



## First evidence of the emergence of novel putative infectious bronchitis virus genotypes in Cuba

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### ABSTRACT

The emergence of new infectious bronchitis virus (IBV) genotypes or serotypes along with the poor cross-protection observed among IBV serotypes have complicated the avian infectious bronchitis (IB) control programs in different geographic regions. In Cuba, the lack of genetic information regarding IBV and the increasing epidemiological importance of this virus in Cuban chicken flocks demand further characterization of IBV isolates. In the present work, studies of genetic diversity and phylogenetic relationships among recent IBV isolates from Cuban chicken flocks showing respiratory disorders were performed. Two putative genotypes genetically different to the Massachusetts genotype H120 strain used in the Cuban vaccination program were found in the flocks assessed. In addition, a potential nephropathogenic IBV isolate was found by first time in Cuba.

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Avian infectious bronchitis (IB) is a highly contagious viral disease of poultry characterized by respiratory signs, nephritis and reduced egg production (Cavanagh and Naqi, 2003). This disease is caused by infectious bronchitis virus (IBV), a member of the genus *Gamma coronavirus*, family *Coronaviridae*, order *Nidovirales* (ICTV, 2009) which is considered a major pathogen in poultry production (OIE, 2008). The IBV genome consists of a single-stranded positive sense RNA molecule of approximately 27.6 Kb which encodes several nonstructural proteins involved in RNA transcription and replication and four structural proteins, spike (S), small membrane (E), membrane (M) and nucleoprotein (N) (Bourisnell et al., 1987). The S glycoprotein is formed by a globular S1 subunit that is anchored in the membrane by the S2 subunit (Cavanagh, 2007). The S1 subunit contains the main virus-neutralization epitopes, which are involved in the induction of neutralizing, serotype-specific haemagglutination inhibiting antibodies, and protective immunity (Cavanagh, 2003; Hodgson et al., 2004). Hence mutations within this genome region may result in the emergence of new viral variants (Moore et al., 1998).

The emergence of new IBV genotypes or serotypes (Lim et al., 2011; Mahmooda et al., 2011; Ducatez et al., 2009) along with the poor cross-protection observed among IBV serotypes (Cowen and Hitchner, 1975) have complicated the IB control programs

(Cavanagh, 2007). In Cuba, the IB control strategy is based on the use of an attenuated live vaccine strain H120 of Massachusetts (Mass) serotype (reviewed in Colas et al., 2010) and despite of the extensive vaccination performed, the presence of IBV in vaccinated flock has been recently revealed by reverse transcriptase-polymerase chain reaction (RT-PCR) detection associated with clinical respiratory disorders in poultry (Acevedo et al., 2010). However, genetic information on IBV in Cuban chicken flocks is not still available.

The nucleotide sequencing followed by phylogenetic analysis based on the IBV S1 complete gene that contains the main virus-neutralization epitopes provide a fast and powerful method for the identification of genotypes and the prediction of serotypes.

Nucleotide sequencing of a diagnostically relevant fragment of the S1 gene is the most useful technique for the differentiation of IBV strains and has become the genotyping method of choice in many laboratories (OIE Manual, 2008). IBV phylogenetic clustering based on partial S1 gene sequences has been considered an accurate and less labor intensive method for the rapid identification of IBV field isolates and variants (Kulkarni and Resurreccion, 2010).

In the view of the lack of genetic information concerning IBV and the increasing epidemiological importance of this virus in Cuban chicken flocks further characterization of IBV isolates was considered necessary. Therefore, the objective of this study was to investigate the genetic diversity and the phylogenetic relationships among IBV isolates associated with respiratory disease from Cuban chicken flocks, based on partial S1 phylogenetic marker.

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| Percentage of nucleotides identity |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |                          |  |
|------------------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|----|--------------------------|--|
|                                    | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   | 13   | 14   | 15   | 16   | 17   |    |                          |  |
| 1                                  |      | 87   | 81.8 | 80.6 | 91.3 | 83.7 | 76.6 | 78   | 76.9 | 80.1 | 80.1 | 76.6 | 82.1 | 81.2 | 78.6 | 80.6 | 79.2 | 1  | Cuba/La Habana/CB6/2009  |  |
| 2                                  | 70.7 |      | 79.2 | 87.8 | 80.9 | 80   | 77.8 | 80.6 | 78.9 | 79.5 | 84.1 | 78.6 | 79.5 | 84.4 | 80.1 | 81.8 | 79.8 | 2  | Cuba/La Habana/CB19/2009 |  |
| 3                                  | 58   | 55.7 |      | 77.8 | 83.8 | 84.8 | 81.5 | 82.4 | 81.2 | 79.8 | 76.9 | 81.5 | 99.7 | 77.2 | 82.7 | 80.4 | 80.1 | 3  | Cuba/La Habana/CB13/2009 |  |
| 4                                  | 60.1 | 77.4 | 54.8 |      | 78.3 | 80   | 77.2 | 81.5 | 79.2 | 78.3 | 87.3 | 79.2 | 77.8 | 85.8 | 79.2 | 80.1 | 82.1 | 4  | Beligian/B1648/1996      |  |
| 5                                  | 78.3 | 56.6 | 62.8 | 53.9 |      | 84.8 | 78.6 | 79.5 | 78.3 | 79.8 | 77.8 | 78   | 84.1 | 78.6 | 78.9 | 80.4 | 81.2 | 5  | USA/DMV/5642/06          |  |
| 6                                  | 30.4 | 24.3 | 32.4 | 24.3 | 28.6 |      | 78.8 | 79.7 | 79.1 | 78.8 | 78   | 79.4 | 85.1 | 78.2 | 80   | 78.5 | 79.4 | 6  | Japan/JP9758/2003        |  |
| 7                                  | 42.1 | 45.1 | 50   | 48.2 | 40.3 | 31   |      | 78.3 | 86.7 | 83.5 | 78   | 86.4 | 81.2 | 78.6 | 92.5 | 79.5 | 81.2 | 7  | USA/ArkDPI/1997          |  |
| 8                                  | 48.2 | 56.2 | 63.3 | 56.2 | 52.2 | 28.6 | 48.6 |      | 79.5 | 78   | 79.5 | 78.6 | 82.7 | 82.1 | 79.2 | 79.5 | 82.4 | 8  | Netherland/D207/1989     |  |
| 9                                  | 49.1 | 55.7 | 58.7 | 55.3 | 50   | 23.2 | 58   | 56.6 |      | 84.7 | 79.2 | 99.1 | 80.9 | 80.4 | 88.1 | 82.4 | 81.2 | 9  | USA/Gray/1983            |  |
| 10                                 | 54.3 | 53   | 56.1 | 50.4 | 54.3 | 24.1 | 53   | 53.9 | 64.6 |      | 79.5 | 84.4 | 79.5 | 79.2 | 85   | 77.5 | 80.9 | 10 | USA/Holte/               |  |
| 11                                 | 57.5 | 66.9 | 50.4 | 69.6 | 52.2 | 23.4 | 44.2 | 50.8 | 53   | 55.7 |      | 78.6 | 76.6 | 88.1 | 78.9 | 79.2 | 82.4 | 11 | Italy/Italy-02/2002      |  |
| 12                                 | 49.1 | 55.7 | 59.6 | 56.2 | 50   | 23.2 | 58   | 53.9 | 97.3 | 64.6 | 52.2 |      | 81.2 | 79.8 | 87.8 | 82.4 | 80.6 | 12 | USA/JMK/1993             |  |
| 13                                 | 58   | 55.7 | 99.1 | 54.8 | 62.8 | 32.1 | 50   | 63.3 | 58.7 | 56.1 | 50.4 | 59.6 |      | 77.5 | 82.4 | 80.6 | 80.4 | 13 | USA/M41                  |  |
| 14                                 | 61.9 | 67.8 | 51.3 | 65.7 | 53.9 | 22.6 | 46.4 | 58.5 | 58.9 | 54.8 | 74.1 | 58   | 51.3 |      | 79.5 | 81.2 | 82.1 | 14 | UK/4/91/1998             |  |
| 15                                 | 50   | 53.9 | 60.5 | 54.4 | 49.1 | 30.1 | 75.8 | 56.6 | 71.4 | 63.7 | 51.3 | 71.4 | 60.5 | 56.2 |      | 80.9 | 82.7 | 15 | USA/Cal99/2003           |  |
| 16                                 | 56.6 | 60.7 | 54.8 | 57.6 | 53.9 | 24.3 | 48.2 | 58   | 63.3 | 51.3 | 53.5 | 63.3 | 54.8 | 60.3 | 59.8 |      | 79.8 | 16 | Brazil/IBV-USP-16/2007   |  |
| 17                                 | 54.8 | 57.1 | 61   | 58.9 | 59.2 | 25.2 | 52.2 | 62.1 | 56.6 | 58.4 | 58   | 54.8 | 61   | 60.3 | 59.2 | 56.2 |      | 17 | China/QXIBV/1999         |  |
| Percentange of amino acid identity |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |                          |  |

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