



Near-Complete Genome Sequence of Infectious Bronchitis Virus Strain VFAR-047 (GI-16 Lineage), Isolated in Peru

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ABSTRACT Here, we report the near-complete genome sequence of the infectious bronchitis virus (IBV) strain VFAR-047, isolated in Peru in 2014. This strain was classified into GI lineage 16 (GI-16) based on both the genome and Spike 1 (S1) sequence analysis. Furthermore, four potential recombination events with other GI-16 and GI-11 strains were identified.

Infectious bronchitis virus (IBV) (*Coronaviridae*, *Gammacoronavirus*) is an economically significant pathogen of the poultry industry worldwide. It causes low egg and meat production and the highly contagious respiratory disease avian infectious bronchitis (1–3) in poultry. IBV has a high mutation and recombination rate, leading to the frequent appearance of new genotypes and antigenic variants worldwide with little or no cross-protection, mainly due to Spike 1 (S1) protein variability (3–5). IBV is classified into six main genotypes (GI to GVI) comprising 32 viral lineages (1 to 32) based on complete nucleotide sequences of the S1 and typical geographical distribution (6). The GI lineage 16 (GI-16), also called the Asia/South America II (A/SaII) genotype, was previously known as the Q1 or CK/CH/LDL/971 type (6, 7). This genotype has been reported in South America (Chile, Colombia, Uruguay, Argentina, and Peru), Asia, and Europe (6, 8–11).

The IBV isolate designated VFAR-047 was isolated from a broiler farm in the north of Lima (Peru) in 2014 (11). Fresh tracheas and kidneys were homogenized and inoculated into the allantoic cavities of specific-pathogen-free embryonated eggs. The allantoic fluid containing IBV was clarified and concentrated in a 20% sucrose gradient by ultracentrifugation. The viral RNA was isolated with an RNeasy midi kit (Qiagen, Germany) and precipitated in ethanol. It was sequenced by Macrogen, Inc. (South Korea) with the HiSeq 2000 platform (Illumina) using the TruSeq stranded total RNA low-throughput (LT) sample prep kit (101 paired ends) (Illumina). The 47,399,834 reads were analyzed, quality checked, and *de novo* assembled using VirusTap (12) and NextGENe (13), producing a genome with a 38% GC content and a coverage of 967.07×. Multiple alignments were performed using Multiple Alignment using Fast Fourier Transform (MAFFT) v7.310 (14), and a phylogenetic tree was generated using the neighbor-joining method in Molecular Evolutionary Genetics Analysis X (MEGA X) (15) with 1,000 bootstrap replications. Recombination analyses were performed using SplitsTree4 v4.14.5 (16) and Recombination Detection Program 4 (RDP4) v.4.95 (17).

The VFAR-047 genome is 27,467 nucleotides (nt) long and was annotated using BLAST comparisons with the nonredundant GenBank database, followed by manual curation. The genome has the typical genetic structure of all IBV strains with 13 open reading frames, organized as follows: 5′-1a-1b-S-3a-3b-E-M-4b-4c-5a-5b-N-6b-3′. The cleavage site of the S protein was typical for A/SaII strains and corresponds to the region between nucleotides 21783 and 21794 (amino acid positions 538 to 541 Arg-Thr-Gly-Arg) (9).

The complete S1 gene (1,617 nt) confirmed that VFAR-047 belongs to lineage GI-16,

Citation Tataje-Lavanda L, Izquierdo-Lara R, Ormeño-Vásquez P, Huamán-Gutiérrez K, Zimic-Peralta M, Fernández-Díaz M. 2019. Near-complete genome sequence of infectious bronchitis virus strain VFAR-047 (GI-16 lineage), isolated in Peru. *Microbiol Resour Announc* 8:e01555-18. <https://doi.org/10.1128/MRA.01555-18>.

Editor Jelle Matthijnsens, KU Leuven

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Received 13 November 2018

Accepted 3 January 2019

Published 31 January 2019

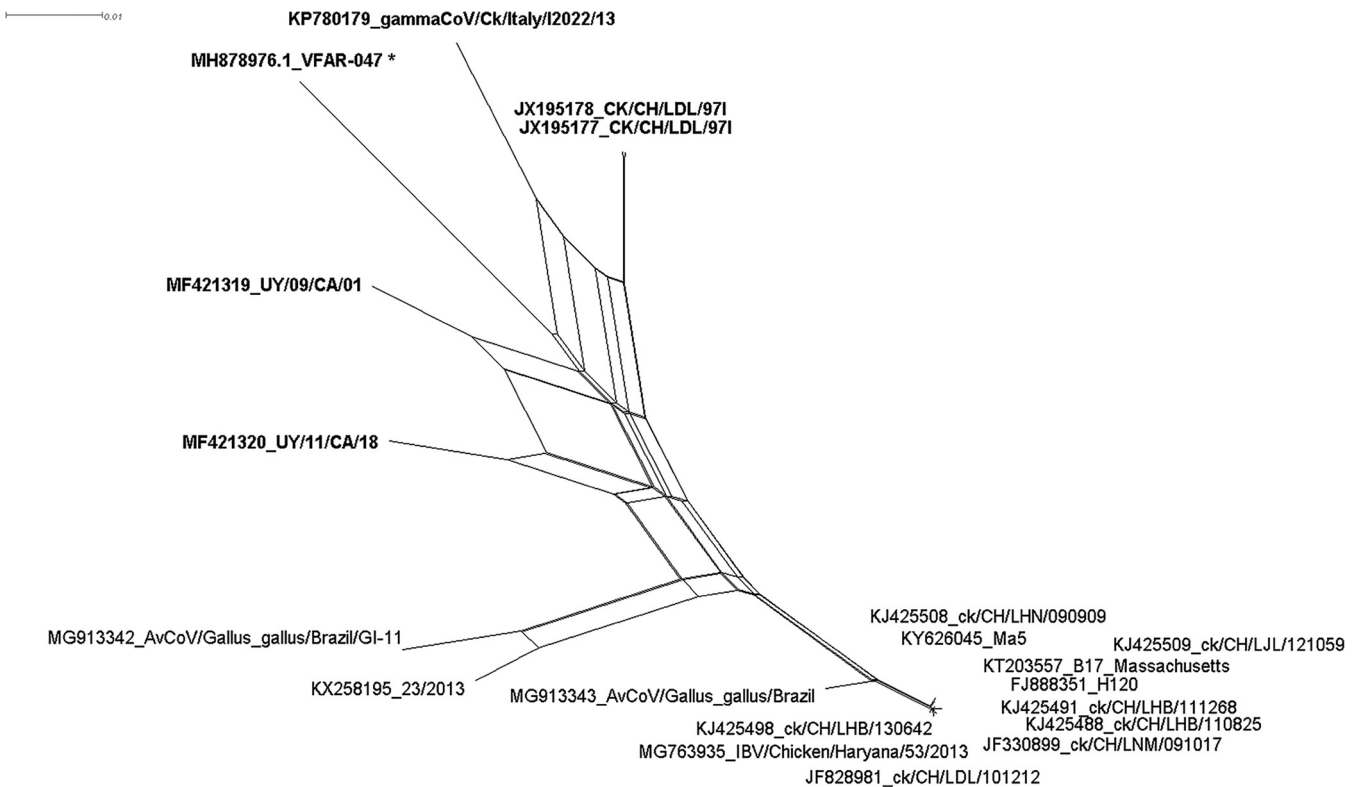


FIG 1 Phylogenetic network (SplitsTree4). The phylogenetic network was constructed using the near-complete genome sequences of 20 IBV strains more closely related to strain VFAR-047 (marked by an asterisk). The multiple reticulate networks indicate historical recombination events (P value = 0.0), and the boxes indicate the likelihood of recombination.

sharing 98% to 93% identity with GI-16 representative strains gammaCoV/Ck/Italy/I2022/13 (GenBank accession number [KP780179](#)), ck/CH/LDL/97I ([JX195177](#) and [JX195178](#)), UY/09/CA/01 ([MF421319](#)), and IZO 28/86 ([KJ941019](#)). Phylogenetic analysis of the near-complete genome showed that VFAR-047 is grouped in the same branch with UY/09/CA/01, UY/11/CA/18 ([MF421320](#)), gammaCoV/Ck/Italy/I2022/13, and CK/CH/LDL/97I, with average nucleotide identities ranging from 93% to 92%. The phylogenetic network (Fig. 1) and the phi test showed statistically significant evidence of recombination ($P = 0.0$). Furthermore, five recombined sequences were identified with high reliability by six methods embedded in RDP4, which include four potential recombination events with GI-16 and GI-11 strains.

The VFAR-047 genome will facilitate future development of new vaccines and other infection control strategies in the poultry industry.

Data availability. The near-complete genome sequence of infectious bronchitis virus isolate VFAR-047 has been deposited in GenBank under the accession number [MH878976](#). Raw data were deposited in the SRA under BioSample number [SAMN10521748](#) and SRA run number [SRR8281084](#), which are part of SRA study number [SRP172861](#).

ACKNOWLEDGMENTS

We thank Ricardo Montesinos, Ángela Montalván, and Edison Huaccachi for their outstanding technical support.

We declare no competing interests.

REFERENCES

- World Organization for Animal Health. 2018. Chapter 2.3.2. Avian infectious bronchitis. In OIE terrestrial manual 2018. World Organization for Animal Health, Paris, France. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.03.02_AIB.pdf.

2. Cavanagh D. 2007. Coronavirus avian infectious bronchitis virus. *Vet Res* 38:281–297. <https://doi.org/10.1051/vetres:2006055>.
3. Lin S-Y, Chen H-W. 2017. Infectious bronchitis virus variants: molecular analysis and pathogenicity investigation. *IJMS* 18:2030. <https://doi.org/10.3390/ijms18102030>.
4. Mo M-L, Li M, Huang B-C, Fan W-S, Wei P, Wei T-C, Cheng Q-Y, Wei Z-J, Lang Y-H. 2013. Molecular characterization of major structural protein genes of avian coronavirus infectious bronchitis virus isolates in southern China. *Viruses* 5:3007–3020. <https://doi.org/10.3390/v5123007>.
5. Thor SW, Hilt DA, Kissinger JC, Paterson AH, Jackwood MW. 2011. Recombination in avian gamma-coronavirus infectious bronchitis virus. *Viruses* 3:1777–1799. <https://doi.org/10.3390/v3091777>.
6. Valastro V, Holmes EC, Britton P, Fusaro A, Jackwood MW, Cattoli G, Monne I. 2016. S1 gene-based phylogeny of infectious bronchitis virus: an attempt to harmonize virus classification. *Infect Genet Evol* 39: 349–364. <https://doi.org/10.1016/j.meegid.2016.02.015>.
7. Han Z, Sun C, Yan B, Zhang X, Wang Y, Li C, Zhang Q, Ma Y, Shao Y, Liu Q, Kong X, Liu S. 2011. A 15-year analysis of molecular epidemiology of avian infectious bronchitis coronavirus in China. *Infect Genet Evol* 11: 190–200. <https://doi.org/10.1016/j.meegid.2010.09.002>.
8. Jackwood MW. 2012. Review of infectious bronchitis virus around the world. *Avian Dis* 56:634–641. <https://doi.org/10.1637/10227-043012-Review.1>.
9. Marandino A, Pereda A, Tomás G, Hernández M, Iraola G, Craig MI, Hernández D, Banda A, Villegas P, Panzera Y, Pérez R. 2015. Phylogenetic analysis of avian infectious bronchitis virus in South America. *J Gen Virol* 96:1340–1346. <https://doi.org/10.1099/vir.0.000077>.
10. Marandino A, Tomás G, Panzera Y, Greif G, Parodi-Talice A, Hernández M, Techera C, Hernández D, Pérez R. 2017. Whole-genome characterization of Uruguayan strains of avian infectious bronchitis virus reveals extensive recombination between the two major South American lineages. *Infect Genet Evol* 54:245–250. <https://doi.org/10.1016/j.meegid.2017.07.009>.
11. Tataje-Lavanda L, Falconi-Agapito F, Montalván Á, Bueno C, Requena D, Fernández-Díaz M. 2016. First evidence of detection of Asia/South America II (A/SAll) infectious bronchitis virus in a commercial broiler flock in Peru. *Vet Rec Case Rep* 4:e000292. <https://doi.org/10.1136/vetreccr-2016-000292>.
12. Yamashita A, Sekizuka T, Kuroda M. 2016. VirusTAP: viral genome-targeted assembly pipeline. *Front Microbiol* 7:32. <https://doi.org/10.3389/fmicb.2016.00032>.
13. SoftGenetics. 2016. NextGene 2.4.2-Next Generation Sequencing Software for Biologists—User Manual. SoftGenetics, State College, PA.
14. Yamada KD, Tomii K, Katoh K. 2016. Application of the MAFFT sequence alignment program to large data—reexamination of the usefulness of chained guide trees. *Bioinformatics* 32:3246–3251. <https://doi.org/10.1093/bioinformatics/btw412>.
15. Kumar S, Stecher G, Li M, Niyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol Biol Evol* 35:1547–1549. <https://doi.org/10.1093/molbev/msy096>.
16. Huson DH, Bryant D. 2006. Application of phylogenetic networks in evolutionary studies. *Mol Biol Evol* 23:254–267. <https://doi.org/10.1093/molbev/msj030>.
17. Martin DP, Murrell B, Golden M, Khoosal A, Muhire B. 2015. RDP4: detection and analysis of recombination patterns in virus genomes. *Virus Evol* 1:vev003. <https://doi.org/10.1093/ve/vev003>.