



Complete Genome Sequence of Avian Coronavirus Strain D274

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ABSTRACT *Avian coronavirus*, the causative agent of avian infectious bronchitis, occurs as multiple genotypes and lineages, and full genomes are not available for the majority of them. This paper reports the (previously unknown) complete genome sequence of strain D274 of this virus (27,599 nucleotides), isolated from chickens in The Netherlands in 1979.

A plethora of types of avian infectious bronchitis virus (IBV) (*Nidovirales: Coronaviridae: Coronavirinae: Gammacoronavirus: Avian coronavirus* [AvCoV]) strains are used in live and inactivated vaccine formulations worldwide to prevent infection with or lessen the symptoms caused by the diverse number of field strains of the virus in chickens (1).

IBV occurs as 6 genotypes with a total of 34 lineages based on the spike S gene and is involved in multisystemic, highly contagious infections of chickens (1, 2). The IBV positive-sense, single-stranded 5' capped RNA genome with circa 27 kb codes for the replicase complex (open reading frame 1 [ORF1]); the four structural proteins spike S, envelope protein E, membrane protein M, and nucleocapsid protein N; and accessory proteins 3a, 3b, 5a, and 5b, with an untranslated region (UTR) at both the 5' and 3' ends of the genome (3, 4).

The Gammacoronavirus/AvCoV/chicken/Netherlands/D274/1979 IBV strain (lineage GI-12) was isolated from tissue samples of chickens collected in 1979 in The Netherlands (5) and is used as an inactivated intramuscular vaccine strain in several countries, including Brazil. An aliquot of a D274 strain used in an inactivated vaccine was clarified at 12,000 × g/15 min at 4°C and then filtered (0.45 μm) and treated with DNase/RNase, and the next total RNA was purified using TRIzol reagent (Life Technologies) and an RNeasy minikit (Qiagen). SuperScript III and Klenow exo-DNA polymerase (Life Technologies) were used to produce double-stranded cDNAs (ds-cDNAs) using total RNA containing 5,000 copies/μl of IBV genome as measured with a quantitative PCR (qPCR) modified from reference 6.

The libraries and sequencing kits used were by Illumina (Nextera XT Index and Nextera XT DNA), and reads were obtained with a NextSeq 500 system (Illumina) using the NextSeq 500 mid-output v2 kit (150 bp). Out of 75,931,144 total reads, 67,015,998 reads were mapped using CLC Genomics Workbench v11.0.1 (Qiagen) with AvCoV isolate 23/2013 (GenBank accession number [KX258195](https://doi.org/10.1128/MRA.01003-18)) as a reference, as *de novo* assembly did not produce a complete and viable genome using the same software.

The D274 genome is 27,599 nucleotides (nt) long [poly(A) tail included] and annotated in the order 5' UTR and leader RNA (nt 1 to 528), ORF1a (nt 529 to 12327), and ORF1ab (nt 529 to 20360) with a ribosomal frameshift, followed by the spike S (nt 20311 to 23802), 3a (nt 23802 to 23975), 3b (nt 23975 to 24169), envelope E (nt 24150 to 24473), membrane M (nt 24442 to 25122), 5a (nt 25481 to 25678), 5b (nt 25675 to 25923), and nucleocapsid N (nt 25866 to 27095) genes and a 3' UTR (nt 27096 to 27599).

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Reads were used to run whole-genome low-variant detection using CLC Genomics Workbench v11.0.1 (Qiagen) ($\geq 100\times$ coverage, ≥ 10 counts, $\geq 5\%$ frequency, 1% significance, and ≥ 20 central and neighborhood qualities) to assess the quasispecies population of D274, i.e., the population of genome variants, including a dominant sequence subjected to natural selection and drift, resulting in only 22 mutations, showing a rather high homogeneity on this D274 sample.

Spike protein sequences, easily obtained by Sanger sequencing, are widely used to speculate about vaccine protection (7), but as the immunity for IBV is not based only on the humoral response to this protein (8, 9), the availability of full genomes for vaccine strains is core to accurately predicting protection markers.

Data availability. The Gammacoronavirus/AvCoV/chicken/Netherlands/D274/1979 complete genome sequence is deposited in GenBank under the accession number [MH021175](https://doi.org/10.1016/j.meegid.2017.08.006). The version described in this paper is the first version, MH021175.1.

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