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Research paper

Phylodynamic analyses of Brazilian antigenic variants of infectious bursal disease virus

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ABSTRACT

Infectious bursal disease virus (IBDV) is a very important pathogen to poultry production and it is classified into three main groups: classical virulent (cvIBDV), very virulent (vvIBDV) and antigenic variants (avIBDV). This last group is composed by five different genetic lineages (recently classified in genogroups G2, G4, G5, G6, and G7) distributed in specific regions around the world. Brazil is one of the biggest poultry producers in the world and the present study aimed to investigate the evolutionary history of avIBDVs of the genogroup G4 in Brazil. A total of 5331 IBDV positive bursa samples, from different Brazilian poultry flocks, were genotyped in a period of ten years (2005 to 2014) and 1888 (35.42%) were identified as local avIBDVs. The highly variable region of the viral protein 2 (hv*vp2*) gene of 28 avIBDVs was sequenced and used in phylogenetic analyses and evaluation of local amino acid signatures. In addition, all complete and partial IBDV *vp2* 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 genogroups occurring in Brazil. Based on a Maximum Likelihood (ML) phylogenetic tree, all Brazilian avIBDVs grouped into the genogroup 4. Bayesian phylodynamics analysis demonstrated the ancestor virus of this group was probably introduced in South America in 1968 (1960 to 1974, 95% HPD) and in Brazil in 1974 (1968 to 1977, 95% HPD) and the most likely source was East Europe (Hungary or Poland). All Brazilian avIBDV sequences, as well as the other genogroup 4 sequences, showed a specific pattern of amino acid: S222, T272, P289, I290, and F296. This report brings new insights about the IBDV epidemiology in Brazil and South America.

1. Introduction

Gumboro disease is an immunosuppressive infection of chickens caused by *Infectious bursal disease virus* (IBDV) that causes considerable economic loss to the poultry industry worldwide. IBDV belongs to the family *Birnavirus*, genus *Avibirnavirus* and has a double-stranded RNA (dsRNA) genome with two segments (A and B) coding for five viral proteins (VPs). VP2 is the main capsid protein displayed at the most external surface of the IBDV particle (Mundt et al., 1995; Vakharia et al., 1994; Van den Berg, 2000). A hypervariable nucleotide region in *vp2* gene (hv*vp2*) codes for a highly variable segment in the VP2 peptide chain, between positions 212 to 324 of the amino acid residues (Bayliss et al., 1990). The high variation in this genomic region is responsible for the antigenic and/or pathogenic diversity among the IBDV strains (Coulibaly et al., 2005; Jackwood et al., 2008; Jackwood and Sommer-Wagner, 2011; Qi et al., 2013; De Haas et al., 2002; Yamaguchi et al., 1996; Schnitzler et al., 1993; van den Berg et al., 1996).

IBDV is divided into two serotypes (1 and 2), but only the first one is pathogenic to chickens (McFerran et al., 1980). According to antigenic and virulence properties, serotype 1 is further subdivided into three main groups: classical virulent (cvIBDV), antigenic variant (avIBDV) and very virulent (vvIBDV) (Eterradossi and Saif, 2008; Müller et al., 2003). A genogroup (G) classification based on a phylogenetic relationship of partial *vp2* gene was recently proposed and IBDV isolates

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were classified into 7 genogroups: G1 and G3 correspond to the cvIBDV and vvIBDV groups, respectively; G2, G4, G5, G6, and G7 are related to different avIBDV strains described around the world (Michel and Jackwood, 2017).

In Brazil, Gumboro disease was first detected in the 1970s (Nakano et al., 1972). The occurrence of vv isolates (which were genetically similar to vvIBDV European strains), and local avIBDV strains was reported in the 1990s and 2000s (Di Fábio et al., 1999a,b; Ikuta et al., 2001). Brazilian avIBDV and cvIBDV isolates were detected in flocks with mild immunosuppression and low mortality. On oppose, vvIBDV was detected in flocks with exacerbated typical signs of Gumboro disease (atrophied and even hemorrhagic bursas, acute nephrosis) and high mortality rates (Di Fábio et al., 1999a, 1999b; Ikuta et al., 2001). Vaccines against vvIBDV ("hot" and "intermediate plus") were released in 1999 and largely used to control severe Gumboro disease. Consequently, all these vaccine strains were widely disseminated in commercial Brazilian poultry flocks in the last decades (Bolis et al., 2003; Moraes et al., 2004).

In order to understand how the virus is spreading, phylogenetic and phylodynamic studies have been performed worldwide (Alfonso-Morales et al., 2013; Banda and Villegas, 2004; Cortey et al., 2012; Hon et al., 2006; Jackwood and Sommer-Wagner, 2007; Li et al., 2015; Vukea et al., 2014). Those studies have focused on the vvIBDV pathotype, due to the importance of the clinical disease caused by this group. Comparisons of the h*vp2* nucleotide and amino acid sequences suggest that viral strains from vvIBDV, isolated in different countries, are highly conserved and genetically clonal (Alfonso-Morales et al., 2013; Brown et al., 1994; Eterradossi et al., 1998; Hon et al., 2006; Rudd et al., 2002; Van den Berg, 2000; Yamaguchi et al., 1997). On the contrary, avIBDV strains present high diversity around the world and clusters with more similarity can be observed according to the geographical region of sample isolation (Ikuta et al., 2001; Jackwood and Sommer-Wagner, 2007; Lupini et al., 2016; Sapats and Ignjatovic, 2000). Studies about local avIBDVs are sparse and usually limited to North America. Only more recently the occurrence of a similar avIBDV group was reported in South America (Hernández et al., 2015; Silva et al., 2013; Vera et al., 2015). The most likely source of cv and vvIBDV are United States and Europe, respectively (Silva et al., 2013). However, data about avIBDV circulation and origin is still scarce in Brazil, the second largest poultry producer country in the world (USDA, 2018). The aim of this study was to characterize the molecular epidemiology of avIBDV in Brazilian commercial poultry flocks. By applying phylodynamics methods we also reconstructed the evolutionary history in time and space of the main avIBDV lineage circulating in Brazil.

2. Material and methods

2.1. IBDV detection and genotyping

A total of 5331 IBDV positive bursa samples, from 2005 to 2014, were analyzed. The samples were collected from broiler, breeder, and layer flocks from different farms in the main poultry-producing regions of Brazil.

Total RNA was extracted from macerated bursas using Prep and Preamp kits according to the manufacturer's protocol (NewGene®, Cachoeirinha, RS, Brazil). All the samples were submitted to IBDV genotyping by Nested-RT-PCR-RFLP procedure (Ikuta et al., 2001). According to the laboratory result, IBDVs were classified into the three main molecular groups: cvIBDV, vvIBDV, and avIBDV.

2.2. IBDV sequencing

To study the genetic diversity of avIBDV, 28 positive bursa samples previously identified as avIBDV group, were selected for sequencing. The h*vp2* was submitted to amplification by Nested-RT-PCR using primers F-IBDV-Seq1 (5′ - GTCCTCAGCYTACCCACATCATATG - 3′) and R-IBDV-Seq2 (5′ - GTTCAGGATTTGGRATCAGCTCG - 3′). Amplification was performed in a Veriti 96 Thermo Cycler (Applied Biosystems Inc., Norwalk, CT) with the following conditions: one cycle at 37 °C for 30 min and 35 cycles at 94 °C for 20 s, 50 °C for 40 s, and 72 °C for 1 min. A nested PCR was carried out with primers F-IBDV-Seq3 (5′ -- GGGTATGTGAGRCTYGGTGACCC - 3′) and R-IBDV-Seq4 (5′- CACCC-CRGCKACCGTAACGAC - 3′) resulting in a 579 bp amplified product. Thermal cycling was performed on the same equipment with the following steps: one cycle at 94 °C for 3 min, 35 cycles at 94 °C for 20 s, 55 °C for 40 s and 72 °C for 1 min, and a final extension cycle at 72 °C for 5 min. The PCR products were visualized after polyacrylamide gel electrophoresis stained with silver nitrate.

The amplified PCR products were sequenced using sense and antisense nested-PCR primers (F-IBDV-Seq3 and R-IBDV-Seq4) and BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems Inc., Norwalk, CT, USA). The sequencing rounds were performed in the thermocycler Veriti 96 (Applied Biosystems Inc., Norwalk, CT, USA) with the same conditions above. All samples were purified by ethanol/EDTA/sodium acetate protocol and the precipitated DNA products were diluted in formamide Hi-Di, denatured (95 °C for 2 min) and injected in the automated DNA sequencing ABI 3130 XL Genetic Analyzer (Applied Biosystems Inc., Norwalk, CT, USA). The nucleotide sequences from both strands were edited, assembled, and analyzed using the Bioedit software package (version 7.0.3.0. available at:). All IBDV sequences generated here were deposited under the GenBank accession numbers MH881013, MH881014, MH881017, MH881018, MH881019, MH881020, MH881022, MH881023, MH881024, MH881028, MH881029, MH881030, MH881031, MH881032, MH881033, MH881034, MH881036, and MH881037.

2.3. Selection of IBDV sequence datasets and phylogenetic analyses

All worldwide available serotype 1 IBDV *vp2* gene sequences with the information of sampling year and country of origin were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/). The avIBDV Brazilian sequences generated in this study were included in the sequence dataset, as well as the reference strains for the seven genogroups (Michel and Jackwood, 2017). According to the length of the sequences available for the *vp2* region, two datasets were created: 1) complete *vp2* gene sequences to perform recombination analysis; 2) a worldwide dataset with all sequences spanning the h*vp2* region to perform phylogenetic and phylodynamics analyses.

Both datasets were aligned with MUSCLE (Edgar, 2004a,b) and visually inspected with AliView (Larsson, 2014) in order to trim out the alignments and delete short sequences. In addition, all the identical sequences were removed. Aiming to identify avIBDV strains in the compiled dataset, a maximum likelihood (ML) phylogenetic tree was constructed with the h*vp2* region by using IQ-TREE program (Nguyen et al., 2014). Prior to the tree reconstruction, ModelFinder application (Kalyaanamoorthy et al., 2017), as implemented in IQ-TREE, was used to select the best-fitted model from the analyzed dataset. Branch support was calculated as the transfer bootstrap expectation (TBE) by applying Booster (Lemoine et al., 2018) to the bootstrap trees generated by IQ-TREE. Finally, trees were visualized and edited in FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

2.4. Recombination analysis

In order to identify possible recombinant isolates, sequences of complete well characterized *vp2* gene were selected as references while others were considered as queries. Recombinant sequences were detected with the Recombination Detection Program v4.72 (RDP4) (Martin et al., 2015). A first screening was performed using the bootscanning (Salminen et al., 1995; Martin et al., 2005) and distance plot (Felsenstein, 1993) methods, which were run manually, using a window size ranging 200 and 300 bp and a step size ranging 10 and

25 bp according to the query sequence, while other parameters were used as a default (Martin et al., 2015). For the positive recombinants, a second analysis was performed. Therefore, a new dataset with reference and query sequences was constructed. RDP (Martin and Rybicki, 2000), BootScan (Martin et al., 2005), MaxChi (Smith, 1992), Chimaera (Posada and Crandall, 2001) and 3Seq (Boni et al., 2007) methods were run automatically as a default (Martin et al., 2015). The Bootscan analysis used the same parameters as the first analysis. The RDP4 was configured to consider only recombinant signal detected by two or more complementary methods, plus a recombinant score equals or greater than 0.600. Analyses were conducted three times to ensure the repeatability of the results. In addition, the BLASTn program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to search the GenBank database for IBDV sequences that were homologous to Brazilian avIBDV strains.

2.5. Phylodynamic analyses

Bayesian phylodynamic analyses were performed with the Brazilian avIBDV cluster and sequences from other countries related to this cluster (G4), as identified in the ML tree. The temporal signal of this cluster 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 reconstruction was performed using BEAST/BEAGLE package v1.7.4 (Drummond et al., 2012; Ayres et al., 2011). Marginal likelihood estimation (MLE) (Baele et al., 2012, 2013) was applied to compare alternative site and clock models in a Bayesian framework. Trees were reconstructed using SRD06 substitution model (Shapiro et al., 2005) and the uncorrelated lognormal (ucld) relaxed molecular clock (Drummond et al., 2006), which outperformed alternative models. Also, the non-parametric Bayesian skygrid coalescent model was applied in the analyses (Gill et al., 2012) to reconstruct the demographic history of G4 avIBDV through time. Furthermore, to investigate the place of origin of the introduction of G4 avIBDV in Brazil, a Bayesian discrete phylogeographic analysis was performed where sequence's sampling locations were used as traits. A symmetrical model of location transitioning with the Bayesian stochastic search variable selection (BSSVS) procedure (Lemey et al., 2009) was applied as implemented in BEAST/BEAGLE package v1.7.4 (Drummond et al., 2012; Ayres et al., 2011).

Markov chain Monte Carlo (MCMC) was run sufficiently long to ensure stationary and adequate effective sample size (ESS) for all the parameters. Tracer software (available in BEAST package) was used to diagnose MCMC, adjust initial burn-in and summarize demographic reconstruction. The Maximum Clade Credibility (MCC) tree was selected from the posterior distribution of trees with TreeAnnotator software (available in BEAST package) and was visualized and edited in FigTree v1.4.3.

2.6. Nucleotide and amino acid analysis

IBDV hv*vp2* sequences obtained in the present study were compared to worldwide sequences (av, vv, and cvIBDV) retrieved from GenBank. Amino acid signatures of the Brazilian avIBDVs, mainly the residues in the hydrophilic peaks, were visually inspected in AliView (Larsson, 2014).

3. Results

3.1. IBDV genotyping and sequencing

From 5331 samples, a total of 2715 (50.93%) were identified as cvIBDV, 728 (13.65%) as vvIBDV and 1888 (35.42%) as Brazilian avIBDV, previously classified as GM15 and GM16 (Ikuta et al., 2001).

From those avIBDV samples, 28 were randomly selected to be sequenced. A 579 bp fragment was successfully obtained from all the samples. From these group, 18 non-identical hv*vp2* sequences were added to further analyses using hv*vp2* gene.

3.2. IBDV datasets

The complete *vp2* gene dataset contained 112 IBDV sequences (all of them from serotype 1) generated from bird samples collected between 1967 and 2014 in five continents: Africa (n = 7; 6.25%), Asia (n = 49; 43.75%), Europe (n = 10; 8.93%), North America (n = 15; 13.39%) and South America (n = 31; 27.68%) (see Supplementary Table 1 for details about this dataset).

The hv*vp2* region dataset was composed by 790 sequences (serotype 1) obtained from GenBank plus 18 non-identical avIBDV sequenced in this study. Those sequences ranged from 1965 to 2015 in six continents: Africa (n = 138; 17.08%), Asia (n = 227; 28.09%), Australia (n = 2; 0.25%), Europe (n = 59; 7.30%), North America (n = 244; 30.20%) and South America (n = 138; 17.08%). In South America, 33 (23.91%) sequences were from Argentina, 3 (2.18%) from Bolivia, 71 (51.45%) from Brazil, 7 (5.07%) from Colombia, 3 (2.18%) from Peru, 9 (6.52%) from Uruguay and 12 (8.69%) from Venezuela (see Supplementary Table 2 for details about this dataset). To compare this dataset with the classification into genogroups, 17 reference sequences (representing all 7 genogroups) were included (Michel and Jackwood, 2017).

3.3. Recombination analysis

The 112 complete *vp2* gene sequences of serotype 1 and one sequence of serotype 2, available at GenBank, were submitted to recombination analysis. It was observed at least seven potential recombinant isolates: AF165150 and AF165151 from South Korea; FJ842492 from Vietnam; GQ166972 from China; KT884452 from India; JN982257 and JN982273 from Brazil. These seven IBDVs presented evidence of cv and vvIBDV recombination. The breakpoints were identified in different positions along the *vp2* gene in the recombinant sequences. According to the position of the breakpoints, four patterns of recombination were found: three sequences showed the pattern vv/cv-1 (KT884452, AF165151, and AF165150), with breakpoints at positions 624 and 625; two sequences the pattern vv/cv-2 (JN982273 and JN982257), with breakpoints positions 604 and 606; one sequence cv/vv-1 (FJ842492), with a breakpoint at position 950; and one sequence the pattern cv/vv-2 (GQ166972), with breakpoint position 408 (see Supplementary Table 3 and Supplementary Material 1).

Another possible breakpoint can be visualized between position 40 and 100 in five sequences analyzed here (GQ166972, JN982257, JN982273, AF165150, and AF165151). However, no statistical support was provided by RDP4 tool for these breakpoints.

3.4. Maximum likelihood phylogenetic analyses

The hv*vp2* region (243 nucleotides long) was used to perform a phylogenetic analysis with a comprehensive dataset of 825 sequences, representative of the worldwide IBDV serotype 1 diversity. The general topology of this tree presented the seven distinct genogroup clusters of the hv*vp2* gene described previously (Fig. 1) (Michel and Jackwood, 2017). All reference sequences were classified into their respective genogroup (from 1 to 7). Most of IBDV sequences (n = 402; 49.75%) clustered into the largest vvIBDV clade (G3), while classical strains clustered into G1 (n = 65; 8.05%). The variant strains clustered into separate groups: G2 grouped sequences from different countries, but mostly of sequences were from United States (n = 241; 29.83%); G5 included only sequences from Mexico (n = 9; 1.11%); G6 included sequences from Italy (n = 3; 0.37%); G4 (n = 85; 10.52%) included all the Brazilian avIBDV sequences and was mainly composed of South American samples. Finally, G7, which should include Australian sequences according to Michel and Jackwood (2017), was composed only

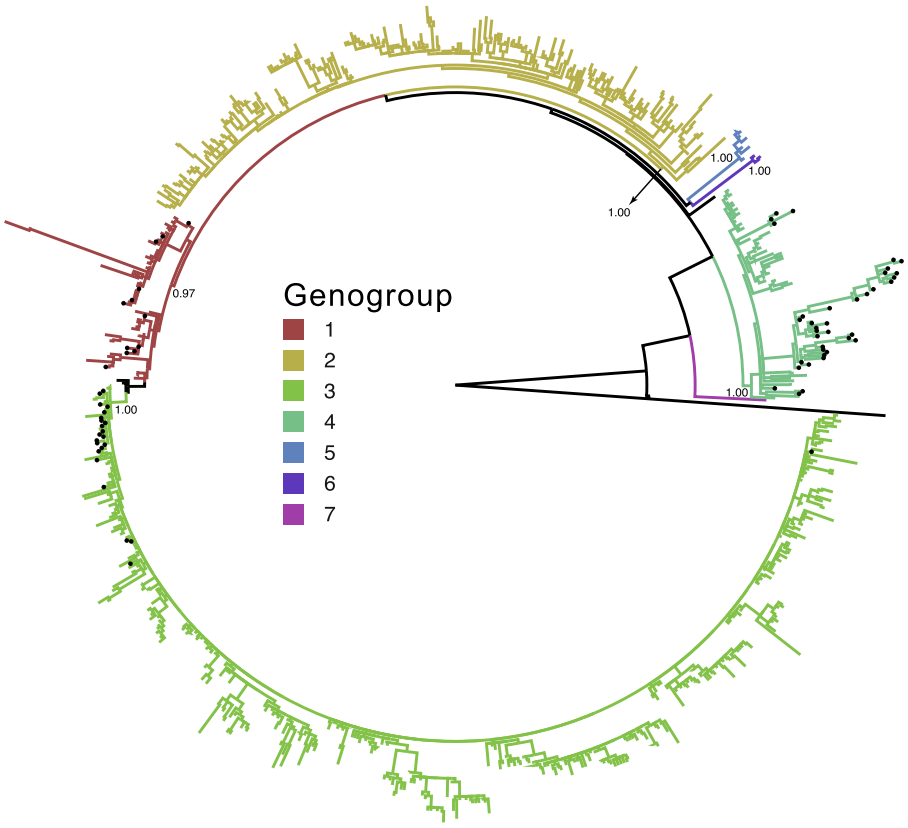

Fig. 1. Circular ML phylogenetic tree of IBDV partial hv*vp2* gene. Sequences from worldwide were selected based on the availability of date and country of sampling information. The analysis involved 825 sequences (serotype 1) of hv*vp2* gene. The classification was performed as proposed by Michel and Jackwood. (2017) by using reference sequences provided by the authors. Branch colors are according to the legend and black colored branches are non-classified sequences. Black dots in external nodes depict Brazilian sequences.

by its reference strain (Fig. 1).

Besides G4, Brazilian sequences were also classified into the genogroups 1 (n = 12) and 3 (n = 22). However, this number is not representative of the prevalence of the Brazilian isolates within each genogroup because identical sequences were removed before the phylogenetic analysis.

Interestingly, G4 presented two separate clusters: 1) only with Brazilian sequences (n = 30), here named G4a; and 2) with isolates predominantly from Argentina (n = 26), Uruguay (n = 5) and Brazil (n = 4), named G4b. This second cluster also included sequences from Hungary (n = 2), Poland (n = 2), Canada (n = 3) and Saudi Arabia (n = 2). Basally to this two groups several sequences from different countries were found: Canada (n = 4), South Korea (n = 2), Colombia (n = 1), Japan (n = 1) and Brazil (n = 3).

3.5. Bayesian phylogenetic analyses

The avIBDV G4 clade was analyzed in TempEst to evaluate the presence of a temporal signal. This procedure revealed that only the Brazilian sub-clade (G4a) had a clock-like behavior on viral phylogenetic reconstructions, showing a regression line R^2 equal to 0.41. The bayesian phylodynamic analysis was applied to this cluster and IBDV estimated rate of evolution was 3.08×10^{-3} substitutions/site/year (1.79 to 4.43×10^{-3}, 95% HPD). The time of the most recent common ancestor (tMRCA) for G4a was 1984 (1970 to 1990, 95% HPD) (Fig. 2). The demographic history of the avIBDV lineage circulating in the G4a clade was reconstructed by using the SkyGrid model. This analysis revealed an increasing population size (N_e) from the middle 1980s until the middle 1990s when the expansion slowed down. IBDV N_e was stable from the 2000s onward, with small fluctuations during this period (Fig. 3).

Temporal and spatial dynamics of the whole G4 was also investigated. Due to the low temporal signals of the whole clade, we used an informative prior on the tMRCA of the G4a clade. This prior was normally distributed with a mean (1984) and a standard deviation (7.3 years) based on the previous analysis of the G4a clade alone. This approach resulted in 1968 (1960 to 1974, 95% HPD) as the tMRCA for the whole G4 clade and in an estimated rate of evolution of 2.62×10^{-3} substitutions/site/year (1.82 to 3.51×10^{-3}, 95% HPD). The phylogeographic analysis estimated Hungary (posterior probability [pp] = 0.52) and Poland (pp = 0.24) as the most likely origins of the avIBDV lineage circulating in South America. In the beginning of the 1970s (1971, 1967 to 1976, 95% HPD) this strain was introduced in Argentina and again the most likely origin was Poland (pp = 0.52) and Hungary (pp = 0.29). From Argentina, the virus was introduced in Uruguay in the middle 2000s (2004, 2003 to 2008, 95% HPD).

In Brazil, avIBDV introduction happened in 1974 (1968 to 1977, 95% HPD), before the estimated genesis of the G4a clade, and the most likely origin also Poland (pp = 0.59) and Hungary (pp = 0.27). Our analysis also revealed a spread of South American avIBDV lineage to countries from other continents. Brazil was estimated as the origin (pp = 0.85) of an introduction into Canada and from Canada, it spread to South Korea, Japan, Saudi Arabia and Colombia from the middle of the 1980s onwards.

3.6. Amino acid hvVP2 signatures

Aiming to identify hvVP2 amino acid signatures of the Brazilian avIBDV, deduced amino acid from all genogroup sequences relative to positions 222 to 306 (Bayliss et al., 1990) were visually inspected in AliView (Larsson, 2014). Many critical residues that constitute some specific patterns for each cluster were detected and related to antigenicity, virulence and cell tropism. Those critical positions and the characteristic patterns are shown in Table 1.

The residues A222, I256, I294, which are characteristic of the vvIBDV (Banda and Villegas, 2004; Hoque et al., 2001), were found in the eight Brazilian field isolates belonging to vvIBDV (SB-6051, SB-7174, SB-9607, SB-9608, SB-7300, SB-1777, SB-3900 and SB-5074).

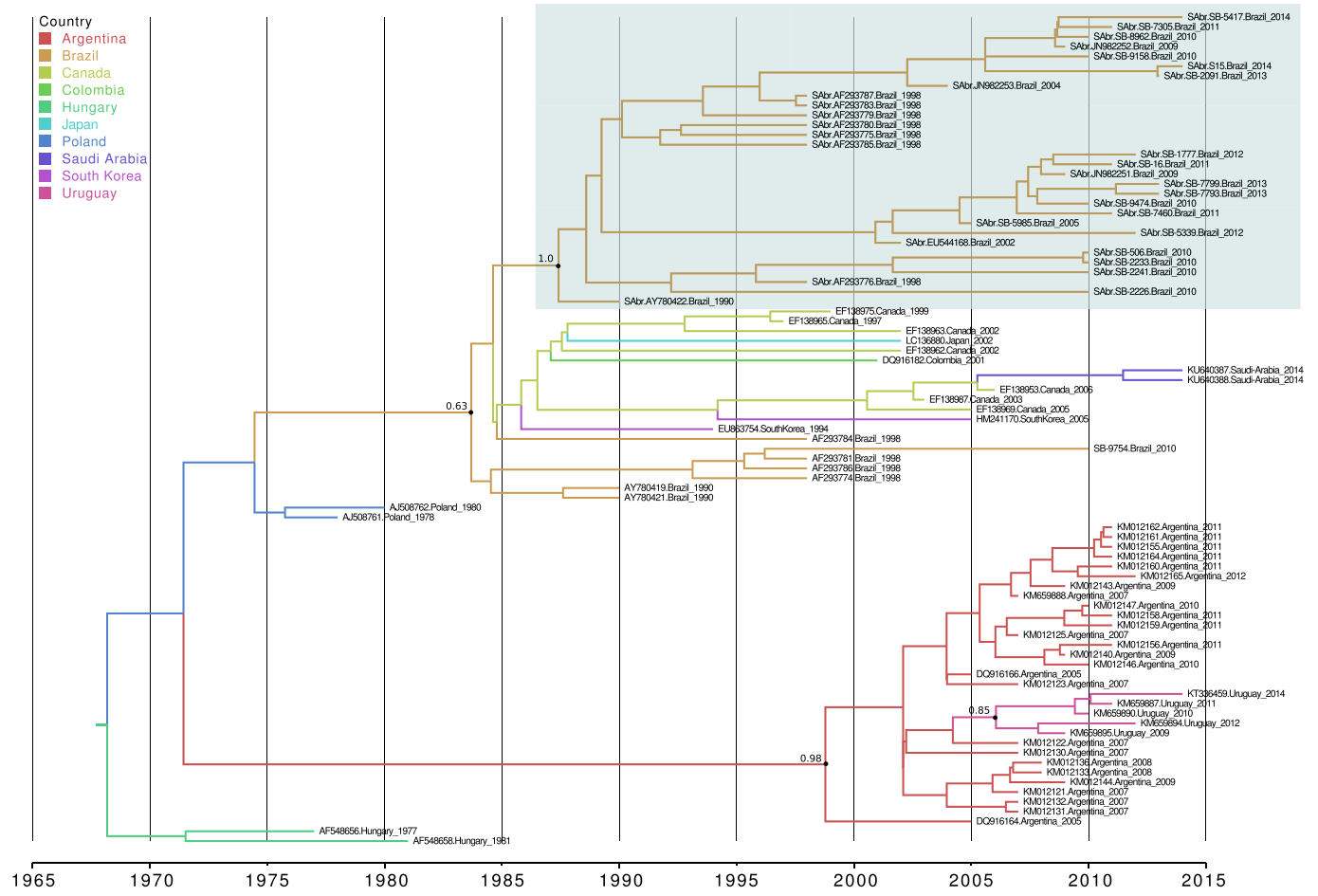

Fig. 2. Time scaled Bayesian phylogenetic tree of avIBDV G4 performed with h*vp2* sequences. Reconstruction of ancestral locations was performed by symmetric transition model with BSSVS. Branches of the tree are colored according to locations annotated to nodes following the legend. The sub-cluster G4a is highlighted in blue and key node supports (posterior probability) are shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Interestingly, all Brazilian vvIBDV strains have Asparagine (N) at position 299 instead of serine (S), like the cvIBDV strains. A comparison between cvIBDVs showed Brazilian sequences with Isoleucine (I) at residue 296 while other countries presented Asparagine (N). Nearly all avIBDV Brazilian sequences revealed five amino acid changes (S222, T272, P289, I290, and F296).

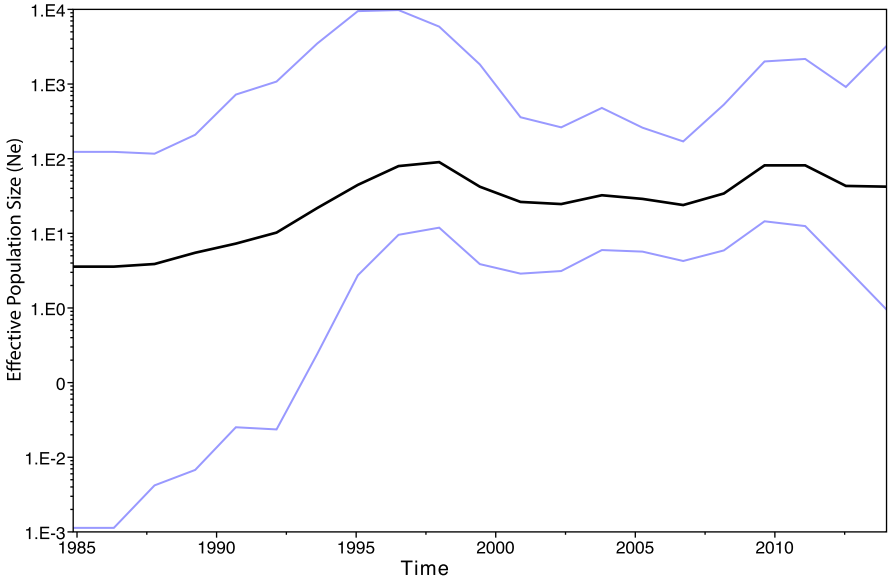

Fig. 3. Bayesian Skygrid plot of avIBDV G4a representing changes in virus effective population size (N_e) through time. Average N_e values are represented by the black line and the 95% HPD ranges are represented by the purple lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
The pattern of the amino acid corresponding to hvVP2 region, according to IBDV genotype.

	VP2 position																	
Genotype	222	242	245	249	253	254	256	270	272	275	279	280	284	289	290	294	296	299
vvIBDV W[a]	A	I	E	Q	Q	G	I	A	I	A	D	N	A	L	M	I	I	S
cvIBDV W[a]	P	V	E	Q	Q/H	G/S	V	A/T	I	A	N	N	A/T	L	M	L	N	N
vvIBDV BR[b]	A	I	E	Q	Q	G/A	I	A	I	A	D	N	A	L	M	I	I	N
cvIBDV BR[b]	P	V	E	Q	Q/H	G	V	A/T	I	A	N/D	N	A/T	L	L/M	L	I	N
NA avIBDV[c]	Q/T	V	E	K	Q/H	S/N	V	A	I	A	N	N	A/T	L	M	L	I	N
SA avIBDV[d]	S	V	G	Q	Q	D/N	V	T	T	A	N	N	A	P	I	L	F	N/S
avIBDV G4-BR1[e]	S	V	E	Q	Q/D	S/D/N	V	T	T	T	N	T	A	P	I	L	F	N
avIBDV G4-BR2[f]	S	V	G	Q	Q	D/N	V	T	T	A	N	N	A	P	I	L	F	N/S

[a] W Sequences from other countries of the world.
[b] BR Sequences from Brazil; vvIBDV: Very virulent IBDV (genogroup 3); cvIBDV: Classical IBDV (genogroup 1).
[c] NA avIBDV: North American Antigenic variant IBDV (genogroup 2).
[d] SA avIBDV: Antigenic variant IBDV from South America (genogroup 4).
[e] avIBDV G4a: Genogroup 4 Lineage a.
[f] avIBDV G4b: Genogroup 4 Lineage b.

4. Discussion

Gumboro disease is a very important issue in poultry production. All groups of IBDV (cv, vv, and av) have been reported in Brazilian flocks (Fernandes et al., 2009; Gomes et al., 2005; Ikuta et al., 2001; Silva et al., 2013), however, molecular and phylogenetic characterization have only focused on vvIBDV and cvIBDV (Gomes et al., 2005; Fernandes et al., 2009; Silva et al., 2013). This scenario probably occurs because classical strains are very common since they have been used as a live vaccine for decades. By its turn, very virulent strains are clinically relevant and disease caused by this group is very exacerbated in non-immunized flocks, causing important economic losses.

On the other hand, the clinical implications of the avIBDVs are not very well established. These strains caused immunosuppression signs, but not classical signs of Gumboro disease, which could be related to secondary infections. However, more definitive experimental trials confirming these findings are still needed. From 5331 samples analyzed in this study, a total of 1888 (35.42%) were classified as avIBDV. Distinct phylogenetic clusters have been reported in different regions around the world, highlighting the importance of the local viral lineages found in the field on the epidemiology of the Gumboro disease. Notably, all avIBDV Brazilian isolates were grouped into a specific cluster, named G4, which presented high similarity and have been detected since the 1990s, showing the occurrence of these genetic groups over the past three decades in Brazilian flocks.

Our ML tree complied the genogroup classification suggested by Michel and Jackwood (2017) with well-supported clades (above 0.9 TBE support). The exception was the G7, where Australian sequences did not group together with the G7 reference sequence. Interestingly, our phylogenetic analysis showed that G1 (cvIBDV) is basally located to G3 (vvIBDV) (Fig. 1), supporting the hypothesis that G3 was originated from G1, as already suggested previously (Van den Berg, 2000). However, the role of genetic reassortment among IBDV strains circulating in commercial and wild animals still needs to be assessed.

It has been suggested that the strong selection pressure generated by intensive vaccination of birds may lead to the emergence of viruses that are able to escape from neutralization antibodies provided by classical strains (van den Berg et al., 1991). The high mutation rate of RNA viruses and the strong selection pressure generated by intensive vaccination of birds can lead to the emergence of viruses with new properties allowing them to persist in immune competent populations (Domingo and Holland, 1997). Historically, these mutations have led to antigenic variation and modifications in virulence of circulating IBDV strains. The evolution of IBDV is a very complex process that happened around the world since the virus was described (Alkie and Rautenschlein, 2016). Identification and characterization of new IBDV isolates as soon as they appear is essential to understand the dynamics of virus evolution and consequently to effective control of the economic losses caused by IBDV infection in poultry-producing countries.

In such scenario, different IBDV genogroups have gradually emerged in the last decades. According to our results, G4 was introduced in South America in 1968 (1960 to 1974, 95% HPD) and the introduction of this strain in Brazil happened few years later (1974, 1968 to 1977, 95% HPD) (Fig. 2). The first report of IBDV in Brazil was made in the 1970s (Nakano et al., 1972) and since then it has been a big concern in the Brazilian poultry industry. Classical strain vaccines started to be used and still being one of the major measures to control the disease. After 1990 different studies started to describe outbreaks of IBDV strains from the genogroups 3 and 4 (Di Fábio et al., 1999a, 1999b; Fernandes et al., 2009; Gomes et al., 2005; Ikuta et al., 2001), exposing the need of new measures to control the economic losses caused by the virus. Our phylodynamics analysis using Skygrid to investigate the demographic history of a specific clade of G4 strain circulating in Brazil (here named G4a) revealed an increasing N_e from the middle 1980s until middle 1990s, and from this point onward followed a stabilization with small fluctuations (Fig. 3). This population dynamic might be explained by the control measures that followed the outbreaks reported in the 1990s, when “hot” vaccines were reintroduced in the field, allied to an improvement in biosecurity measures.

According to Silva et al. (2013), cv and vvIBDV strains were originated from The United States and The Netherlands, respectively, probably due to the importation of birds from these countries. The current study provides insights into the possible routes leading to the emergence and expansion of avIBDV G4 in South America. Our results revealed that the origin of this viral lineage is likely to be East Europe (Poland or Hungary according to our sampling). However, the circumstances of this IBDV lineage entrance in South America remains unclear. It has been hypothesized the entrance of the vvIBDV was by imported tulip bulbs (fertilized by contaminated chicken litter) from Netherlands (Di Fábio, 2002, apud Muniz et al., 2018). However other vehicles could also carry IBDV strains since the viral particle is very resistant in the outside environment (Eterradossi and Saif, 2008; Ingrao et al., 2013). The current study also revealed the spreading of the avIBDV G4 beyond South America, where Canada played a role disseminating it to Asia after receiving an introduced lineage from Brazil. Interesting to note that the diversity of avIBDV lineages in South America seems to follow a similar pattern that the one observed for IBV (*Infectious bronchitis virus*), where lineages from Brazil grouped apart from lineages from Argentina and Uruguay, the last two belonging to the same group (Fraga et al., 2018). Despite we have analyzed all partial h*vp2* IBDV sequences available in GenBank, it is important to note that our results on the phylogeographic analysis can be biased due

to limited sampling.

The av. Brazilian isolates contain some characteristic residues in VP2, for instance, the residues S222, T272, P289, I290, and F296. Those residues are also present in sequences from Argentina, Uruguay, Canada, Saudi Arabia, Poland, and Hungary (Domanska et al., 2004; Hernández et al., 2015; Ojkic et al., 2007; Remorini et al., 2006; Vera et al., 2015). Notably, the amino acid residues T/Q222, K249 and S254, which have been identified as being critical for the North American antigenic variant phenotype (Brandt et al., 2001), were also present in Brazilian sequences. Neutralizing antibodies have been shown to bind to VP2 within a restricted region, called the variable domain, between amino acids 206 and 350, which is highly hydrophobic but has short hydrophilic regions at each end (Bayliss et al., 1990).

Regardless of all the measures to control IBDV infection, outbreaks are frequently observed. Those outbreaks are due to failures in the vaccination programs or to the emergence of new strains that can break through the protection given by the classical vaccines. Therefore, the genetic characterization of the viruses responsible for recent and past outbreaks is necessary to evaluate the control programs, as well as to understand the epidemiology and evolution of IBDV (Hernández et al., 2015; Ito et al., 1990; Lasher and Davis, 1997; Ojkic et al., 2007; Sapats and Ignjatovic, 2000; Snyder, 1990).

5. Conclusions

The most common IBDV genogroup circulating in Brazil is the G4, which was characterized in this study. This lineage has spread in the last ~45 years in flocks of poultry production in the continent. Specifically, for Brazilian avIBDV sequences, a unique and conserved molecular signature at hvVP2 was observed: 222S, 272T, 289P, 290I, and 296F. The results presented here are also useful for understanding the regional dynamic of transmissions between countries and the overall evolutionary history of the virus.

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Data availability

All data retrieved, generated and presented in this article is available in the Supplementary material.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.04.020.

References

Alfonso-Morales, A., Martínez-Pérez, O., Dolz, R., Valle, R., Perera, C.L., Bertran, K., Frías, M.T., Majó, N., Ganges, L., Pérez, L.J., 2013. Spatiotemporal phylogenetic analysis and molecular characterization of infectious bursal disease viruses based on the VP2 hyper-variable region. PLoS One 8 (6), e65999. https://doi.org/10.1371/journal.pone.0065999.

Alkie, T.N., Rautenschlein, S., 2016. Infectious bursal disease virus in poultry: current status and future prospects. Vet. Med. 7, 9. https://doi.org/10.2147/VMRR.S68905.

Ayres, D.L., Darling, A., Zwickl, D.J., Beerli, P., Holder, M.T., Lewis, P.O., Huelsenbeck, J.P., Ronquist, F., Swofford, D.L., Cummings, M.P., Rambaut, A., Suchard, M.A., 2011. BEAGLE: an application programming interface and high-performance computing library for statistical phylogenetics. Syst. Biol. 61 (1), 170–173. https://doi.org/10.1093/sysbio/syr100.

Baele, G., Lemey, P., Bedford, T., Rambaut, A., Suchard, M.A., Alekseyenko, A.V., 2012. Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. Mol. Biol. Evol. 29 (9), 2157–2167. https://doi.org/10.1093/molbev/mss084.

Baele, G., Li, W.L.S., Drummond, A.J., Suchard, M.A., Lemey, P., 2013. Accurate model selection of relaxed molecular clocks in Bayesian phylogenetics. Mol. Biol. Evol. 30 (2), 239–243. https://doi.org/10.1093/molbev/mss243.

Banda, A., Villegas, P., 2004. Genetic characterization of very virulent infectious bursal disease viruses from Latin America. Avian Dis. 48 (3), 540–549. https://doi.org/10.1637/7157-12304R.

Bayliss, C.D., Spies, U., Shaw, K., Peters, R.W., Papageorgiou, A., Müller, H., Boursnell, M.E.G., 1990. A comparison of the sequences of segment A of four infectious bursal disease virus strains and identification of a variable region in VP2. J. Gen. Virol. 71 (6), 1303–1312. https://doi.org/10.1099/0022-1317-71-6-1303.

Bolis, D.A., Paganini, F.J., Simon, V.A., Zuanaze, M.F., Scanavini Neto, H., Correa, A.R.A., Ito, N.M.K., 2003. Gumboro disease: evaluation of serological and anatomopathological responses in vaccinated broiler chickens challenged with very virulent virus strain. Braz. J. Poult. Sci. 5 (2), 137–146. https://doi.org/10.1590/S1516-635X2003000200008.

Boni, M.F., Posada, D., Feldman, M.W., 2007. An exact nonparametric method for inferring mosaic structure in sequence triplets. Genetics. https://doi.org/10.1534/genetics.106.068874.

Brandt, M., Yao, K., Liu, M., Heckert, R.A., Vakharia, V.N., 2001. Molecular determinants of virulence, cell tropism, and pathogenic phenotype of infectious bursal disease virus. J. Virol. 75 (24), 11974–11982. https://doi.org/10.1128/JVI.75.24.11974-11982.2001.

Brown, M.D., Green, P., Skinner, M.A., 1994. VP2 sequences of recent European 'very virulent' isolates of infectious bursal disease virus are closely related to each other but are distinct from those of 'classical' strains. J. Gen. Virol. 75 (3), 675–680. https://doi.org/10.1099/0022-1317-75-3-675.

Cortey, M., Bertran, K., Toskano, J., Majó, N., Dolz, R., 2012. Phylogeographic distribution of very virulent infectious bursal disease virus isolates in the Iberian Peninsula. Avian Pathol. 41 (3), 277–284. https://doi.org/10.1080/03079457.2012.682562.

Coulibaly, F., Chevalier, C., Gutsche, I., Pous, J., Navaza, J., Bressanelli, S., Delmas, B., Rey, F.A., 2005. The birnavirus crystal structure reveals structural relationships among icosahedral viruses. Cell 120 (6), 761–772. https://doi.org/10.1016/j.cell.2005.01.009.

van Loon, A.A.W.M., De Haas, N., Zeyda, I., Mundt, E., 2002. Alteration of amino acids in VP2 of very virulent infectious bursal disease virus results in tissue culture adaptation and attenuation in chickens. J. Gen. Virol. 83 (1), 121–129. https://doi.org/10.1099/0022-1317-83-1-121.

Di Fabio, J., 2002. Laboratorios JF. Campinas. Personal communication.

Di Fábio, J., Castro, A.G.D., Gardin, A.Y., Rossini, L.I., Toquin, L.I.D., Eterradossi, N., 1999a. Very virulent IBD spreads to South America. World Poult. 15 (9).

Di Fábio, J., Rossini, L.I., Eterradossi, N., Toquin, M.D., Gardin, Y., 1999b. European-like pathogenic infectious bursal disease viruses in Brazil. Vet. Rec. 145 (7), 203.

Domanska, K., Mato, T., Rivallan, G., Smietanka, K., Minta, Z., De Boisseson, C., Toquin, D., Lomniczi, B., Palya, V., Eterradossi, N., 2004. Antigenic and genetic diversity of early European isolates of infectious bursal disease virus prior to the emergence of the very virulent viruses: early European epidemiology of infectious bursal disease virus revisited? Arch. Virol. 149 (3), 465–480. https://doi.org/10.1007/s00705-003-0230-6.

Domingo, E.J.J.H., Holland, J.J., 1997. RNA virus mutations and fitness for survival. Annu. Rev. Microbiol. 51 (1), 151–178. https://doi.org/10.1146/annurev.micro.51.1.151.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4 (5), e88. https://doi.org/10.1371/journal.pbio.0040088.

Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29 (8), 1969–1973. https://doi.org/10.1093/molbev/mss075.

Edgar, R.C., 2004a. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32 (5), 1792–1797. https://doi.org/10.1093/nar/gkh340.

Edgar, R.C., 2004b. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinf. 5 (1), 113. https://doi.org/10.1186/1471-2105-5-113.

Eterradossi, N., Saif, Y.M., 2008. Infectious bursal disease. In: Saif, Y.M. (Ed.), Diseases of Poultry, 12ª Ed. https://doi.org/10.1002/9781119421481.

Eterradossi, N., Arnauld, C., Toquin, D., Rivallan, G., 1998. Critical amino acid changes in VP2 variable domain are associated with typical and atypical antigenicity in very virulent infectious bursal disease viruses. Arch. Virol. 143 (8), 1627–1636. https://doi.org/10.1007/s007050050404.

Felsenstein, J., 1993. PHYLIP (Phylogeny Inference Package), Version 3.5 c.

Fernandes, M.J.B., Simoni, I.C., Vogel, M.G., Harakava, R., Rivas, E.B., Oliveira, M.B.C., Kanashiro, A.M.I., Tessari, E.N.C., Gama, N.M.S.Q., Arns, C.W., 2009. Molecular characterization of Brazilian infectious bursal disease virus isolated from 1997 to 2005. Avian Dis. 53 (3), 449–454. https://doi.org/10.1637/8531-112808-Reg.1.

Fraga, A.P., Gräf, T., Pereira, C.S., Ikuta, N., Fonseca, A.S.K., Lunge, V.R., 2018. Phylodynamic 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.

Gill, M.S., Lemey, P., Faria, N.R., Rambaut, A., Shapiro, B., Suchard, M.A., 2012. Improving Bayesian population dynamics inference: a coalescent-based model for multiple loci. Mol. Biol. Evol. 30 (3), 713–724. https://doi.org/10.1093/molbev/mss265.

Gomes, A.D., Abreu, J.T., Redondo, R.A.F., Martins, N.R.S., Resende, J.S., Resende, M., 2005. Genotyping of infectious bursal disease virus strains by restriction fragment length polymorphism analysis of the VP1, VP2, and VP3 genes. Avian Dis. 49 (4), 500–506. https://doi.org/10.1637/7351-030205R.1.

Hernández, M., Tomás, G., Marandino, A., Iraola, G., Maya, L., Mattion, N., Hernández, D., Villegas, P., Banda, A., Panzera, Y., Pérez, R., 2015. Genetic characterization of south American infectious bursal disease virus reveals the existence of a distinct worldwide-spread genetic lineage. Avian Pathol. 44 (3), 212–221. https://doi.org/10.1080/03079457.2015.1025696.

Hon, C., Lam, T., Drummond, A., Rambaut, A., Lee, Y., Yip, C., Zeng, F., Lam, P., Ng, P.T.W., Leung, F.C.C., 2006. Phylogenetic analysis reveals a correlation between the expansion of very virulent infectious bursal disease virus and reassortment of its genome segment B. J. Virol. 80 (17), 8503–8509. https://doi.org/10.1128/JVI.00585-06.

Hoque, M.M., Omar, A.R., Chong, L.K., Hair-Bejo, M., Aini, I., 2001. Pathogenicity of Ssp I-positive infectious bursal disease virus and molecular characterization of the VP2 hypervariable region. Avian Pathol. 30 (4), 369–380. https://doi.org/10.1080/03079450120066377.

Ikuta, N., El-Attrache, J., Villegas, P., Garcia, M., Lunge, V.R., Fonseca, A.S.K., Oliveira, C., Marques, E.K., 2001. Molecular characterization of Brazilian infectious bursal disease viruses. Avian Dis. 297–306. https://doi.org/10.2307/1592968.

Ingrao, F., Rauw, F., Lambrecht, B., van den Berg, T., 2013. Infectious Bursal disease: a complex host–pathogen interaction. Dev. Comp. Immunol. 41 (3), 429–438. https://doi.org/10.1016/j.dci.2013.03.017.

Ito, N.M.K., Noronha, A.M.B., Dagli, M.L.Z., Gaviolle, M.C., Rossini, L.I., Matsuguma, L.K., 1990. Doença infecciosa bursal: estudo de caso. Braz. J. Vet. Res. Anim. Sci. 27 (1), 99–110. https://doi.org/10.11606/issn.0000-0000.27199-110.

Jackwood, D.J., Sommer-Wagner, S., 2007. Genetic characteristics of infectious bursal disease viruses from four continents. Virology 365 (2), 369–375. https://doi.org/10.1016/j.virol.2007.03.046.

Jackwood, D.J., Sommer-Wagner, S.E., 2011. Amino acids contributing to antigenic drift in the infectious bursal disease Birnavirus (IBDV). Virology 409 (1), 33–37. https://doi.org/10.1016/j.virol.2010.09.030.

Jackwood, D.J., Sreedevi, B., LeFever, L.J., Sommer-Wagner, S.E., 2008. Studies on naturally occurring infectious bursal disease viruses suggest that a single amino acid substitution at position 253 in VP2 increases pathogenicity. Virology 377 (1), 110–116. https://doi.org/10.1016/j.virol.2008.04.018.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., Jermiin, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Methods 14 (6), 587. https://doi.org/10.1038/nmeth.4285.

Larsson, A., 2014. AliView: a fast and lightweight alignment viewer and editor for large datasets. Bioinformatics 30 (22), 3276–3278. https://doi.org/10.1093/bioinformatics/btu531.

Lasher, H.N., Davis, V.S., 1997. History of infectious Bursal disease in the USA: the first two decades. Avian Dis. 11–19. https://doi.org/10.2307/1592439.

Lemey, P., Rambaut, A., Drummond, A.J., Suchard, M.A., 2009. Bayesian phylogeography finds its roots. PLoS Comput. Biol. 5 (9), e1000520. https://doi.org/10.1371/journal.pcbi.1000520.

Lemoine, F., Entfellner, J.B.D., Wilkinson, E., Correia, D., Felipe, M.D., Oliveira, T., Gascuel, O., 2018. Renewing Felsenstein's phylogenetic bootstrap in the era of big data. Nature 556 (7702), 452. https://doi.org/10.1038/s41586-018-0043-0.

Li, Z., Qi, X., Ren, X., Cui, L., Wang, X., Zhu, P., 2015. Molecular characteristics and evolutionary analysis of a very virulent infectious bursal disease virus. Sci. China Life Sci. 58 (8), 731–738. https://doi.org/10.1007/s11427-015-4900-x.

Lupini, C., Giovanardi, D., Pesente, P., Bonci, M., Felice, V., Rossi, G., Morandini, E., Cecchinato, M., Catelli, E., 2016. A molecular epidemiology study based on VP2 gene sequences reveals that a new genotype of infectious bursal disease virus is dominantly prevalent in Italy. Avian Pathol. 45 (4), 458–464. https://doi.org/10.1080/03079457.2016.1165792.

Martin, D., Rybicki, E., 2000. RDP: detection of recombination amongst aligned sequences. Bioinformatics 16 (6), 562–563. https://doi.org/10.1093/bioinformatics/16.6.562.

Martin, D.P., Posada, D., Crandall, K.A., Williamson, C., 2005. A modified bootscan algorithm for automated identification of recombinant sequences and recombination breakpoints. AIDS Res. Hum. Retrovir. 21 (1), 98–102. https://doi.org/10.1089/aid.2005.21.98.

Martin, D.P., Murrell, B., Golden, M., Khoosal, A., Muhire, B., 2015. RDP4: detection and analysis of recombination patterns in virus genomes. Virus Evol. 1 (1). https://doi.org/10.1093/ve/vev003.

McFerran, J.B., McNulty, M.S., McKillop, E.R., Connor, T.J., McCracken, R.M., Collins, D.S., Allan, G.M., 1980. Isolation and serological studies with infectious bursal disease viruses from fowl, turkeys and ducks: demonstration of a second serotype. Avian Pathol. 9 (3), 395–404. https://doi.org/10.1080/03079458008418423.

Michel, L.O., Jackwood, D.J., 2017. Classification of infectious bursal disease virus into genogroups. Arch. Virol. 162 (12), 3661–3670. https://doi.org/10.1007/s00705-017-3500-4.

Moraes, H.L.S., Salle, C.T.P., Padilha, A.P., do Nascimento, V.P., G F de, S., Pereira, R.A., Artencio, J.O., de O Salle, F., 2004. Infectious bursal disease: evaluation of pathogenicity of commercial vaccines from Brazil in specific pathogen free chichens. Revista Brasileira de Ciência Avícola 6 (4), 243–247. https://doi.org/10.1590/S1516-635X2004000400009.

Müller, H., Islam, M.R., Raue, R., 2003. Research on infectious bursal disease—the past, the present and the future. Vet. Microbiol. 97 (1–2), 153–165. https://doi.org/10.1016/j.vetmic.2003.08.005.

Mundt, E., Beyer, J., Müller, H., 1995. Identification of a novel viral protein in infectious bursal disease virus-infected cells. J. Gen. Virol. 76 (2), 437–443. https://doi.org/10.1099/0022-1317-76-2-437.

Muniz, E.C., Verdi, R., Jackwood, D.J., Kuchpel, D., Resende, M.S., Mattos, J.C.Q., Cookson, K., 2018. Molecular epidemiologic survey of infectious bursal disease viruses in broiler farms raised under different vaccination programs. J. Appl. Poult. Res. 27 (2), 253–261. https://doi.org/10.3382/japr/pfx043.

Nakano, M.P., Masc, Saliba S., Nobre, D., Narimatsu, M.N., 1972. Ocorrência da "Doença de Gumboro" no Brasil. Diagnóstico Anátomo-Patológico. Biológico 38, 60–61.

Nguyen, L., Schmidt, H.A., von Haeseler, A., Minh, B.Q., 2014. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. 32 (1), 268–274. https://doi.org/10.1093/molbev/msu300.

Ojkic, D., Martin, E., Swinton, J., Binnington, B., Brash, M., 2007. Genotyping of Canadian field strains of infectious bursal disease virus. Avian Pathol. 36 (5), 427–433. https://doi.org/10.1080/03079450701598408.

Posada, D., Crandall, K.A., 2001. Evaluation of methods for detecting recombination from DNA sequences: computer simulations. Proceedings of the National Academy of Sciences 98 (24), 13757–13762. https://doi.org/10.1073/pnas.241370698.

Qi, X., Zhang, L., Chen, Y., L Gao, G. Wu, Qin, L., Wang, Y., Ren, X., Gao, Y., Gao, H., Wang, X., 2013. Mutations of residues 249 and 256 in VP2 are involved in the replication and virulence of infectious bursal disease virus. PLoS One 8 (7), e70982. https://doi.org/10.1371/journal.pone.0070982.

Rambaut, A., Lam, T.T., Carvalho, L.M., Pybus, O.G., 2016. Exploring the temporal structure of heterochronous sequences using TempEst (formerly Path-O-Gen). Virus Evol. 2 (1), vew007. https://doi.org/10.1093/ve/vew007.

Remorini, P., Calderón, M.G., Aguirre, S., Periolo, O., La Torre, J., Mattion, N., 2006. Characterization of infectious bursal disease viruses from Argentina. Avian Dis. 50 (2), 245–251. https://doi.org/10.1637/7447-092605R.1.

Rudd, M.F., Heine, H.G., Sapats, S.I., Parede, L., Ignjatovic, J., 2002. Characterisation of an Indonesian very virulent strain of infectious bursal disease virus. Arch. Virol. 147 (7), 1303–1322. https://doi.org/10.1007/s00705-002-0817-3.

Salminen, M.O., Carr, J.K., Burke, D.S., Mccutchan, F.E., 1995. Identification of breakpoints in intergenotypic recombinants of HIV type 1 by bootscanning. AIDS Res. Hum. Retrovir. 11 (11), 1423–1425. https://doi.org/10.1089/aid.1995.11.1423.

Sapats, S.I., Ignjatovic, J., 2000. Antigenic and sequence heterogeneity of infectious bursal disease virus strains isolated in Australia. Arch. Virol. 145 (4), 773–785. https://doi.org/10.1007/s00705005067.

Schnitzler, D., Bernstein, F., Müller, H., Becht, H., 1993. The genetic basis for the antigenicity of the VP2 protein of the infectious bursal disease virus. J. Gen. Virol. 74 (8), 1563–1571. https://doi.org/10.1099/0022-1317-74-8-1563.

Shapiro, B., Rambaut, A., Drummond, A.J., 2005. Choosing appropriate substitution models for the phylogenetic analysis of protein-coding sequences. Mol. Biol. Evol. 23 (1), 7–9. https://doi.org/10.1093/molbev/msj021.

Silva, F.M.F., Vidigal, P.M.P., Myrrha, L.W., Fietto, J.L.R., Silva Jr., A., Almeida, M.R., 2013. Tracking the molecular epidemiology of Brazilian infectious bursal disease virus (IBDV) isolates. Infect. Genet. Evol. 13, 18–26. https://doi.org/10.1016/j.meegid.2012.09.005.

Smith, J.M., 1992. Analyzing the mosaic structure of genes. J. Mol. Evol. 34 (2), 126–129. https://doi.org/10.1007/BF00182389.

Snyder, D.B., 1990. Changes in the field status of infectious bursal disease virus. Avian Pathol. 19 (3), 419–423. https://doi.org/10.1080/03079459008418695.

United States Department of Agriculture (USDA), Foreign Agricultural Service, 2018. Livestock and Poultry: World Markets and Trade. https://apps.fas.usda.gov/psdonline/circulars/livestock_poultry.pdf, Accessed date: 30 August 2018.

Vakharia, V.N., He, J., Ahamed, B., Snyder, D.B., 1994. Molecular basis of antigenic variation in infectious bursal disease virus. Virus Res. 31 (2), 265–273. https://doi.org/10.1016/0168-1702(94)90009-4.

Van den Berg, T., 2000. Acute infectious bursal disease in poultry: a review. Avian Pathol. 29 (3), 175–194. https://doi.org/10.1080/03079450050045431.

van den Berg, T.P., Gonze, M., Meulemans, G., 1991. Acute infectious bursal disease in poultry: isolation and characterisation of a highly virulent strain. Avian Pathol. 20 (1), 133–143. https://doi.org/10.1080/03079459108418748.

van den Berg, T.P., Gonze, M., Morales, D., Meulemans, G., 1996. Acute infectious bursal disease in poultry: immunological and molecular basis of antigenicity of a highly virulent strain. Avian Pathol. 25 (4), 751–768. https://doi.org/10.1080/03079459608419179.

Vera, F., Craig, M.I., Olivera, V., Rojas, F., König, G., Pereda, A., Vagnozzi, A., 2015. Molecular characterization of infectious bursal disease virus (IBDV) isolated in Argentina indicates a regional lineage. Arch. Virol. 160 (8), 1909–1921. https://doi.org/10.1007/s00705-015-2449-4.

Vukea, P.R., Willows-Munro, S., Horner, R.F., Coetzer, T.H.T., 2014. Phylogenetic analysis of the polyprotein coding region of an infectious south African bursal disease virus (IBDV) strain. Infect. Genet. Evol. 21, 279–286. https://doi.org/10.1016/j.meegid.2013.11.017.

Yamaguchi, T., Ogawa, M., Inoshima, Y., Miyoshi, M., Fukushi, H., Hirai, K., 1996. Identification of sequence changes responsible for the attenuation of highly virulent infectious bursal disease virus. Virology 223 (1), 219–223. https://doi.org/10.1006/viro.1996.0470.

Yamaguchi, T., Ogawa, M., Miyoshi, M., Inoshima, Y., Fukushi, H., Hirai, K., 1997. Sequence and phylogenetic analyses of highly virulent infectious bursal disease virus. Arch. Virol. 142 (7), 1441–1458. https://doi.org/10.1007/s007050050171.