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Research paper

Phylodynamic analysis and molecular diversity of the avian infectious bronchitis virus of chickens in Brazil

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ABSTRACT

Avian infectious bronchitis virus (IBV) is the etiological agent of a highly contagious disease, which results in severe economic losses to the poultry industry. The spike protein (S1 subunit) is responsible for the molecular diversity of the virus and many sero/genotypes are described around the world. Recently a new standardized classification of the IBV molecular diversity was conducted, based on phylogenetic analysis of the S1 gene sequences sampled worldwide. Brazil is one of the biggest poultry producers in the world and the present study aimed to review the molecular diversity and reconstruct the evolutionary history of IBV in the country. All IBV S1 gene sequences, with local and year of collection information available on GenBank, were retrieved. Phylogenetic analyses were carried out based on a maximum likelihood method for the classification of genotypes occurring in Brazil, according to the new classification. Bayesian phylogenetic analyses were performed with the Brazilian clade and related international sequences to determine the evolutionary history of IBV in Brazil. A total of 143 Brazilian sequences were classified as GI-11 and 46 as GI-1 (Mass). Within the GI-11 clade, we have identified a potential recombinant strain circulating in Brazil. Phylodynamic analysis demonstrated that IBV GI-11 lineage was introduced in Brazil in the 1950s (1951, 1917–1975 95% HPD) and population dynamics was mostly constant throughout the time. Despite the national vaccination protocols, our results show the widespread dissemination and maintenance of the IBV GI-11 lineage in Brazil and highlight the importance of continuous surveillance to evaluate the impact of currently used vaccine strains on the observed viral diversity of the country.

1. Introduction

Infectious bronchitis (IB) is an acute and highly contagious viral disease that affects domestic fowl (*Gallus gallus*) worldwide (Cavanagh, 2007). The etiological agent is the avian infectious bronchitis virus (IBV), a *Gammacoronavirus* from the family *Coronaviridae* (ICTV, 2016). IBV genome is a single positive sense RNA strand with approximately 27.6 Kb in length that encodes four structural proteins - nucleocapsid (N), membrane (M), envelope (E), and spike (S) - in addition to an RNA-dependent RNA polymerase and numerous accessory proteins (Jackwood, 2012). Among all the structural proteins, S is the most important for antigenic and immunogenic reasons. It is cleaved into the subunits S1 and S2 with approximately 535 and 625 amino acids, respectively. S1 glycoprotein is important in adsorption to the cellular receptor and virus entry into the host cell, besides inducing neutralizing antibodies. S1 gene is highly variable among the different viral strains and is directly related to the diversity of IBV antigenic and genetic groups (Cavanagh, 2007; Toro et al., 2012).

IBV genetic diversity, mainly in the S1 gene, was demonstrated in different poultry-producing regions of the world. Historically, Massachusetts (Mass) and Connecticut (Conn) serotypes were the first isolates in the 1940s and 1950s, respectively (Schalk and Hawn, 1931; Jungherr et al., 1956). Since then, several new IBV sero/genotypes have been identified and associated with the disease around the world (Cook et al., 2012; De Wit et al., 2011). However, for decades, the identification and classification of IBV genetic types were mostly performed without clear criteria regarding the nomenclature, methods to compare viral molecular data and exact genetic region to be analyzed.

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Consequently, subclades of the closely related virus, sometimes circulating in small geographical regions, have been assigned as new genotypes and more than 50 genetic groups were already reported (De Wit et al., 2011). Recently, a more definitive phylogeny-based classification system was proposed for the assignment of IBV strains. This study classified IBV in six genotypes, which are further divided into 32 genetic lineages, and provides reliable reference sequences (lineage prototypes) to guide the viral classification (Valastro et al., 2016).

Currently, Brazil is the main exporter and second-biggest producer of chicken meat in the world (USDA, 2016). In 2016, more than 13 million tons of chicken was produced and over 4 million tons of chicken products were exported to all continents. This high production is obtained in intensive raising systems, favoring the dissemination of respiratory infections (Bermudez, 2008) such as IBV, which causes great economic losses in Brazilian poultry flocks (broilers, breeders and layers) (Balestrin et al., 2014; Carranza et al., 2017; Colvero et al., 2015).

IB has been described in Brazil since the 1950s. However, a better genetic characterization of the field IBV isolates has started to be performed only in the last decade. It is known that a Brazilian variant (BR-I) is widely disseminated in the main poultry-producing regions of the country. Similar strains were also observed to circulate in Argentina and Uruguay and the whole genetic cluster was also identified as South America I (SA-I) (Marandino et al., 2015) and recently renamed as GI-11 (Valastro et al., 2016). Moreover, subclades of GI-11 were reported by distinct studies (Fraga et al., 2013; Villarreal et al., 2010) but the origin of these lineages and the role of recombination are still to be investigated.

Moreover, IBV molecular diversity in Brazil has been investigated by using diverse methods on different regions of S1 gene, making it difficult to compare results from different studies and impairing epidemiological surveillance efforts to track down IB outbreaks. In the view of the recently proposed system of IBV classification, the present study investigated the lineages circulating in Brazil and the role of recombination for the current observed genetic diversity. This study also applied phylodynamic methods to estimate the time of the most common recent ancestor and demographic history of the IBV field variants in Brazil and related sequences from South America.

2. Material and methods

2.1. Sequence dataset compilation and maximum likelihood (ML) analysis

All available IBV S1 gene sequences from Brazil were downloaded from GenBank. Alignment was performed with Mafft (Katoh and Standley, 2013) and visually inspected in AliView (Larsson, 2014). A reference sequence dataset for the genotypic classification of IBV was used as provided by Valastro et al. (2016). Due to different sizes in length and sequences that cover distinct and not overlapping regions of S1 gene, the genotyping of Brazilian sequences was performed in separate datasets when necessary.

To further analyze the global circulation of IBV strains isolated in Brazil, all international IBV S1 sequences with information for country and date of sampling were downloaded from GenBank. Alignment was performed with Mafft and trimmed aiming to keep the highest number of sequences from Brazil. RAxML (Stamatakis, 2014) was used to remove identical sequences and construct maximum likelihood (ML) trees. The general time reversible model (GTR) with gamma-distributed rate heterogeneity plus a proportion of invariable sites (GTR + G + I) was used as the optimal nucleotide substitution model as identified in the jModelTest program (Posada, 2008).

2.2. Recombination analyses

Analyses of recombination were performed for sequences grouped within the GI-11 lineage. The S1 gene fragment analyzed corresponds to nucleotide positions 8 to 550, according to the H120 reference strain (M21970 - accession code). Simplot software (Lole et al., 1999) was used applying the bootscanning method. Neighbor-Joining (NJ) trees were constructed under Kimura two-parameter model with sliding windows of 100, 160 and 200 base pairs (bp) with incremental steps of 20 bases. Query sequences were compared against reference sequences for each lineage defined by Valastro et al. (2016). S1 gene regions showing patterns of recombination were used as a query in a BLAST search to identify the source of potential recombination fragments. The top 10 hits for each query were downloaded and added to the sequence dataset in case they had not been yet analyzed.

2.3. Phylodynamic analyses

The temporal signal of the sequences to be submitted to phylodynamic analysis was investigated with TempEst software (Rambaut et al., 2016). Sequences outliers in the regression of root-to-tip divergence versus sampling time were excluded. Time-scaled phylogenetic tree estimation was performed using BEAST/BEAGLE software (Ayres et al., 2012; Drummond et al., 2012) through the Cipres Science Gateway (https://www.phylo.org). BEAST software allows for the combination of different clock, substitution, and demographic models, demanding an appropriate model test approach. In the current study, marginal likelihood estimation (MLE) (Baele et al., 2012, 2013) was applied to compare alternative models in a Bayesian framework. Trees were reconstructed using SRD06 substitution model (Shapiro et al., 2005) and the uncorrelated gamma distributed (ucgd) relaxed molecular clock (Drummond et al., 2006), which outperformed alternative models. IBV demographic history in Brazil was investigated by applying the non-parametric Bayesian Skygrid coalescent model, which estimates the product of viral effective population size (N_e) and generation time throughout evolutionary history (Gill et al., 2013). To avoid making assumptions regarding IBV generation time, here we refer to estimates of effective population size as relative genetic diversity. In addition, we tested with MLE the best demographic parameter that described the IBV population history.

Monte Carlo Markov Chains (MCMC) were run sufficiently long to ensure stationary and adequate effective sample size (ESS) for the main parameters. Tracer software (available at: http://beast.bio.ed.ac.uk/Tracer) was used to diagnose MCMC, adjust initial burn-in and to perform the Skygrid demographic reconstruction. TreeAnnotator was used to summarize the maximum clade credibility (MCC) tree from the posterior distribution of trees and the MCC tree was visualized and edited in FigTree (available at: http://tree.bio.ed.ac.uk/software/figtree/).

3. Results

3.1. The molecular diversity of IBV in Brazil

In order to classify the IBV lineages circulating in Brazil, a preliminary analysis was performed including all Brazilian IBV sequences available in GenBank and the reference dataset provided by Valastro et al. (2016). A total of 192 IBV Brazilian sequences for S1 gene was obtained. These sequences varied in size and covered different regions of S1 gene. A total of 140 sequences (73%) covered the first portion of S1 region, approximately between positions 190 and 700 (including hypervariable regions 1 and 2 located between nucleotides 112 and 423); 27 sequences (14%) covered the middle of the gene between positions 740 and 1090 (including hypervariable region 3 located between nucleotides 820 and 1161); and 25 sequences (13%) spanned the whole S1 gene. ML tree reconstruction using Valastro et al. (2016) reference sequences classified 143 (74.5%) Brazilian sequences as GI-11 lineage (former SA-I group), 46 (24%) as GI-1 (former Mass serotype), 2 (1%) as GI-13 (former 4/91 serotype) and 1 (0.5%) as GI-9 (former Ark serotype).