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Genotyping of the infectious bursal disease virus (IBDV) based on the hypervariable region of the *vp2* gene in broiler chickens from the State of Rio de Janeiro, Brazil[1]

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ABSTRACT.- Godinho DAO, Lima PPABM, Paulino PG, Silva NM, Pimenta RL, Machado LS, Santos HA. **Genotyping of the infectious bursal disease virus (IBDV) based on the hypervariable region of the *vp2* gene in broiler chickens from the State of Rio de Janeiro, Brazil.** *Pesquisa Veterinária Brasileira 46:e07709, 2026.* Departamento de Epidemiologia e Saúde Pública, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Rodovia BR-465 Km 07, Seropedica, RJ 23890-000, Brazil. E-mail: huarrisson@yahoo.com.br

Infectious bursal disease virus (IBDV) causes immunosuppression in broilers by affecting the bursa of Fabricius. IBDV strains were classified into seven genogroups based on the amino acid composition of the hypervariable region of the VP2 capsid protein. This study aimed to detect and genotype IBDV strains in bursal samples from broilers in Rio de Janeiro and evaluate the genetic variability of the *vp2* gene. Sixty-five bursal tissue samples from broilers suspected to have IBD were collected from four farms in Rio de Janeiro and stored in RNAlater. RNA was extracted using TRIzol reagent, and genotyping was performed using RT-qPCR. The VP2 hypervariable region was amplified using conventional PCR, purified, and sequenced. Phylogenetic analysis was conducted using the maximum likelihood method with the Kimura 2-parameter substitution model. IBDV was detected in 75.38% (49/65) of the samples, with 8.16% (4/49) classified as very virulent (vvIBDV) and 91.84% (45/49) as non-vvIBDV strains. This study seems to be the first report of G-3 infection in the state of Rio de Janeiro. Sequence comparison identified multiple missense mutations, including 20 in genogroup 4 and eight in genogroup 3, relative to the reference strain Edgar-USA. The genetic distances among the isolates suggest potential viral evolution and adaptation. These findings highlight the genetic diversity of IBDV in this region and reinforce the importance of continuous molecular surveillance of the virus. The detection of vvIBDV in a previously unreported area underscores the need for monitoring strategies to mitigate the impact of emerging strains on poultry production. Further studies focusing on antigenic variation and vaccine efficacy are essential to ensure effective control of IBDV.

INDEX TERMS: Genotyping, infectious bursal disease virus (IBDV), broiler chickens, Rio de Janeiro.

RESUMO.- [**Genotipagem do vírus da doença infecciosa da bursa (VDIB) com base na região hipervariável do gene *vp2* em frangos de corte do estado do Rio de Janeiro, Brasil.**] O vírus da doença infecciosa da bursa (VDIB) causa imunossupressão em frangos de carne, afetando a bursa de Fabricius. As estirpes do VDIB foram classificadas em sete genogrupos com base na sua composição de aminoácidos na região hipervariável da proteína VP2 do capsídeo viral. O objetivo deste estudo foi detectar e realizar a genotipagem de cepas de VDIB em amostras de bursa de frangos de corte no Rio de Janeiro e avaliar a variabilidade genética do gene *vp2*. Sessenta e cinco amostras de tecido da bursa de Fabricius de frangos com suspeita de DIB foram coletadas em quatro granjas do Rio de Janeiro e armazenadas em RNAlater. O RNA foi extraído usando o reagente TRIzol, e a genotipagem foi realizada usando RT-qPCR. A região hipervariável da VP2 foi

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amplificada por PCR convencional, purificada e sequenciada. A análise filogenética foi efetuada utilizando o método da máxima verosimilhança com o modelo de substituição Kimura-2 parâmetros. O VDIB foi detectado em 75,38% (49/65) das amostras, com 8,16% (4/49) classificadas como muito virulentas (vvVDIB) e 91,84% (45/49) como estirpes não vvVDIB. Este estudo parece ser o primeiro relato de infeção por vvVDIB no estado do Rio de Janeiro. A comparação de sequências identificou múltiplas mutações de sentido trocado, incluindo 20 mutações no genogrupo 4 e oito no genogrupo 3 em relação à estirpe de referência Edgar-USA. As distâncias genéticas entre os isolados sugerem uma evolução potencial e adaptação viral. Estes resultados realçam a diversidade genética do VDIB nesta região e reforçam a importância da vigilância molecular contínua. A deteção do vvVDIB numa área anteriormente não registada sublinha a necessidade de estratégias de monitorização para mitigar o impacto das estirpes emergentes na produção avícola. São essenciais mais estudos centrados na variação antigênica e na eficácia da vacina para garantir medidas de controle eficazes contra o VDIB.

TERMOS DE INDEXAÇÃO: Genotipagem, vírus da doença infecciosa da bursa (VDIB), frango de corte, Rio de Janeiro.

INTRODUCTION

The Brazilian poultry industry has experienced significant modernization, driving advancements in production and helping Brazil achieve the status of the world's largest exporter of broiler meat in 2023, with an impressive 5.14 million tons sold (Bordin et al. 2021, ABPA 2024). To maintain leadership and meet the growing global demand, the sector has invested heavily in genetic improvement, nutrition, health, and management practices (Fukayama et al. 2005). One of the key pillars supporting this growth is the implementation of robust biosecurity programs aimed at safeguarding flock health and contributing to the emergence of a structured and competitive poultry industry. However, immunosuppressive pathogens that compromise chicken resistance to field challenges pose a significant threat to productivity, potentially increasing production costs (Tessari et al. 2000, Alves 2005). Among these pathogens, infectious bursal disease virus (IBDV), the causative agent of Gumboro disease, induces immunosuppression in broilers by triggering apoptosis in bursal cells, a critical site for the maturation and production of B lymphocytes (Kim et al. 1999, Withers et al. 2005, Zhou et al. 2010).

Only serotype 1 of IBDV causes immunosuppression, typically affecting broilers under three weeks of age. Infected birds exhibit bursal lesions such as atrophy, necrosis, and hemorrhage, along with pectoral muscle discoloration or hemorrhage (Sharma et al. 2000). IBDV is a non-enveloped virus with an icosahedral capsid and two double-stranded RNA genomic segments, A and B (Böttcher et al. 1997, Nagarajan & Kibenge 1997). Segment A encodes several proteins, including VP2, the main external protein of the viral capsid, which induces neutralizing antibodies, is linked to the host's immune response, and plays a central role in the molecular characterization of IBDV (Islam et al. 2021, Qiao et al. 2021).

Traditionally, IBDV strains have been categorized as classical (cvIBDV), variant (avIBDV), or very virulent (vvIBDV), based on their pathogenic and antigenic characteristics (Van den Berg et al. 2004). However, ongoing mutations, reassortment, and recombination events have led to the emergence of novel strains, complicating this classification. Consequently, IBDV is now grouped into seven genogroups based on amino acid variations in the hypervariable region of the VP2 capsid protein (hvVP2): classical (G-1), antigenic variant (G-2), virulent virus (G-3), distinct lineage (G-4), variant/classical recombinant (G-5), ITA (G-6), and Australian (G-7)(Michel & Jackwood 2017, Ali Khan et al. 2019). Recombination and rearrangement of the VP2 sequence can alter virulence and antigenicity (Fernández-Arias et al.1997, Rodríguez-Lecompte et al. 2005, Hon et al. 2008).

The classic IBDV strain was first identified in the USA in 1957. A variant strain emerged in Delaware in 1985, evading vaccine protection and spreading across North America and Australia. In 1989, a highly transmissible and virulent strain (G-3) emerged in Belgium and quickly spread across Europe, Asia, Africa, and South America (Zhang et al. 2022). IBDV isolates and local strains were reported in Brazil during the 1990s and the early 2000s, with the avIBDV genotype being the most prevalent. This has a significant impact on disease epidemiology and poses challenges for sanitary control (Fraga et al. 2019, Tomás et al. 2020).

To better understand the circulating IBDV strains in Brazil, several studies have investigated the genetic diversity of the VP2 protein across different states, including Minas Gerais, São Paulo, Paraná, Goiás, Mato Grosso, and Santa Catarina. These studies have reported the presence of classical (cv), very virulent (vv), and attenuated (av) IBDV strains circulating throughout the country (Paula et al. 2004, Gomes et al. 2005, Fernandes et al. 2009, Silva et al. 2013, Fraga et al. 2019). Notably, the presence of vvIBDV has been reported in the states of Goiás (Paula et al. 2004), São Paulo, Paraná (Fernandes et al. 2009), and Santa Catarina (Silva et al. 2013). To date, no similar studies have been conducted in the state of Rio de Janeiro.

The poultry industry has invested heavily in biosecurity and vaccination programs to minimize the impact of infectious diseases. However, the circulation of different IBDV variants, including highly virulent strains, poses a constant challenge to effective disease control. In this context, this study aimed to detect and genetically classify IBDV in bursa samples and evaluate the molecular variability of VP2 in strains found in broiler chickens in the state of Rio de Janeiro.

MATERIALS AND METHODS

Ethical approval. The animal experimental protocol was approved by the Ethics Committee on Animal Use of the "Universidade Federal Rural do Rio de Janeiro" (CEUA/UFRRJ), under protocol number CEUA 2729280316 (ID 000366).

Samples. Sixty-five bursa of Fabricius samples from broilers (*Gallus gallus*) suspected of having IBDV were collected from four farms in the state of Rio de Janeiro. Samples were collected from June to December 2016. The broilers were vaccinated subcutaneously on day 1 with Transmune (Ceva®) and Vaxxitek (Merial®) (Table 1). Four flocks of Cobb broilers strain showing clinical signs such as anorexia, ruffled feathers, tremors, watery diarrhea, and prostration were slaughtered at 37 to 42 days of age, weighing 1.9 to 2.5 kg. After euthanasia, the birds were necropsied, and bursa samples with macroscopic alterations, such as increased size, color changes, hemorrhagic spots, and/or edema, were selected.

Each sample, weighing approximately 100 mg, was stored in RNAlater Stabilization Solution (Thermo Fisher Scientific, Waltham/MA, USA) at -80 °C until RNA extraction.

RNA extraction. Total RNA was extracted using TRIzol reagent (Thermo Fisher Scientific, Waltham/MA, USA). The samples were thawed slowly, washed twice with DEPC-treated water, and macerated using liquid nitrogen. Next, 1.0 mL of TRIzol was added to each sample, and the samples were incubated at room temperature for 7 min. Subsequently, 200 μL of chloroform was added, followed by vortexing for 15 s, a 3-minute incubation at room temperature, and centrifugation at 12,000 × g for 15 min at 4 °C. After centrifugation, 450 μL of the aqueous phase was collected and mixed with 500 μL of isopropanol. The mixture was gently inverted, incubated at room temperature for 10 min, and centrifuged at 12,000 × g for 10 min at 4 °C. The supernatant was discarded, and the pellet was washed with 950 μL of 75% ethanol (in DEPC-treated water) and centrifuged at 7,400 × g for 5 min at 4 °C. After removing the supernatant, the tubes were inverted and incubated at room temperature for ~45 min. The pellet was resuspended in 100 μL diethyl pyrocarbonate (DEPC)-treated water. RNA concentration and purity were assessed using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham/MA, USA), and RNA integrity was evaluated by electrophoresis on a 1% agarose gel.

Molecular assay. Reverse transcription was performed using the High-Capacity RNA-to-cDNA Kit (Thermo Fisher Scientific, Waltham/MA, USA) according to the manufacturer's instructions. The 20 μL reaction mix contained 1 μL of 20X Enzyme Mix (MuLV and RNase inhibitor), 4 μL of 2X RT Buffer Mix (including dNTPs, random octamers, and oligo dT-16), and 1 μg of total RNA (4 μL of RNA sample). The reactions were performed in a Veriti® thermocycler (Thermo Fisher Scientific, Waltham/MA, USA) under the following conditions: 37 °C for 60 min, followed by 95 °C for 5 min. The resulting cDNA was used for reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and PCR analyses.

All samples were analyzed using PCR targeting the broiler cytochrome B (*cytB*) gene as an internal control. The PCR reaction was performed in a final volume of 25 μL, which included 1X AmpliTaq Gold™ 360 Master Mix (Thermo Fisher Scientific, Waltham/MA, USA), 600 nM *cytB* primers, and 10 ng/μL cDNA (Vilela et al. 2024). The thermocycling conditions were as follows: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 57 °C for 30 s, and 72 °C for 30 s.

RT-qPCR was used to detect and differentiate very virulent IBDV strains (vv-IBDV) from non-vvIBDV strains (classic and variant) (Tomás et al. 2012). The assay employs two minor groove binding (MGB) probes targeting a single nucleotide polymorphism (SNP) within a highly conserved genomic region. Each reaction, with a final volume of 12 μL, contained 0.9 μM of primers F178 and R272, 0.2 μM of MGB probes Pv and Pn (Table 2), 6 μL of TaqMan® Genotyping Master Mix (Thermo Fisher Scientific, Waltham/MA, USA), and 30 ng/μL cDNA. Reactions were performed on a StepOnePlus instrument (Applied Biosystems) under the following thermocycling conditions: 5-minute incubation at 50 °C, followed by 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C, ending with a final step at 70 °C for 5 min.

The very virulent IBDV strains detected by qPCR were amplified using PCR targeting the hypervariable region of the *vp2* gene (nucleotides 587–1229), as described by Liu et al. (1998). Each reaction, with a final volume of 25 μL, contained 1x PCR buffer, 3 mM $MgCl_2$, 0.4 mM dNTPs, 0.5 μM primers (Table 2), 1.5 U of Platinum Taq DNA polymerase, and 60 ng/μL of cDNA. Amplification was performed in an Eppendorf thermocycler (Eppendorf, Hamburg, Germany) under the following conditions: initial denaturation at 95 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 3 min.

Amplified products were subjected to 2% agarose gel electrophoresis. Electrophoresis was conducted at 110 V for 60 min in Tris-acetic acid-EDTA (TAE) buffer. The gels were stained with 0.5 μg/ml ethidium bromide (SIGMA) and visualized under ultraviolet light; photographs were taken with a L-Pix Touch equipment (Loccus Biotecnologia, São Paulo, BR).

The attenuated vaccine strain IBDV GBV-8 (Biovet®) was used as a positive control for both reactions. Ultrapure water (Thermo Fisher Scientific, Waltham/MA, USA) was used as a negative control during sample processing to prevent cross-contamination during qPCR and PCR. DNA extraction, PCR reagent preparation, DNA addition to the mix, and electrophoresis were conducted in separate rooms, following a unidirectional workflow to minimize contamination risk.

Amplicon purification and sequencing. The PCR products were purified using ExoSAP-IT™ PCR Product Cleanup (Thermo Fisher Scientific, Waltham/MA, USA) and sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). Sequencing was performed bidirectionally using the same primers as those used for RT-PCR (Table 2). Sequencing was performed at the "*Centro de Estudos do Genoma Humano* e Células-Tronco" (Human Genome Studies Center) of the "Universidade de São Paulo" (USP) using an ABI 3730 DNA Analyzer (Applied Biosystems, Perkin Elmer, CA, USA).

Phylogenetic analyses. The complete VP2 protein-coding genomic sequences available in the GenBank database[4] were selected for phylogenetic analysis and were aligned with the sequences obtained in the present study using the ClustalW program (Thompson et al. 1994). Nucleotide sequences were translated into amino acids using CLC Main Workbench v.7.9 software. Polypeptide sequences from the hypervariable region of VP2 were used for viral lineage characterization.

Phylogenetic analysis was performed using the MEGAX program (Kumar et al. 2018), employing the Kimura 2-parameter (K2P) substitution model with gamma distribution (two rate categories), using the maximum likelihood method and 1000 bootstrap repetitions to assess the robustness of the generated trees.

Table 1. Identification of poultry farms evaluated in the study, number of bursa of Fabricius samples collected per farm, and type of vaccination program used against infectious bursal disease virus (IBDV) in the State of Rio de Janeiro

Farms	Samples of the bursa of Fabricius	Vaccination
A	20	Transmune (Ceva®)
B	20	Transmune (Ceva®)
C	10	Transmune (Ceva®)
D	15	Vaxxitek

Table 2. Primers and probes sequences used in the study and their binding positions on the reference sequence

Primer/Probe	Sequence 5'–3'	Position
F178	GAG CCT TCT GAT GCC AAC AAC	178-198
R272	TCA AAT TGT AGG TCG AGG TCT CTGA	272-248
Pv	FAM-ACA CCC TAG AGA GAA GC-MGB	222-236
PN	VIC-ACA CCC TGG AGA AGC-MGB	222-236
VP2F	TCA CCG TCC TCA GCT TAC	587-604
VP2R	TCA GGA TTT GGG ATC AGC	1212-1229

F = Forward, R = reverse.

[4] Acessed on June 16, 2025. http://www.ncbi.nlm.nih.gov/Genbank/

The obtained sequences were deposited in the GenBank database and subsequently used to construct a phylogenetic tree alongside strains previously available in GenBank (Table S1) (Ali Khan et al. 2019).

RESULTS

A total of 75.38 % (49/65) of the samples collected in the state of Rio de Janeiro tested positive for IBDV. Among these, 8.16 % (4/49) were classified as very virulent (vvIBDV), whereas 91.84 % (45/49) were identified as non-vvIBDV according to qPCR analysis. Four vvIBDV-positive and eight non-vvIBDV-positive samples were successfully amplified by RT-PCR targeting the *vp*2 gene, generating 642 bp fragments.

Sequencing showed that the four vvIBDV isolates shared identical VP2 sequences, whereas sequence variations were observed among the non-vvIBDV isolates. Five representative VP2 sequences were deposited in GenBank under accession numbers PQ415647–PQ415651; one amplicon was excluded because of low-quality chromatograms.

Phylogenetic analysis was performed using the newly obtained sequences and 69 reference sequences from the GenBank database. Four sequences (PQ415647, PQ415348, PQ415649, and PQ415650) clustered within genogroup 4 (G-4), whereas PQ415651 was grouped within genogroup 3 (G-3) (Fig. 1). Within G-4, PQ415348, PQ415649, and PQ415650 were 100 % identical, whereas PQ415647 shared 99.81 % identity with the other G-4 representatives.

The G-3 isolate PQ415651 (Brazil-RJ) exhibited a genetic distance of approximately 6% from the classical IBDV strain Edgar-USA (A33255; G-1). This strain also showed a 11.65% genetic distance from the G-4 isolate PQ415647-50. Its closest

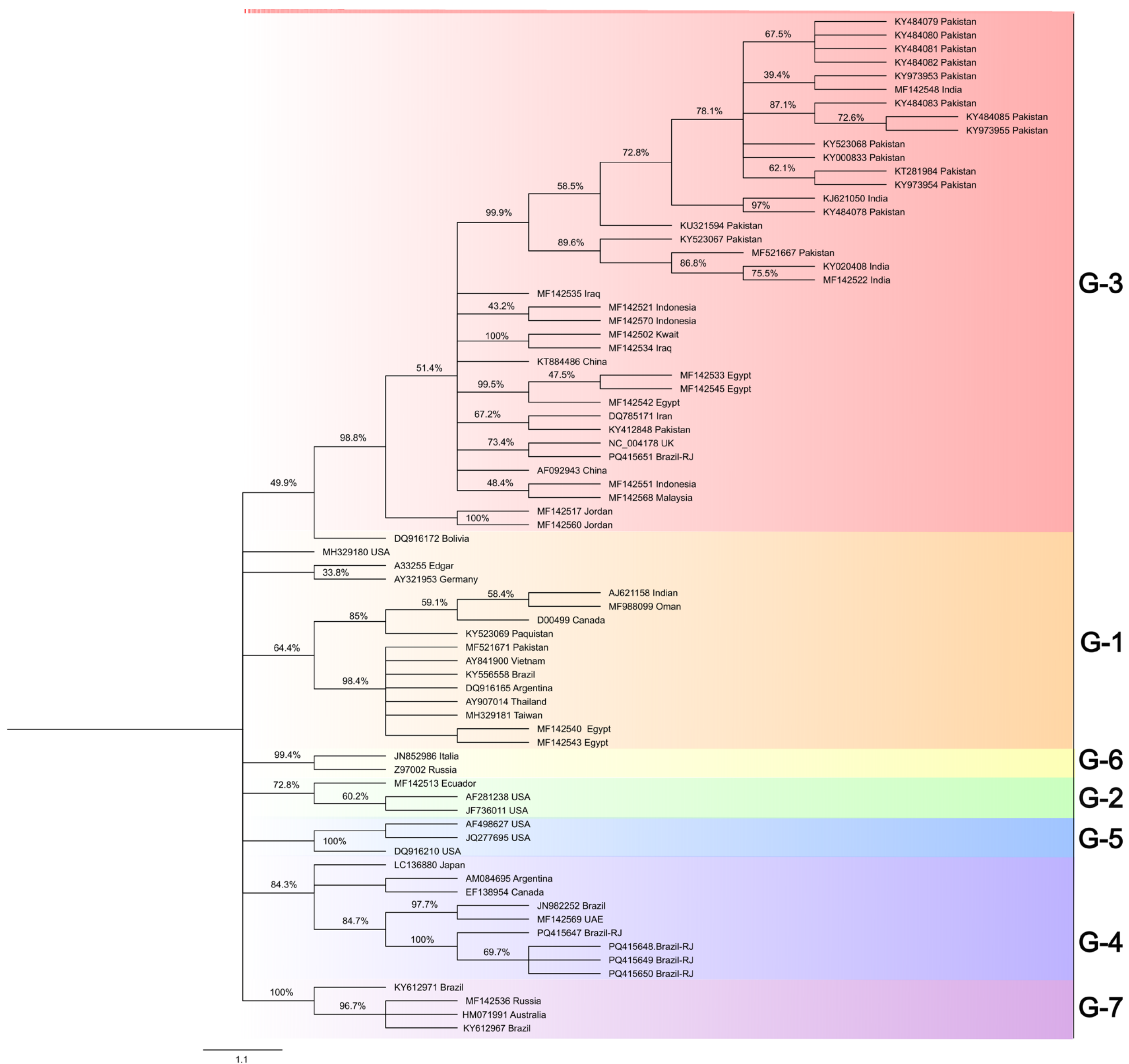

Fig. 1. Phylogenetic analysis of the infectious bursal disease virus (IBDV) in broiler chickens from the state of Rio de Janeiro, Brazil. The phylogenetic tree was constructed with the MEGA X software, employing the Kimura 2-parameter (K2P) substitution model with gamma distribution, using the maximum likelihood method and 1,000 bootstrap replicates to assess the robustness of the clusters. The tree was unrooted.

relative was the UK strain NC_004178 (G-3), with a genetic distance of only 0.38%. When compared with Brazilian sequences included in the phylogenetic analysis, the G-1 strain KY556558 had a 6% distance from PQ415651, and the G-4 strain JN982252 showed a 10% distance. G-7 strains (KY612671 and KY612967) were more distant, with 15% and 13.78% divergence, respectively.

The Edgar-USA strain exhibited 8.95% genetic divergence from the G-4 samples of this study (PQ415647, PQ415348, PQ415649, and PQ415650). Within G-4, the Brazilian strain JN982252 differed by 7%, whereas the Brazilian G-1 strain KY556558 differed by 9%.

Because G-3 is broadly associated with very virulent IBDV (vvIBDV), the G-3 isolate PQ415651 was further compared with the Korean vvIBDV strain (AF508177) and classical Edgar-USA strain (A33255) (Table 3). The PQ415651 strain exhibited eight missense substitutions in VP2 relative to the classical Edgar-USA strain reference. Notably, it carried classical vvIBDV-associated amino acid signatures within the VP2 hypervariable region — 222A, 256I, 294I, and 299S — as described for vvIBDV by Aliyu et al. (2021) and Mosad et al. (2024). The combined presence of these molecular markers, together with its G-3 phylogenetic placement, supports the classification of PQ415651 as vvIBDV.

In contrast, the G-4 isolates displayed 20 missense substitutions compared with the classical Edgar strain (Table 4); however, they did not exhibit the hallmark vvIBDV VP2 molecular signatures nor cluster with highly virulent lineages. Therefore, these isolates were designated as non-vvIBDV isolates. In total, 61 nucleotide substitutions were detected across the sequenced samples, none of which resulted in amino acid changes, indicating that they represented silent mutations.

DISCUSSION

Infectious bursal disease (IBD) primarily affects the bursa of Fabricius, leading to acute mortality or immunosuppression in flocks during the early weeks of life (Yilmaz et al. 2019). As the world's second-largest broiler meat producer and leading exporter, Brazil has a critical need for epidemiological surveillance of immunosuppressive diseases, including IBDV, to safeguard poultry health (Tomás et al. 2019, ABPA 2024). The prevention of IBDV infection is primarily accomplished through biosecurity measures and prophylactic vaccination on poultry farms. However, the emergence of new strains in several countries has led to immunosuppression, even in vaccinated herds (Pikuła et al. 2023).

From 2011 to 2014, a study of 246 bursa samples from broilers aged 25 to 35 days, collected across Brazil's five macro-regions, found a 30% (75/246) prevalence of IBDV, including field and vaccine strains, through molecular analysis of the *vp2* gene (Muniz et al. 2018). Of these, 25% (19/75) were vvIBDV strains, 27% (20/75) were variant strains, and the remaining samples were classified as vaccine strains, highlighting the circulation of diverse IBDV strains across Brazil. Specifically, 14.66% (11/75) of the positive samples originated from southeastern Brazil (excluding Rio de Janeiro), of which 18% (2/11) were vvIBDV strains, 27% (3/11) were variants, and the remainder were vaccine strains. In contrast, the present study identified an 8.16% (4/49) prevalence of vvIBDV in 49 broiler bursa samples from Rio de Janeiro, representing the first detection of vvIBDV in this state. Silva et al. (2013) previously reported vvIBDV positivity rates of 25% (4/29) in Minas Gerais and 63.63% (11/29) in São Paulo in broiler farms from 1997 to 2009.

The circulation of vvIBDV in Brazil draws special attention to the potential of the virus for genetic rearrangements and recombination, which can modify its virulence, antigenicity, and pathogenicity (Hon et al. 2006, Jackwood et al. 2007, Sreedevi et al. 2007, Jackwood et al. 2008, Li et al. 2009, Pikuła et al. 2018, Molini et al. 2019). The co-circulation of different IBDV genogroups (G-3 and G-4) in the same region could increase the likelihood of genetic reassortment, potentially leading to the emergence of new strains with unpredictable biological properties. Several studies have tracked the distribution and genetic variation of IBDV since the 1970s to understand its

Table 3. Alignment of amino acid sequences in the hypervariable region of VP2 between the G-3 sample and reference strains Koren vvIBDV (GenBank: AF508177) and Edgar (classical IBDV)

	Position in the alignment																								
Lineage	5	124	213	222	242	249	251	254	256	270	272	275	279	280	286	289	290	294	299	305	318	321	323	359	451
AF508177	Q	I	D	A	I	Q	S	G	I	A	I	A	D	N	T	L	M	I	S	I	G	A	D	T	L
Edgar	T	V	*	P	*	*	*	*	V	T	*	*	*	*	*	*	*	L	N	*	*	*	*	*	I
Sample PQ415651	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

vvIBDV = Very virulent infectious bursal disease virus; Q = glutamina, I = isoleucine, D = aspartate, A = alanine, S = serine, G = glycine, N = asparagine, T = threonine, L = leucine, M = methionine, V = valine, P = proline, F = phenylalanine, K = lysine; * Represents the same amino acid residue.

Table 4. Alignment of amino acid sequences in the VP2 hypervariable region, comparing G-4 study samples with the Edgar reference strain (classical IBDV), highlighting sequence similarities and differences

	Position in the alignment																								
Lineage	5	124	213	222	242	251	254	256	270	272	275	278	279	280	289	290	294	296	299	305	318	321	323	359	451
Edgar	T	V	D	P	I	S	G	V	T	I	A	S	D	N	L	M	L	I	N	I	G	A	D	T	I
Sample PQ415647-50	Q	I	N	S	V	N	S	*	*	T	T	A	A	T	P	I	*	F	*	V	*	T	E	K	L

IBDV = Infectious bursal disease virus; T = threonine, V = valine, D = aspartate, P = proline, I = isoleucine, S = serine, G = glycine, A = alanine, N = asparagine, L = leucine, M = methionine, Q = glutamina, F = phenylalanine, K = lysine; * Represents the same amino acid residue.

impact on the poultry industry (Nakano et al. 1972, Saukas et al. 1978, Jackwood & Nielsen 1997). The VP2 hypervariable region (aa 206–350) has been widely used for typing IBDV strains because it encodes antigenic and virulence determinants (Sahithi et al. 2019). According to Michel & Jackwood (2017), IBDV comprises seven major genogroups, some of which are geographically restricted and others globally dispersed. In this classification, G-1 corresponds to cvIBDV, G-3 to vvIBDV, and genogroups G-2, G-4, G-5, G-6, and G-7 to different avIBDV strains.

Fraga et al. (2019) evaluated 5,331 positive bursa samples collected from broiler, breeder, and layer flocks in Brazil, finding 50.93% (2,715/5,331) cvIBDV strains, 35.42% (1,888/5,331) avIBDV strains, and 13.65% (728/5,331) as vvIBDV strains. Consistent with this study, the present investigation identified genogroups G1, G3, and G4 in Rio de Janeiro, confirming the diverse genetic landscape of IBDV in Brazil.

Comparative analysis with the classical Edgar-USA strain (A33255) revealed multiple nucleotide substitutions in VP2 (Table 3), possibly reflecting selective pressure from widespread vaccination. These vaccination practices may exert a selection pressure, contributing to the emergence of antigenically divergent field strains. These antigenic changes resulting from mutations in viral epitopes can increase the virulence of circulating strains, as reported by Fraga et al. (2019).

This study collected samples from farms using the live attenuated intermediate vaccine Transmune (Ceva®) for one-day-old chicks. The attenuated viral strains included in their formulation induce microscopic changes in the bursa of Fabricius, similar to those caused by less virulent strains found in the field, thus stimulating the immune response of birds (Lupini et al. 2016). But it may also promote the emergence of more virulent strains. The use of live attenuated vaccines may exert evolutionary pressure on circulating viruses, potentially promoting the emergence of new strains (Hanley et al. 2011). These observations reinforce the need for continuous molecular surveillance of the virus. Phylogenetic studies of IBDV strains worldwide can provide valuable insights into the circulation of major genogroups and the adaptability of the virus to evolutionary pressure. In Brazil, previous studies reported the presence of cv, av, and vv IBDV strains circulating in the country across different states (Paula et al. 2004, Gomes et al. 2005, Fernandes et al. 2009, Silva et al. 2013, Muniz et al. 2018, Fraga et al. 2019); neither study included samples from Rio de Janeiro. This study bridges this gap by revealing the presence of vvIBDV and avIBDV strains in southeastern Brazil.

Although a newer classification system based on segments A and B has been proposed (Islam et al. 2021, Wang et al. 2021, Gao et al. 2023), VP2 genotyping remains essential for understanding virus evolution, because most historical and contemporary datasets are VP2-based. The proximity of poultry farms increases the likelihood of exposure to altered strains via horizontal transmission, thereby promoting the spread of viral variants (Guzmán et al. 2022). The G-3 strain in this study showed classical vvIBDV amino acid signatures (222A, 242I, 256I, 294I, and 299S), consistent with previous reports (Michel & Jackwood 2017, Dey et al. 2019, Nour et al. 2023), supporting its classification as vvIBDV. In contrast, G-4 isolates displayed different substitution patterns, indicating genomic divergence among local strains that could influence their virulence.

Highly virulent strains often exhibit a hypervariable region enriched with serine residues, which enhances intra- and intermolecular interactions via hydrogen bonding. These interactions are less prominent in avirulent strains, where serine substitutions impose such constraints (Lachheb et al. 2021). Some amino acid substitutions may influence virulence by altering protein interactions or surface properties (Li et al. 2015). Additionally, VP2 hypervariable region analysis supports the hypothesis that IBDV was introduced into Brazil from Europe, with vvIBDV G-3 originating in the Netherlands and avIBDV G-4 in Eastern Europe (Silva et al. 2013, Ikuta et al. 2023). This genetic similarity explains the close phylogenetic relationship between the RJ and UK strains within genogroup 3.

According to genogroup classification, certain G-3 viruses lack the typical amino acids found at positions 222, 242, 256, 294, and 299, which are commonly found in vvIBDV strains (Michel & Jackwood 2017, Dey et al. 2019). However, in this study, the G-3 sample exhibited amino acid substitutions at these positions, which is consistent with the vvIBDV profile, suggesting that these variations may influence virulence. Similarly, Nour et al (2023) identified new G3 strains with amino acid profile characteristics of vvIBDV. Similar amino acids were observed at the corresponding sites.

Substituting certain amino acids with alanine can create a more hydrophobic environment, potentially enhancing interactions between the virus and its target cells. This increase in hydrophobicity can facilitate stronger binding between the virus and specific receptors on the host cell (Li et al. 2015). These molecular characteristics suggest that the amino acid changes observed in this study may be associated with increased virulence and a more efficient infection process in the host.

Moreover, the amino acids threonine, lysine, serine, isoleucine, and aspartate at positions 222, 249, 254, 286, and 318, respectively, play crucial roles in determining IBDV antigenic variants (Gao et al. 2023). These positions were previously identified in Brazilian sequences classified as G4 by Fraga et al. (2019). In contrast to other Brazilian G4 strains, the strains in this study exhibited amino acid profiles consistent with the G4 classification only at position 254, highlighting some divergence.

Additional analyses of amino acids in hvVP2 from the samples in this study revealed positions unique to some virulent viral strains. This study also identified silent mutations that did not alter amino acids but could be linked to reassortment and mutation processes driven by vaccination pressure and interactions with the circulating strains. This study emphasizes the importance of ongoing molecular surveillance and genetic mapping of IBDV, particularly in hypervariable regions. This is crucial for developing effective vaccination protocols and control strategies against this disease.

Given the recent emergence of new strains in Rio de Janeiro, it is crucial to differentiate and identify IBDV isolates to select appropriate vaccines, maintain adequate antibody levels in poultry, and ensure effective protection. The genetic differences observed in the VP2 hypervariable region highlight the need for molecular tools to better understand the antigenic variation and pathogenicity of IBDV. The genetic diversity and unique amino acid profiles identified in this study highlight the importance of developing region-specific or updated vaccines that better match circulating strains. The genetic differences

observed between field and vaccine strains may result in reduced cross-protection, potentially leaving vaccinated flocks vulnerable to infection. These findings emphasize the need for continuous surveillance and updating of vaccination strategies to ensure the effective control of IBDV in poultry populations.

CONCLUSION

Virulent strains of infectious bursal disease virus (IBDV) have been identified in broiler flocks in the state of Rio de Janeiro, exhibiting high genetic similarity among themselves and clear distinctions from international strains. These findings highlight the need for continuous surveillance and in-depth research on the epidemiology and evolution of IBDV to safeguard poultry health and minimize economic losses associated with the disease. Understanding the genetic diversity and evolutionary dynamics of IBDV is crucial for developing targeted control strategies and optimizing vaccination protocols. This approach will contribute to sustainable poultry production and reinforce Brazil's position as a global leader in the poultry industry.

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Data availability statement. The VP2 gene sequences generated in this study were deposited in the GenBank database (NCBI; https://www.ncbi.nlm.nih.gov/genbank/) under accession numbers PQ415647–PQ415651. Reference sequences used for phylogenetic analyses were retrieved from GenBank (Table S1).

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