with tuberculosis at the human-livestock-wildlife interface. Comp Immunol Microbiol Infect Dis **36:**263–268. http://dx.doi.org/10.1016/j.cimid.2012.07.005.
13. **Daszak P, Cunningham AA, Hyatt AD**. 2000. Wildlife ecology—emerging infectious diseases of wildlife—threats to biodiversity and human health. Science **287:**443–449. http://dx.doi.org/10.1126/science.287.5452.443.
14. **Devlin JM, Vaz PK, Coppo MJ, Browning GF**. 2016. Impacts of poultry vaccination on viruses of wild bird. Curr Opin Virol **19:**23–29. http://dx.doi.org/10.1016/j.coviro.2016.06.007.
15. **Ayala AJ, Dimitrov KM, Becker CR, Goraichuk IV, Arns CW, Bolotin VI, Ferreira HL, Gerilovych AP, Goujgoulova GV, Martini MC, Muzyka DV, Orsi MA, Scagion GP, Silva RK, Solodiankin OS, Stegniy BT, Miller PJ, Afonso CL**. 2016. Presence of vaccine-derived Newcastle disease viruses in wild birds. PLoS One **11:**e0162484. http://dx.doi.org/10.1371/journal.pone.0162484.