Emergence of *Avian coronavirus* genotype GI-11 in Colombia

Nelson F. Santana-Clavijo 1 · Paulo E. Brandão 1

Received: 8 June 2020 / Accepted: 17 October 2020
© Sociedade Brasileira de Microbiologia 2020

**Abstract**

*Avian coronavirus* (AvCoV/IBV) is a virus with high morbidity, which can cause respiratory, digestive, renal, and reproductive diseases in chickens. Molecular detection and sequencing are the main tool for identification and classification of AvCoV. Thirty-six samples were collected in three broiler farms from different regions in Colombia, due to mortality increase; ten samples were positive using RT-qPCR targeted to the 5′ UTR of AvCoV, and one sample was positive and had its partial S gene sequenced. Phylogenetic analysis revealed that this strain belongs to the GI-11 lineage, similar to the Brazilian cluster. Several lineages have already been described in Colombia but, to the best of our knowledge, this is the first time that GI-11 has been detected in this country, which suggests that this subtype may be more widespread in South America than previously thought.

**Keywords** *Avian coronavirus* · Subtype GI-11 · Morbidity · Phylogenetic analyses · Brazilian cluster

*Avian coronavirus* (AvCoV, host-type avian infectious bronchitis virus, IBV) is the causative agent of highly contagious diseases for chickens, placing a significant economic burden on the poultry industry worldwide [9, 12]. AvCoV belongs to order Nidovirales, family Coronaviridae, subfamily Orthocoronavirinae, genus Gammacoronavirus, and subgenus Igacovirus [16]. The viral genome is single-stranded RNA, positive sense, with 30 kb, which comprises two untranslated regions (UTRs) at the 5′ and 3′ ends [19, 28], two overlapping open read frames (ORFs) encoding the polyproteins 1a and 1ab (15 nonstructural proteins), and eight ORFs that codes for structural proteins (S, E, M, N) and accessory proteins (3a, 3b, 5a, 5b) [7, 23].

The spike protein is a glycoprotein responsible for binding to host receptors and determines the tropism and host range of the virus [30]; this glycoprotein has two subunits, S1 that is anchored to viral membrane by S2 subunit [1]. Subunit S1 contains the epitopes involved in the induction of serotype-specific neutralizing antibodies, but cross protection is poor and most of these serotypes differ from each other by 20–25% at amino acid level in S1 subunit [1, 26, 29]. Nucleotide heterogeneity is more prevalent in the S1 portion of the S gene and is largely contained in three different hypervariable regions (HVR) (aa 38–67, 91–141, 275–287). The analysis of complete or partial S1 gene nucleotide sequence has been conventionally used to determine viral genetic types, and more than 50 different antigenic and genetic types of AvCoV have been recognized [13, 17, 23].

In Colombia, where the only IBV strain used in vaccines belongs to the GI-1 lineage “Massachusetts,” few studies have been conducted on AvCoV. In 1963, this virus was isolated from samples from two geographical regions in embryonated eggs for the first time, from broilers and layers with respiratory signs [20]; in 2003, field isolates from broilers and layers of five different regions were studied based on spike gene S1 (HVR 1–2), and showed the presence of genotypes GI-1, GI-16, GI-20, and GVI-1 [2]. Serological studies were conducted in two regions of Colombia: Santander, in which antibodies were detected in fighting roosters in 2005, 42.4% of which were positive without vaccination [14]; and in Cundinamarca, where two studies were carried out on farms with previously molecular detection of AvCoV [3, 10], when 85.72% of samples were positive in poultry with respiratory signs and vaccinated against AvCoV. In 2016, AvCoV samples from four cities in the central region of Colombia (Tolima) were isolated and sequenced, and the study reported a low nucleotide identity between South American strains (< 75%), and high...
identity with Cuban strains (82 to 99%) [8]. In Colombia, there are very few molecular studies of AvCoV circulating strains. Thus, the aim of this study is to report the emergence of the GI-11 lineage in Colombia, which has been reported only in Argentina, Brazil, and Uruguay [21, 22, 25].

A total of 36 samples from broilers (twelve per farm) were collected from three farms that had been showing an increased mortality up to 10%, located in three different geographic regions of Colombia (Cundinamarca, Santander, and Valle del Cauca). Samples were collected using Whatmann® FTA® cards, between December 2018 and January 2019, from broilers vaccinated at days 4 and 14 with a GI-1 strain. From the three farms, three FTA cards were collected/received per farm, sampling 10 birds/farm; as each FTA card used had 4 spots (for one sample/spot), a total of 12 samples were collected from each farm, making a total of 36 samples, pooling lungs, tracheas, kidneys, and cecal tonsils from 2 to 3 birds in each respective spot, and from each farm samples of ten broilers collected, due to a recommendation for the use of FTA cards with pooled samples (three or four birds per card). These samples were from lungs (four, L), trachea (two, T), kidney (three, K), and cecal tonsils (three, CT) per farm. Total

Fig. 1 Maximum likelihood phylogenetic tree for partial spike protein region S1 HVR3 (nt 705–1097) of AvCoV. The figure showing genotypes I to VI and the respective lineages. The GI-11 detected in this study is in bold. The bar represents number of substitutions per site.
RNA was extracted from FTA® Cards, cutting ¼ of each card and using PureLink RNA™ (Invitrogen), where the samples of lung and trachea were pooled for extraction and molecular tests (L+T).

Screening real-time PCR was made using the method developed by Callison et al. [6] to detect region 5′ UTR of AvCoV. Positive samples were subjected to a nested PCR for partial amplification of S1 subunit gene of the spike protein of IBV (nt position 677-1097 Z83979) using the primers reported by Worthington et al. [27] for the amplification of HVR 3. Amplicons were sequenced bidirectionally using BigDye Terminator v3.1 Cycle Sequencing Kit™ (Applied Biosystems) and an ABI-3500 Genetic Analyzer™ (Applied Biosystems), following the manufacturer’s instructions. Positions with Phred scores ≥20 were assembled with BioEdit 7.0.5.3 (Ibis Biosciences, Carlsbad, CA, USA), and the final sequence was used to build a maximum likelihood tree (Tamura-Nei model and GTR+G) with 1000 bootstrap replicates using MEGA 7 [18], with a dataset from complete genome available of AvCoV using the classification suggested for Valastro et al. [23], but aligned for HV3/S1 region of virus.

From the tested samples, 10 were positive (Cundinamarca L+T: 1 Ct 34.52, CT: 1 Ct 27.7, and K: 1 Ct 37.13; Santander L+T: 1 Ct 32.32, CT: 1 Ct 31.28; and Valle del Cauca L+T: 2 Ct 31.91 ± 0.43, CT: 2 Ct 30.05 ± 1.74, and K: 1 Ct 30.82) with mean Ct 31.81 ± 2.9, and a partial S1 sequence was obtained for one of them (cecal tonsil/Cundinamarca Ct 27.7) (GenBank accession number MK896657) which was assigned to genotype GI lineage 11 and the sample MK896657, which showed a significative bootstrapping in relation with the sequences in the study. The bar represents the number of substitutions per site.
classification based on Valastro et al. [23]; nucleotide identities showed a variation between 58.1 and 85.9%. Since bootstrapping was less than 75%, another phylogenetic tree was constructed following the same method for the first tree and using the phylogenetic analysis proposed by De Wit et al. [12], in which MK896657 was located close, but outside the cluster Brazil I (Fig. 2), and the nucleotide identities ranged from 0.778 to 0.929. Fourteen amino acid residues changes were observed: S255D, S257L, R261K, D282Y/H, F288L/P/S, Y303H/C, C321V, V332K, F332Y, W347L, G348W, F352L, I354V, and F156L with references to Brazilian strains (Genbank access KP202366-372, AF093793-4, AF169859-860, M21970, AY561713, AY851295, X15832, and KY626044).

GI-11 probably emerged in the 1960s and was believed to be restricted to Argentina, Brazil, and Uruguay [21]. GI-11 represents 74% of AvCoV types in Brazil [11, 15] and was implicated in nephrosis, orchitis, respiratory, and enteric diseases [12, 24].

Regarding the farms sampled in this study, increased mortality was the sign reported for the broilers; AvCoV is a known cause of mortality in broilers [4] and GI-11 has been shown to cause a significant economic burden in broilers [9], showing that this lineage is more widespread than previously considered.

The first full genome of AvCoV GI-11 was published in 2016 [5], showing a 27,615-nt genome with a gene arrangement similar to other AvCoVs, but with a phylogeny that confirmed its divergence from the other lineages. Obtaining the full genome for the strain reported herein will allow for an in-depth understanding of the phytogeography and evolution of this lineage in South America.

It is important to highlight that Alvarado et al. [2] based their study on portion of S1 gene that corresponds to HVR 1 and 2, while the sequence obtained herein maps to HVR 3, impairing a comparison between both studies. On the other hand, the sequences reported in 2016 [8] are not yet available on GenBank, but only their phylogenetic relationships were mentioned in that report.

Whether GI-11 is to become a concern for the poultry industry in Colombia still remains to be determined, as additional data is needed regarding its distribution in this country and its pathogenic consequences, as well as robust scientific data, before changes in vaccine strains are seriously considered.

In summary, the present study indicates that Avian coronavirus be widespread in South America, with few countries that have yet reported it, which makes it necessary to clarify how this subtype has spread throughout most of the continent, taking into account the poultry trade between the South American countries and their sanitary protocols.

**Funding** This work was funded by CNPq (Brazilian National Board for Scientific and Technological Development) grant numbers 307291/2017-0 and 400604/2016-7 and CAPES (Coordination for the Improvement of Higher Education Personnel, Brazil) Finance Code 001, which had no role in the study design, collection, analysis and interpretation of data, writing of the report or decision to submit the article for publication.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**References**

1. Abolnik C (2015) Genomic and single nucleotide polymorphism analysis of infectious bronchitis coronavirus. Infect Genet Evol 32: 416–426. https://doi.org/10.1016/j.meegid.2015.03.033
2. Alvarado IR, Villegas P, Mossos N, Jackwood MW (2006) Molecular characterization of avian infectious bronchitis virus strains isolated in Colombia during 2003. Avian Dis 49:494–499. https://doi.org/10.1637/7202-050304r.1
3. Álvarez D, Usma J, Jaime J, Vera V (2009) Dinámica Serológica Del Virus De Bronquitis Departamento De Cundinamarca. Rev Med Vet y Zootecnia 56:105–112
4. Bande F, Arshad SS, Omar AR, Bejo MH, Abubakar MS, Abba Y (2016) Pathogenesis and diagnostic approaches of avian infectious bronchitis. Adv Virol 2016:1–11. https://doi.org/10.1155/2016/4621659
5. Brandão PE, Ayres GRR, Torres CA, Villarreal LYB, Hora AS, Taniwaki A (2016) Complete genome sequence of a Brazil-type avian coronavirus. 39:15–16. https://doi.org/10.1128/genomeA.01135-16.Copyright
6. Callison SA, Hilt DA, Boynton TO, Sample BF, Robison R, Swayne DE, Jackwood MW (2006) Development and evaluation of a real-time Taqman RT-PCR assay for the detection of infectious bronchitis virus from infected chickens. J Virol Methods 138:60–65. https://doi.org/10.1016/j.jviromet.2006.07.018
7. Cavanagh D (2005) Coronavirus in poultry and other birds. Avian Pathol 34:439–448. https://doi.org/10.1080/03079450500367682
8. Cifuentes-Rincón A, Lopes PD, Sanmiguel RA (2016) Genotipificación de variantes del virus de bronquitis infecciosa aviar en el departamento del Tolima, Colombia. Rev MVZ Cordoba 21:5500–5510. https://doi.org/10.21897/mvzv.824
9. Colvero LP, Villarreal LYB, Torres CA, Brandão PE (2015) Assessing the economic burden of avian infectious bronchitis on poultry farms in Brazil. OIE Rev Sci Tech 34:993–999. https://doi.org/10.21050/rst.34.3.2411
10. Córdoba Argoti G, Vera Alfonso VJ, Correa Jaime J, Ramírez Nieto GC (2015) Comportamiento del virus de la bronquitis infecciosa aviar en aves con sintomatología respiratoria provenientes de granjas de producción del Departamento de Cundinamarca. Nova 13:47. https://doi.org/10.22490/24629448.1705
11. de Fraga AP, Gräf T, Pereira CS, Ikuta N, Fonseca ASK, Lunge VR (2018) Phylogenetic analysis and molecular diversity of the avian infectious bronchitis virus of chickens in Brazil. Infect Genet Evol 61:77–83. https://doi.org/10.1016/j.meegid.2018.03.014
12. De Wit JJ, Brandao P, Torres CA, Koopman R, Villarreal LY (2015) Increased level of protection of respiratory tract and kidney by combining different infectious bronchitis virus vaccines against challenge with nephropathogenic Brazilian genotype subcluster 4 strains. Avian Pathol 44:352–357. https://doi.org/10.1080/03079457.2015.1058916
13. de Wit JJ, Cazaban C, Dijkman R, Ramon G, Gardin Y (2018) Detection of different genotypes of infectious bronchitis virus and of infectious bursal disease virus in European broilers during an epidemiological study in 2013 and the consequences for the diagnostic approach. Avian Pathol 47:140–151. https://doi.org/10.1080/03079457.2017.1387231

14. Diaz J, Rios H, & Moreno O (1). Determinación serológica para las enfermedades de Newcastle y bronquitis infecciosa en las aves de combate de Bucaramanga. Spei Domus, 1(1). Recuperado a partir de https://revistas.ucc.edu.co/index.php/sp/article/view/555

15. Fraga AP, Balestrin E, Ikuta N, Fonseca ASK, Spilki FR, Canal CW, Lunge VR (2013) Emergence of a new genotype of avian infectious bronchitis virus in Brazil. Avian Dis 57:225–232. https://doi.org/10.1637/10346-090412-reg.1

16. Franzo G, Legnardi M, Tucciarone CM, Drigo M, Martini M, Cecchinato M (2019) Evolution of infectious bronchitis virus in the field after homologous vaccination introduction. Vet Res 50:92. https://doi.org/10.1186/s13567-019-0713-4

17. Jackwood MW, Hall D, Handel A (2012) Molecular evolution and emergence of avian gammacoronaviruses. Infect Genet Evol 12:1305–1311. https://doi.org/10.1016/j.meegid.2012.05.003

18. Lizowska A, Sajewicz-Krukowska J, Fusaro A, Pikula A, Domanska-Blicharz K (2017) First characterization of a Middle-East GI-23 lineage (Var2-like) of infectious bronchitis virus in Europe. Virus Res 242:43–48. https://doi.org/10.1016/j.virusres.2017.09.010

20. Luque Forero G, Yepez MM, Morales Granados A (1963) Aislamiento del virus de la bronquitis infecciosa de las aves en Colombia. Rev la Fac Med Vet y Zootec 26:1024–1027

21. Marandino A, Tomás G, Panzer 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

22. Marandino A, Vagnozzi A, Craig MI, Tomás G, Techera C, Panzer Y, Vera F, Pérez R (2019) Genetic and antigenic heterogeneity of infectious bronchitis virus in South America: implications for control programmes. Avian Pathol 48:270–277. https://doi.org/10.1080/03079457.2019.1583315

23. 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

24. Villarreal LYB, Brandão PE, Chacón JL, Assayag MS, Maiorka PC, Raffi P, Sainedberg ABS, Jones RC, Ferreira AJP (2007a) Orchitis in roosters with reduced fertility associated with avian infectious bronchitis virus and avian metapneumovirus infections. Avian Dis 51:900–904. https://doi.org/10.1637/7815-121306-reg.1

25. Villarreal LYB, Brandão PE, Chacón JL, Sainedberg ABS, Assayag MS, Jones RC, Ferreira AJP (2007b) Molecular characterization of infectious bronchitis virus strains isolated from the enteric contents of Brazilian laying hens and broilers. Avian Dis 51:974–978. https://doi.org/10.1637/7983-041307-1

26. Winter C, Schwegmann-Wellens C, Cavanagh D, Neumann U, Hettler G (2006) Sialic acid is a receptor determinant for infection of cells by avian infectious bronchitis virus. J Gen Virol 87:1209–1216. https://doi.org/10.1099/vir.0.81651-0

27. Worthington KJ, Currie RJW, Jones RC (2008) A reverse transcriptase-polymerase chain reaction survey of infectious bronchitis virus genotypes in Western Europe from 2002 to 2006. Avian Pathol 37:247–257. https://doi.org/10.1080/03079450801986529

28. Yang D, Leibowitz JL (2015) The structure and functions of coronavirus genomic 3’ and 5’ ends. Virus Res 206:120–133. https://doi.org/10.1016/j.viruses.2015.02.025

29. Zhang T, Han Z, Xu Q, Wang Q, Gao M, Wu W, Shao Y, Li H, Kong X, Liu S (2015) Serotype shift of a 793/B genotype infectious bronchitis coronavirus by natural recombination. Infect Genet Evol 32:377–387. https://doi.org/10.1016/j.meegid.2015.03.034

30. Zhua Z, Zhanga Z, Chena W, Caia Z, Gea X, Zhua H, Jiang T, Tane W, Penga Y (2018) Predicting the receptor-binding domain usage of the coronavirus based on kmer frequency on spike protein. Infect Genet Evol 61:183–184

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.