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VETERINARY MICROBIOLOGY - SHORT COMMUNICATION

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

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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 heterogenicity 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 determinate 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 fight 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

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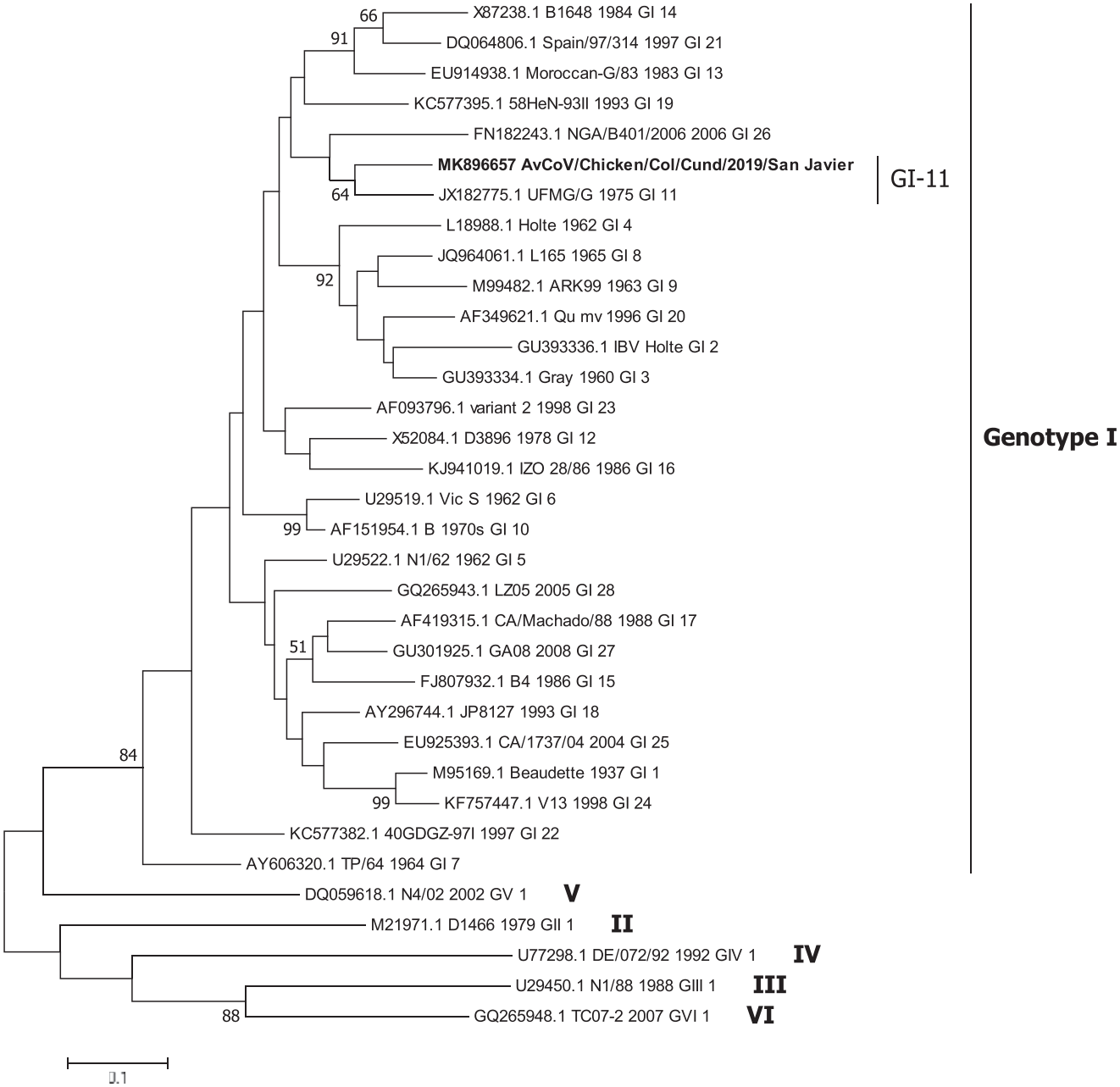

Fig. 1 Maximum likelihood phylogenetic tree for partial spike protein region S1 HVR3 (nt 705–1097) of AvCoV. The figure showing genotypes I to VI and the respective lineages. The GI-11 detected in this study is in bold. The bar represents number of substitutions per site

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

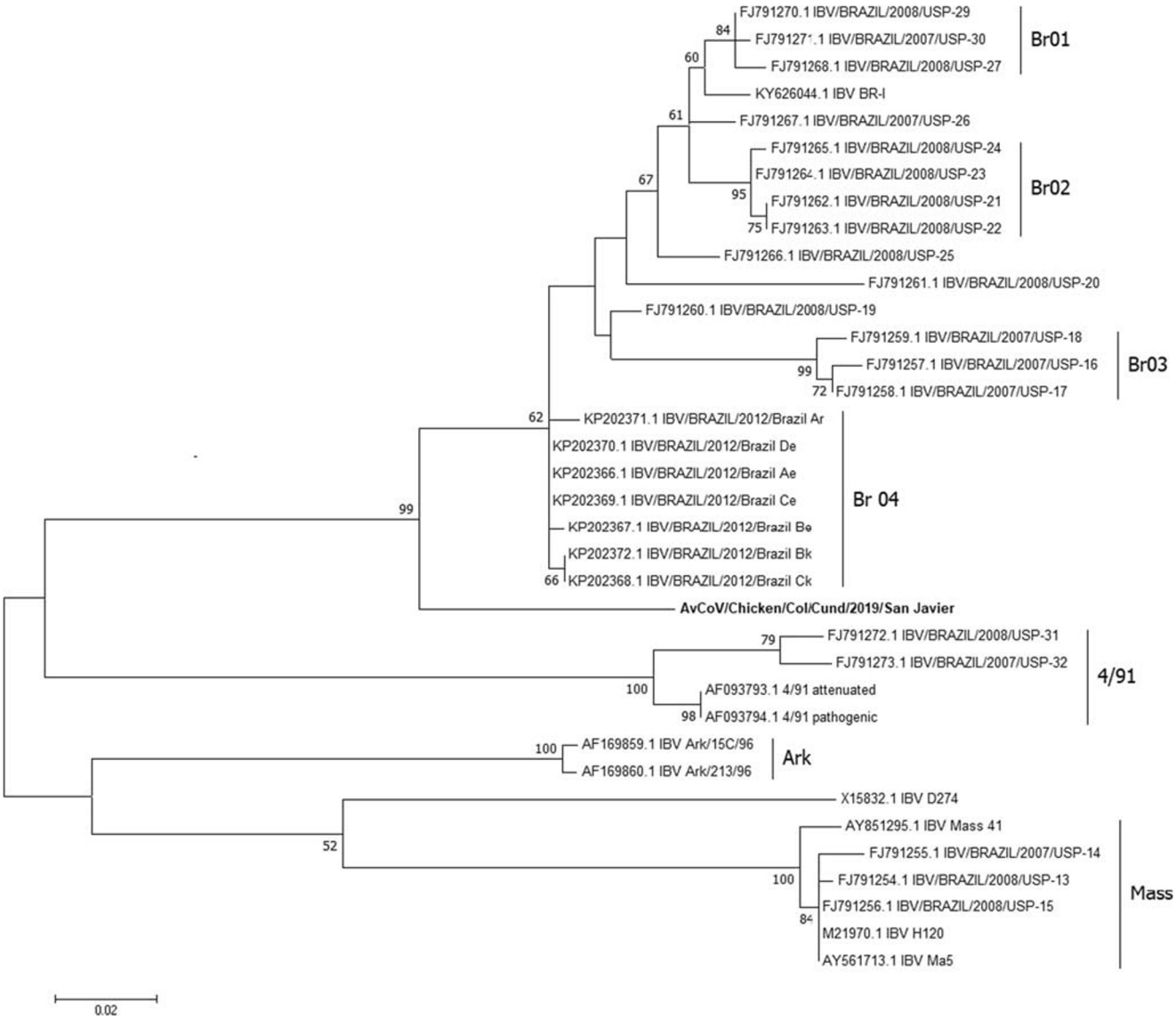

Fig. 2 Maximum likelihood phylogenetic tree for the partial spike protein region S1 HVR3 (nt 705–1097) of AvCoV. The figure shows the genotypes detected in Brazil in the respective lineage on genotype GI-11 and the sample MK896657, which showed a significative bootstrapping in relation with the sequences in the study. The bar represents the number of substitutions per site

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 (Fig. 1) using a

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 phylogenic 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, V323K, 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 phylogenic 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.

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Compliance with ethical standards

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

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