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Case Report

First Isolation, Molecular Identification, and Phylogenetic Characterization of A3B5 Very Virulent Infectious Bursal Disease Virus in Pullets in Chile

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Abstract: Infectious bursal disease virus (IBDV) is an important pathogen affecting the poultry industry worldwide. IBDV serotype 1, including classical virulent strains (cvIBDV), variant strains (varIBDV), and very virulent strains (vvIBDV), is pathogenic for chickens. IBDV mainly infects immature B-lymphocytes in the bursa of Fabricius, weakening the humoral immune response and leading to secondary infections and increased morbidity and mortality. The Laboratory of Avian Pathology received ten live 8-week-old pullets from a laying hen operation experiencing increased mortality, prostration, diarrhea, and sudden death. Upon necropsy, the affected birds presented swollen, hemorrhagic, and edematous bursa of Fabricius, as well as hemorrhage in the breast and thigh muscles. RT-PCR confirmed that the samples from the bursa of Fabricius were positive for IBDV. Phylogenetic analysis of the VP1 and VP2 gene nucleotide sequences classified the strain, isolated in embryonated chicken eggs, as the A3B5 genotype. Amino acid sequence analysis of the VP2 hypervariable region revealed the presence of amino acid residues commonly found in vvIBDV. Additional studies are required to investigate the epidemiological situation of this genotype in Chile and to evaluate current vaccination plans and their effectiveness against new variants.

Keywords: Avian infectious bursal disease virus; IBDV; poultry; very virulent; phylogenetic analysis

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1. Introduction

Infectious bursal disease (IBD) or "Gumboro Disease" is an acute, highly contagious, immunosuppressive disease affecting young chicks. It causes significant economic losses worldwide due to mortality, increased susceptibility to infection, and poor vaccination response [1–3]. The disease is caused by the infectious bursal disease virus (IBDV), a small non-enveloped viral particle with a 60 to 70 nm diameter that belongs to the Avibirnavirus genus within the *Birnaviridae* family [4]. The virus genome comprises double-stranded RNA divided into A and B segments. The longer segment A (3.2 kb) encodes VP2–VP3–VP4 polyprotein and a nonstructural protein VP5, while the shorter segment B (2.9 kb) encodes VP1, which has RNA-dependent RNA polymerase activity [2]. The capsid consists of 260 VP2 trimers. Each VP2 monomer has three distinct domains: base domain (B), shell domain (S), and projection domain (P) [5]. The P domain contains a hypervariable (hv) region between aa positions 206 and 350 [6], which includes four exposed loops identified as P_{BC}, P_{DE}, $P_{FG,}$ and P_{HI} [7]. The VP2 protein is the main structural protein related to virulence, cellular tropism, production of neutralizing antibodies, and prediction of phylodynamic relationships [8]. Cross-neutralization assays have identified two IBDV serotypes [9]. Serotype 2 is nonpathogenic for chickens, and there are no cross-reactions between the two serotypes [10]. Serotype 1 includes classical virulent strains (cvIBDV) [11], variant strains (varIBDV) [12], and very virulent strains (vvIBDV) [13]. As a non-enveloped virus, IBDV is highly resistant to environmental conditions, facilitating its persistence and spread in poultry farms [14].

The virus primarily targets the bursa of Fabricius (BF), infecting immature B lymphocytes in the target organ, inducing inflammation and subsequent atrophy and decreasing the B lymphocyte count. The resulting weakened immune response [15] reduces vaccine-induced immunity and predisposes birds to secondary infections, increasing morbidity and mortality in infected birds [16]. Macroscopically, on day 3–4 PI, the bursa increased in size and weight due to edema and hyperemia. Necrotic foci and petechial or ecchymotic hemorrhages are usually observed on the mucosal surface, resulting in a "purple grape" appearance. Microscopically, severely reduced lymphocytes, heterophil infiltrates, and interstitial hyperplasia are observed, along with erythrocyte infiltration, which produces atrophy and bleeding of the bursa [17].

IBDV first appeared in Gumboro, Delaware, USA, in 1957 and is commonly known as the classical virulent strain (cvIBDV). The disease was called "avian nephrosis" due to kidney damage in affected birds [18]. In 1984, variant IBDV (varIBDV) strains emerged in Gumboro County [19]. Very virulent strains (vvIBDV) were later identified in Holland [11], Belgium [20], Brazil [21], and the USA [22]. These vvIBDV strains cause 60% to 70% mortality in susceptible 3- to 10-week-old layer pullets [1]. According to molecular epidemiology studies, approximately 60–76% of IBDV isolates in several countries worldwide can be classified as very virulent [23].

The emergence of new strains with different characteristics led to proposals for new classification schemes, some of which are based exclusively on changes in the hypervariable region of VP2 (hvVP2), making it possible to classify IBDV strains into seven genogroups [24]. These were subsequently complemented with an analysis of the VP1 (segment B) sequence since both contribute to IBDV pathogenicity [25]. The combined classification scheme detects reassortment in the IBDV segments. It identifies nine genogroups for Segment A [A1 (classical), A2 (US antigenic variant), A3 (very virulent), A4 (dIBDV), A5 (atypical Mexican), A6 (atypical Italian), A7 (early Australian), A8 (Australian variant), and A9 (attenuated)] and five genogroups for Segment B [B1 (classical-like), B2 (very virulent-like), B3 (early Australian-like), B4 (Polish and Tanzanian), and B5 (Nigerian)]. Given IBDV's bi-segmented genome, up to 45 different genotypes could be identified if all genogroups are present [26–28].

The vvIBDV strains have been identified in Poland [29], China [30], Finland [31], Nigeria [32], Egypt [33], and Western European countries [34]. In Latin America, they have been identified in Brazil, the Dominican Republic, Venezuela [35], and Argentina [36]. In Chile, cvIBDV strains of the A1B1 genotype and a varIBDV strain of the A2B1 genotype have been identified [37]. This is the first report on the isolation, molecular identification, and phylogenetic characterization of a very virulent strain of infectious bursal disease virus in Chile.

2. Materials and Methods

2.1. Examination and Sampling of the Pullets

The Universidad de Chile's Avian Pathology Laboratory received ten live, 8-week-old pullets from a laying hen farm experiencing increased mortality, prostration, diarrhea, and sudden death in Chile's Metropolitan Region. The birds were humanely euthanized by cervical dislocation for subsequent necropsy. The flocks had been vaccinated at the hatchery for *Salmonella*, infectious bronchitis, and Marek's disease. No IBD vaccine had been administered. Bursa samples were collected, sliced, and homogenized in phosphate-buffered saline (PBS; pH 7.2) 10% containing 200 U/mL penicillin and 0.2 mg/mL streptomycin. Homogenates were vortexed for 10 s, subjected to three freeze–thaw cycles, and centrifuged at 3000× *g* for 10 min at 4 °C. The supernatant was filtered through a 0.22 µm membrane, transferred to a sterile tube and stored at −80 °C for further analysis.

2.2. Viral Isolation and Propagation in Specific Pathogen-Free Embryonated Chicken Eggs (SPF-ECE)

Fertile 9- to 11-day-old SPF-ECE were inoculated via chorioallantoic membrane (CAM) using a dose of 0.1 mL per egg with bursa homogenate. They were subsequently incubated at 37 °C and 60–70% humidity. The chicken embryos were candled daily; those that died within the first 48 h post-inoculation were discarded. Then, the CAM from the affected ECE was collected and homogenized in sterile PBS. The resulting supernatant was filtered through a 0.22 µm membrane, placed in sterile tubes, and stored at −80 °C for molecular analysis [38].

2.3. Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Total RNA from bursa homogenates and CAM was extracted using the PureLink™ Viral RNA/DNA Mini Kit (Invitrogen, Waltham, MA, USA) according to the manufacturer's instructions. RNA was eluted in 50 µL nuclease-free water to amplify IBDV gene fragments from segments A and B. For viral identification and phylogenetic analyses, cDNA was obtained using SuperScript III Reverse Transcriptase (Invitrogen, Waltham, MA, USA) according to the manufacturer's instructions. The PCR was conducted in a final volume of 25 µL containing 10 mM of each primer, 2.5 µL of 10x High Fidelity PCR buffer, 10 mM dNTP Mix, 50 mM $MgSO_4$, 0.5 U of Platinum™ Taq DNA Polymerase High Fidelity (Invitrogen, Waltham, MA, USA), 5 µL of cDNA and ultrapure water under the following conditions: 2 min at 94 °C, 35 cycles of 1 min at 94 °C, 30 s at 61 °C, 45 s at 72 °C; and, finally, an additional extension step at 72 °C for 10 min. The PCR products were analyzed by 2% agarose gel electrophoresis using GelRed® Nucleic Acid Stain (Millipore, Burlington, MA, USA) and visualized using a Trans-Lum SOLO transilluminator (Biotop, Jing'an District, Shanghai, China). The primer sequences used are listed in Table 1.

Table 1. Primers used in this study.

Segment	Gen	Primer	Sequence (5′ to 3′)	PCR Product (bp)	Reference
A	VP2	U3	TGTAAAACGACGGCCAGTGCATGCGGTATGTGAGGCTTGGTGAC	604	[39]
		L3	CAGGAAACAGCTATGACCGAATTCGATCCTGTTGCCACTCTTTC		
B	VP1	+290	TGTAAAACGACGGCCAGTGAATTCAGATTCTGCAGCCACGGTCTCT	642	[40]
		−861	CAGGAAACAGCTATGACCCTGCAGTTGATGACTTGAGGTTGATTTTG		

2.4. VP1 and VP2 Sequence Analysis and Phylogenetic Analysis

The amplified PCR products from the VP1 and VP2 genes were purified using a PureLink™ Quick Gel Extraction Kit (Invitrogen, Waltham, MA, USA) according to the manufacturer’s instructions. Austral Omics (Valdivia, Los Ríos Region, Chile) provided the sequencing services. Sanger sequencing was performed using the chain termination method with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Invitrogen, Waltham, MA, USA) according to the manufacturer’s instructions. Sequences from this study’s VP1 and VP2 genes were assembled and translated into amino acid sequences using Bi-oedit v.7.2.5 software [41]. VP1 and VP2 gene sequences were retrieved from GenBank and included in the analysis. The datasets were aligned using the MAFFT v.7 online software (http://mafft.cbrc.jp/alignment/server/large.html (accessed on 25 June 2024)) [42]. The VP2 dataset included 74 sequences, whereas the VP1 dataset consisted of 57 representative sequences from the previously described genogroups for segments A and B [26]. These figures include sequences from reported Chilean isolates [37]. A maximum-likelihood tree was created using PhyML 3.0 [43], and a bootstrap expectation (TBE) process with 1000 replicates supported the robustness of the nodes. The tree was visualized and edited using FigTree v. 7.4.4 software [44] (https://code.google.com/archive/p/beast-mcmc/ (accessed on 28 June 2024)).

MEGA 11 software [45] estimated evolutionary divergence in the VP1 and VP2 sequences. The data are expressed as a relationship between the two groups listed in the headers of each table in terms of the average difference (by group) in the amino acids per site. A bootstrapping procedure (500 replicates) produced standard error estimates, and analysis used the Poisson correction model. The rate of variation between the two sites was modeled with gamma distribution (shape parameter = 4).

To analyze the VP2 hypervariable region (hvVP2), the sequence of the strain isolated in this study was compared with previously published Chilean IBDV sequences, GenBank sequences for the A1, A2, A3, A4, A5, A6, A7, A8, and A9 genogroups (accession numbers AY012683, JX134483, MF996499, MN313611, MF142574, JN852985, AF381001, and AF148081, MZ687401, respectively), and sequences from live attenuated vaccines: D78 (accession number AJ586963) and ViBursa CE (accession number EU162089).

3. Results

3.1. Examination of the Pullets

The postmortem analysis of the pullets in the laboratory reveals dehydration and macroscopic lesions in the bursa of Fabricius, which appear swollen, hemorrhagic, and edematous (Figure 1a,b). Hemorrhages were also found in the breast and thigh muscles of some affected birds (Figure 1c). The kidneys were swollen, whitish, and contained urate deposits. Lesions were compatible with infectious bursal disease. No other significant lesions were observed in any other organ.

Figure 1. Gross lesions in affected pullets. (**a**) Swollen, hemorrhagic, and edematous bursa of Fabricius; (**b**) swollen, hemorrhagic, and edematous bursae of Fabricius removed from affected birds; (**c**) hemorrhages in pectoral muscle. Bar = 1 cm.

3.2. Viral Isolation

Bursal samples were propagated via the chorioallantoic route on embryonated chicken eggs to isolate the IBDV. Forty-eight hours post-inoculation, the dead embryos exhibited lesions of dwarfing (Figure 2a), congestion, edema, hemorrhage, a pale appearance of the heart, and a swollen, greenish liver (Figure 2b). The chicken embryo negative controls showed no significant lesions (Figure 2c).

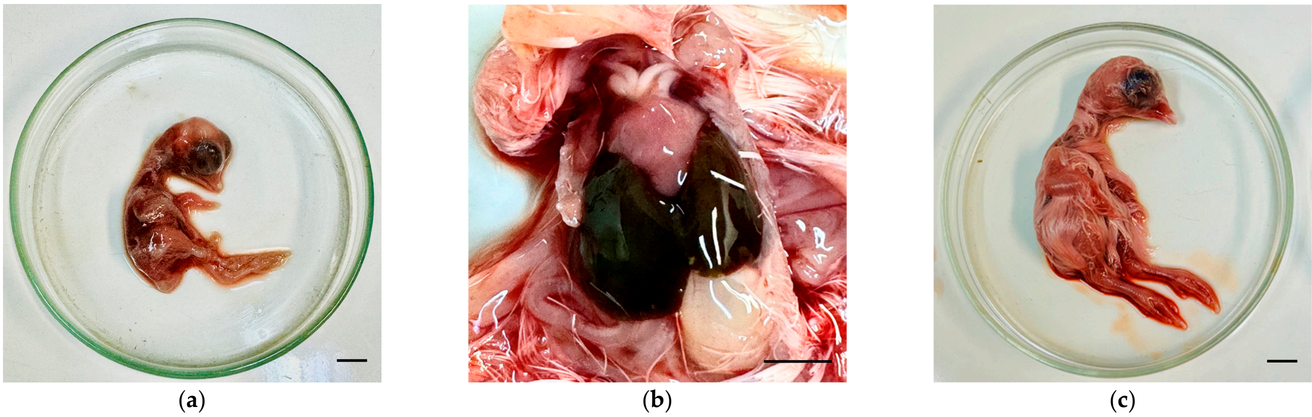

Figure 2. Isolation of the IBDV strain in 10-day-old chick embryo. (**a**) Chicken embryo with dwarfism; (**b**) swollen, greenish-colored liver; (**c**) chicken embryo negative control. Bar = 1 cm.

3.3. Identification of the IBDV

The samples from the pullets◎BF were positive for IBDV infection, amplifying 642 and 604 bp corresponding to VP1 and VP2 genes, respectively. The samples obtained from the CAM of SPF-ECE infected with bursa homogenate were also positive for both genes (Figure 3), confirming the presence of the virus in the affected bursa and its successful isolation in embryonated chicken eggs.

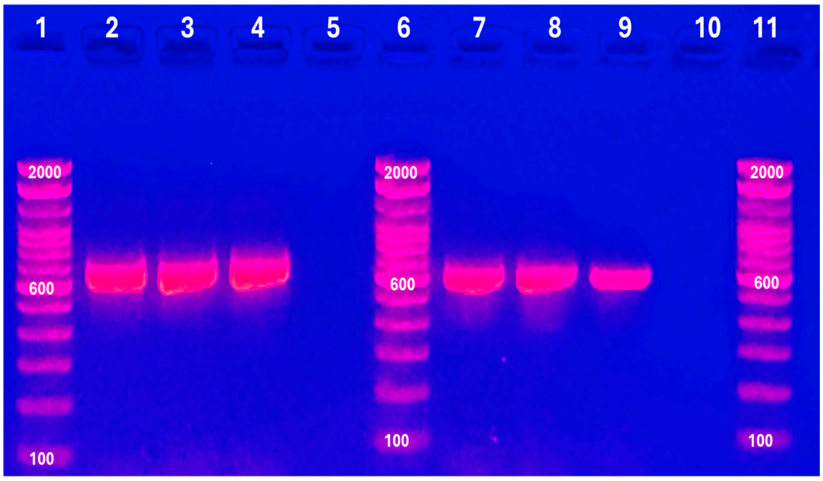

Figure 3. PCR amplicons of VP1 (lanes 2–4) and VP2 (lanes 7–9). Lane 1: 100 bp DNA ladder. Lane 2: Bursa homogenate sample. Lane 3: CAM sample. Lane 4: Positive control. Lane 5: Negative control. Lane 6: 100 bp DNA ladder. Lane 7: Bursa homogenate sample. Lane 8: CAM sample. Lane 9: Positive control. Lane 10: Negative control. Lane 11: 100 bp DNA ladder.

3.4. Phylogenetic Analysis of VP1 and VP2

Analysis of the VP1 sequence shows that the isolated virus could be classified as genogroup B5, which contains previously characterized strains from Nigeria (Figure 4). Analysis of the VP2 sequence classifies it within genogroup A3, which contains strains previously characterized as very virulent in several countries [20] (Figure 5). Genotyping analysis based on the partial sequences of both segments classified the detected IBDV strain as the A3B5 genotype. The sequences obtained in this study were designated as 16124_CL_2024 and submitted to GenBank. The accession numbers assigned to VP1 and VP2 were PP824543 and PP824544, respectively.

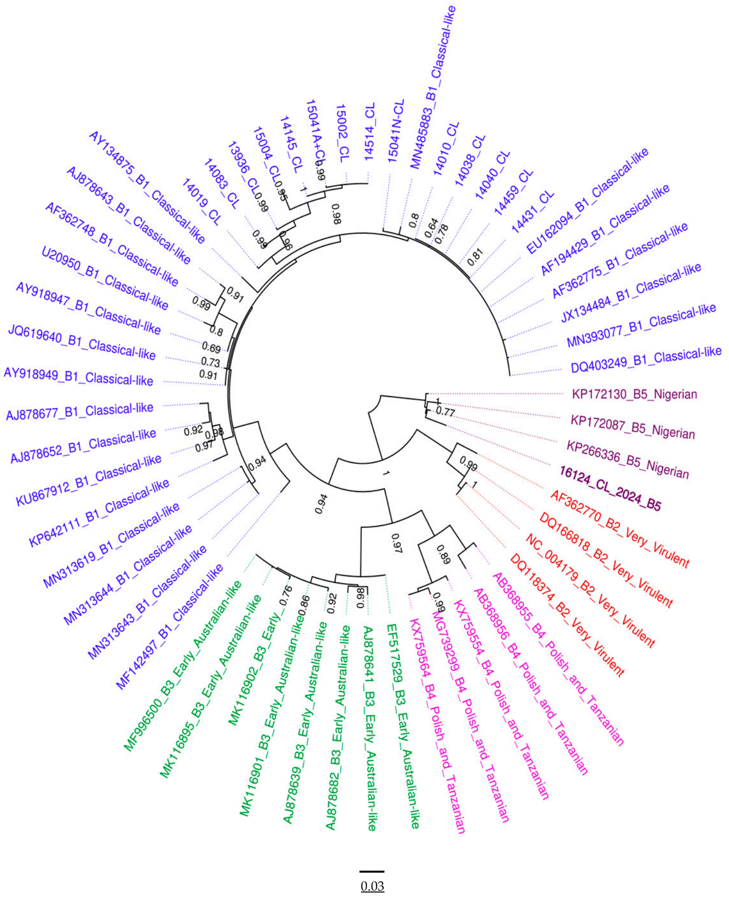

Figure 4. Maximum-likelihood tree (polar format) with 1000 bootstrap replicates. The tree was constructed using 57 VP1 sequences, including the one isolated in this study and other Chilean IBDV sequences. Genogroups were highlighted as follows: B1 = blue, B2 = red, B3 = green, B4 = light violet, B5 = dark violet.

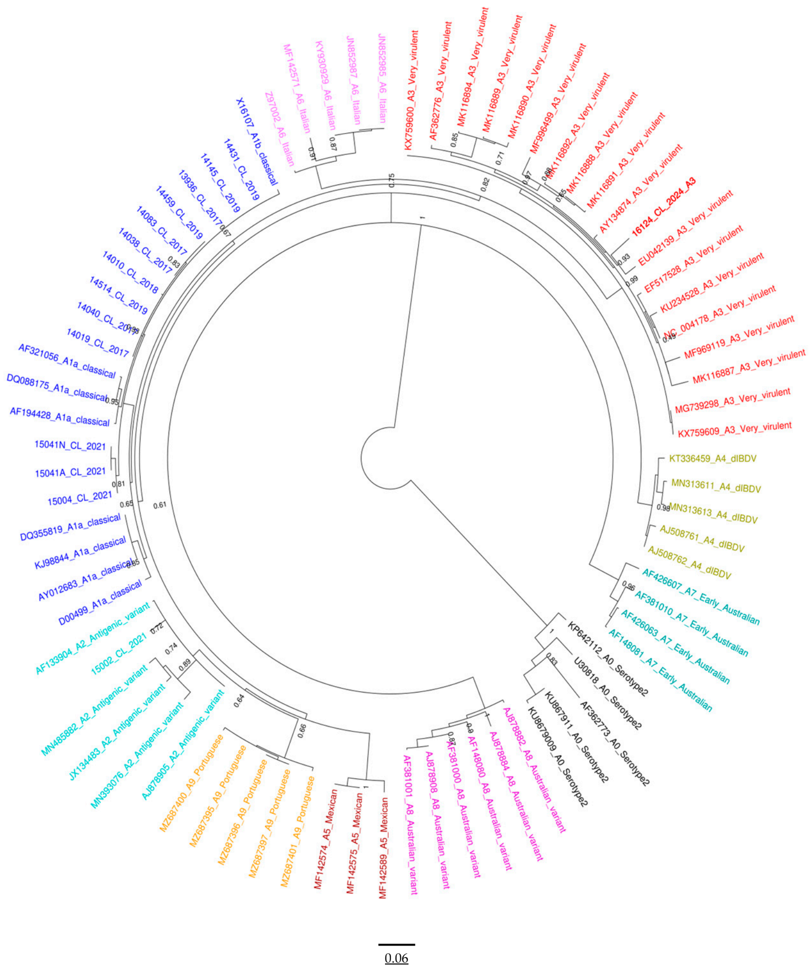

Figure 5. Maximum-likelihood tree (polar format) with 1000 bootstrap replicates. The tree was constructed using 79 VP2 sequences, including the one isolated in this study and other Chilean IBDV sequences. Genogroups were highlighted as follows: A0 = black, A1 = blue, A2 = light blue, A3 = red, A4 = yellow, A5 = dark red, A6 = light purple, A7 = green, A8 = dark purple, A9 = orange.

3.5. Nucleotide Sequence Analysis of Segments A and B

Continuing nucleotide sequence analysis for the VP1 and VP2 genes, the consensus sequences obtained for segments A and B were compared to the GenBank database using the BLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi (accessed on 26 July 2024)).

This analysis determined that the nucleotide sequences shared significant identity with those from strains obtained primarily in Brazil and the USA. A summary of the main results is presented in Table 2.

Table 2. Closest BLAST matches against NCBI database of IBDV segments A and B.

Segment	Gene	Accession Number	% Identity	Accession Number	Country	Year
A	VP2	PP824544	95.31%	JN982259	Brazil	2013
			95.31%	JF811919	Brazil	2016
			95.31%	DQ286035	Brazil	2008
B	VP1	PP824543	97.64%	JN982250	Brazil	2013
			97.30%	JN982249	Brazil	2013
			97.13%	DQ679811	USA	2008

3.6. Amino Acid Sequence Analysis for Segments A and B

When comparing the amino acid distance between the different genogroups and the strain isolated in this study, based on segment A, less divergence was observed with genogroup A3 (0.0102) than with other genogroups (A1 to A2, A4 to A9 average = 0.1342) (Table 3). When comparing the amino acid distances of different genogroups based on segment B and the strain isolated in this study, a close relationship was observed with genogroup B5 (0.0365), with greater divergence from the rest of the genogroups (B1 to B4 average = 0.1648) (Table 4).

Table 3. Amino acid distance between IBDV genogroups based on segment A [1].

	A0	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**CL1**	**CL2**
A0		0.0674	0.0779	0.0714	0.0715	0.0769	0.0733	0.0719	0.0641	0.0778	0.0701	0.0717
A1	0.4360		0.0254	0.0210	0.0243	0.0284	0.0270	0.0267	0.0339	0.0279	0.0124	0.0206
A2	0.4924	0.1110		0.0281	0.0315	0.0281	0.0331	0.0346	0.0373	0.0232	0.0283	0.0282
A3	0.4679	0.0794	0.1157		0.0299	0.0332	0.0261	0.0298	0.0307	0.0325	0.0252	0.0035
A4	0.4708	0.0957	0.1324	0.1207		0.0333	0.0329	0.0300	0.0358	0.0329	0.0268	0.0299
A5	0.4993	0.1106	0.1021	0.1314	0.1307		0.0338	0.0388	0.0409	0.0278	0.0305	0.0329
A6	0.4535	0.1115	0.1396	0.1004	0.1388	0.1308		0.0325	0.0335	0.0347	0.0299	0.0265
A7	0.4769	0.1130	0.1601	0.1223	0.1220	0.1695	0.1347		0.0321	0.0379	0.0288	0.0300
A8	0.3857	0.1569	0.1767	0.1340	0.1538	0.1875	0.1381	0.1366		0.0405	0.0366	0.0311
A9	0.4906	0.1071	0.0708	0.1238	0.1210	0.0872	0.1368	0.1610	0.1823		0.0311	0.0321
CL1	0.4479	0.0481	0.1150	0.0921	0.1009	0.1133	0.1146	0.1202	0.1620	0.1146		0.0252
CL2	0.4643	0.0716	0.1078	0.0102	0.1153	0.1249	0.0968	0.1169	0.1297	0.1153	0.0860	

[1] The number of per-site amino acid substitutions based on the average of all inter-group sequence pairs. Standard error estimates are shown above the diagonal. "CL1" indicates the group of Chilean viruses previously isolated. "CL2" indicates the virus isolated in this study.

Table 4. Amino acid distance between IBDV genogroups based on segment B [1].

	B1	**B2**	**B3**	**B4**	**B5**	**CL2**	**CL1**
B1		0.0220	0.0199	0.0199	0.0244	0.0267	0.0075
B2	0.1273		0.0221	0.0238	0.0231	0.0244	0.0227
B3	0.1241	0.1361		0.0165	0.0271	0.0295	0.0205
B4	0.1310	0.1492	0.1036		0.0249	0.0287	0.0204
B5	0.1484	0.1385	0.1633	0.1498		0.0090	0.0249
CL2	0.1625	0.1466	0.1804	0.1699	0.0365		0.0272
CL1	0.0470	0.1305	0.1320	0.1369	0.1540	0.1642	

[1] The number of per-site amino acid substitutions based on the average of all inter-group sequence pairs. Standard error estimates are shown above the diagonal. "CL1" indicates the group of Chilean viruses previously isolated. "CL2" indicates the virus isolated in this study.

3.7. Analysis of VP2 Hypervariable Region (hvVP2)

The amino acid sequence for the VP2 hypervariable region of the strain isolated in this study was compared with previously published Chilean IBDV sequence; representative sequences recovered from GenBank of A1, A2, A3, A4, A5, A6, A7, A8, and A9 genogroups; and sequences from live attenuated vaccines D78 and ViBursa CE. The VP2 amino acid sequences of the strain isolated in this study had the markers typical of vvIBDV (222A, 242I, 256I, 279D, 294I, and 299S) [20] (Figure 6).

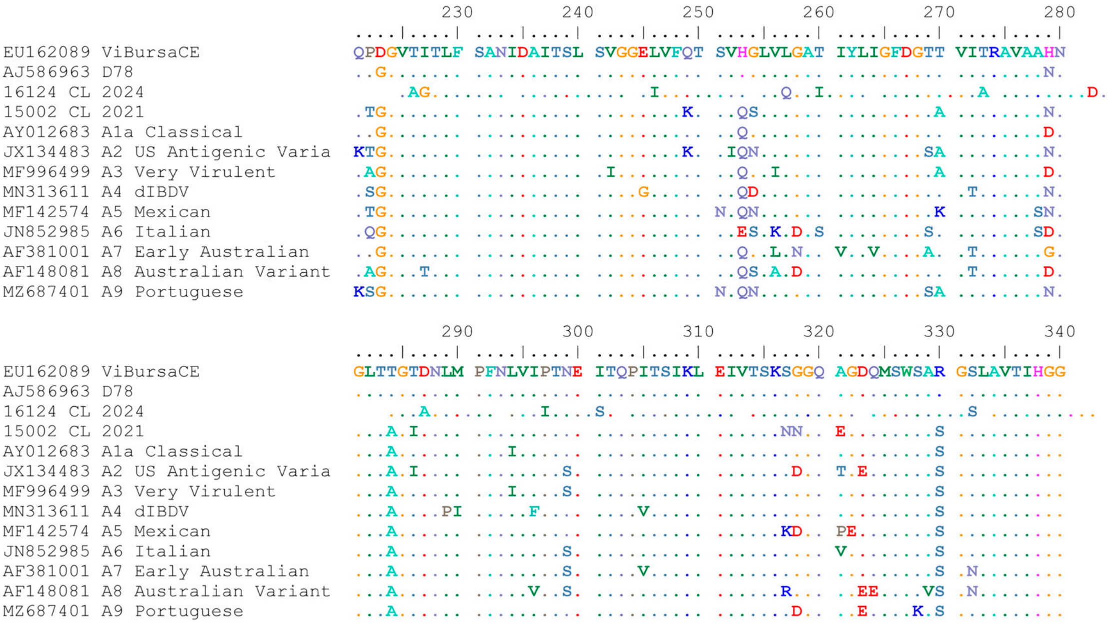

Figure 6. Amino acid differences in hvVP2 (aa 220–340) between the virus isolated in this study, representative viruses of each genogroup, and two vaccines currently used in Chile. The dots indicate that the sample had the same amino acid as the query sequence. Abbreviations: A—alanine, D—aspartic acid, E—glutamic acid, F—phenylalanine, G—glycine, H—histidine, I –isoleucine, K—lysine, L—leucine, Q—glutamine, N—asparagine, P—proline, S—serine, R—arginine, T—threonine, V—valine, Y—tyrosine.

4. Discussion

IBD causes significant losses for the poultry industry. The cvIBDV, varIBDV, and vvIBDV strains have spread worldwide, and the presence of IBDV in Chile, specifically the classic (A1B1) and variant (A2B1) strains, has been previously confirmed [37]. However, this is the first report of a very virulent IBDV (vvIBDV). IBDV vaccination is widely used in poultry production worldwide, and live-attenuated vaccines based on classical-type virus strains are common. Despite vaccination, IBD is continuously observed [46].

The pullets examined in this study showed swollen, hemorrhagic, and edematous bursa of Fabricius, similar to that previously characterized in vvIBDV strains [47]. The vvIBDV isolated in fertile 9- to 11-day-old SPF-ECE resulted in dwarfing and greenish liver in the inoculated dead embryos, similar to previous observations [48]. The presence of IBDV was confirmed in the bursa of Fabricius sample and the CAM by RT-PCR using a set of primers previously designed to amplify the hv region of the VP2 gene and the VP1 gene of IBDV strains [39,40].

According to the phylogenetic analysis of the nucleotide sequences of the VP2 and VP1 genes (segment A and B, respectively), the isolated IBDV strain was classified within the A3B5 genotype using the scheme proposed by Islam et al. [26] and modified by Wang et al. [28]. Genogroup A3 (previously G3) has been observed in strains from the Netherlands, France, Poland, China, Finland, Vietnam, and other countries [48]. In Latin America, it has also been characterized in Argentina [36], Brazil [49], and Uruguay [50]. The hvVP2 sequences of this virus are genetically similar to those of the vvIBDV strains EU042139 and AY134874 of Chinese origin [51]. Meanwhile, genogroup B5 has only been identified in Nigeria [52]. The VP1 sequence of this virus is genetically similar to the vvIBDV strains KP172130, KP172087, and KP266336 of Nigerian origin. The A3B5 genotype identified in this study is part of a monophyletic cluster formed exclusively by Nigerian strains [53]. The origin of this new A3B5 genotype in Chile remains unknown. There are no data on the occurrence of this genotype in Latin America. More studies and greater GenBank database availability of sequences originating in the region are required to clarify this issue.

The high percentage identity of the VP1 and VP2 genes (average of 97.36% and 95.31%, respectively) between the virus isolated in this study and other IBDVs previously reported (mainly in Brazil) could indicate a possible origin, probably due to the importation of sub-clinically infected birds from nearby countries. This needs to be properly determined. Previous reports have indicated that vvIBDV strains have been introduced in Brazil, mainly from the Netherlands [54] and that very virulent IBDV isolates in Latin America emerged from virulent strains from Europe or Asia. This could partly explain the transmission route of the virus from Africa and Asia to America [35].

Based on segments A and B, the estimated amino acid distance between IBDV genogroups confirmed that this isolated IBDV strain belongs to the A3B5 genotype and was classified as very virulent. Amino acid analysis of the hvVP2 region revealed that the 16,124 CL strain contained the conserved residues (A222, I242, I256, I294, and S299) typical of vvIBDV strains [24]. Residues at positions 222, 242, 256, 279, 294, and 299 are involved in antigenicity, virulence, cell tropism, ability to replicate in chicken embryonic fibroblasts, and pathogenicity [39,54 The hypervariable region of VP2 (aa 210–350) in 16,124 CL was completely identical to the vvIBDV strain MF996499 from Pakistan [55], confirming observations in the pathological findings of the affected birds, phylogenetic analysis, and analysis of the nucleotide sequences of the VP1 and VP2 genes. To the best of our knowledge, this is the first report of vvIBDV infection in Chile.

5. Conclusions

The presence of very virulent IBDV strain in Chile has been confirmed for the first time. Phylogenetic analysis based on segments A and B classified the strain within the A3B5 genotype, previously identified only in strains isolated from Nigeria. However, the high percentage of nucleotide identity in both genes relative to strains isolated in America suggests that the strain may have been introduced in Chile from nearby countries via the importation of sub-clinically infected birds. The flock was not adequately vaccinated against IBD; the emergence of new variants reinforces the importance of vaccination to prevent the spread, mutation, and reassortment of IBDV strains. Despite the widely distributed immunoprophylaxis, live-attenuated vaccines based on classic strains, as commonly used in Chile, may not adequately protect against vvIBDV strains. As a result, developing next-generation vaccines—e.g., approaches based on bioinformatics and reverse vaccinology [56]—is essential to effectively and safely controlling the disease in susceptible birds. The presence of three genotypes of IBDV in Chile (A1B1, A2B1, and A3B5) has been confirmed. Given the IBDV strain's reassortment capacity, co-circulation of several different genotypes in the same region, simultaneous co-infection of the same host, and vaccination failures could favor the emergence of a new reassortment of viruses. Epidemiological surveillance and pathogenicity studies of vvIBDV strains are necessary to evaluate the effectiveness of the vaccines currently available in Chile.

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