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Epidemiologic and Genetic Assessment of Infectious Bursal Disease Virus in Egypt: A Study of Strain Diversity and Evolution

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Abstract | Infectious Bursal Disease Virus (IBDV) is one of the most significant viruses that responsible for immunosuppression and high mortality in young chicks. In Egypt, the prevalence of IBDV is complicated as multiple strains co-circulating, including classical, variant, and very virulent (vvIBDV). This study aims to investigate the genetic variability and reassortment interactions between co-circulating IBDV strains in Egyptian field, with a particular focus on the VP1(segment B) and VP2 (Segment A) gene sequences. Samples of bursal tissue were obtained from poultry farms located in the governorates of Dakahlia, Kafr Elsheikh, Menoufia, and Qalyubia in Egypt. Following extraction of total RNA from these samples, the partial segments of IBDV VP1 and VP2 were amplified using reverse transcription polymerase chain reaction (RT-PCR). Followed by sequencing of these segments, and a phylogenetic analysis of the strains was performed to evaluate the genetic divergence and IBDV reassortment. The genetic analysis revealed that the variant strains were more frequent (80%) than the classical (15%) and vvIBDV (5%) strains. The VP1 and VP2 phylogenetic analysis revealed three distinct groups: classical, variant, and vvIBDV strains. Furthermore, there was no evidence of genetic re-assortment between the circulating strains that were examined. To achieve more effective control of IBD, the findings brought light the significance of developing new vaccination strategies for the Egyptian poultry industry that are tailored to the variant strains that are currently circulated. The molecular analysis must be performed regularly to track the IBDV strains and their evolution, in addition, to assessing the effectiveness of vaccines.

Keywords: Phylogenetic analysis, Strains, Infectious bursal disease, VP1, VP2, Chicken

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INTRODUCTION

Infectious bursal disease (IBD) is a highly contagious viral disease that affects young chicks and has a significant impact on the worldwide poultry industry. This disease is caused by IBDV which is bisegmented double-stranded RNA, a member of the Birnaviridae family. IBD virus tropism includes but is not limited to immature B lymphoid cells in the bursa of Fabricius, which leads to severe immunosuppression increased direct mortality rates from the disease itself and indirect secondary infections. The most significant impact of IBD is the effectiveness of vaccination

programs used to control other diseases (Eterradossi *et al*., 2020). The infection with variant IBD associated with severe immunosuppression which reflect on exaggerated losses because of the effect on immune cells and the lower level of humoral immune response due to effect on B-lymphocytes from other infection like Avian influenza (low pathogenic and Highly pathogenic viruses), Newcastle disease, Infectious bronchitis, Coryza, Salmonella, Cholera (Eid *et al*., 2019; Sultan *et al*., 2019; Elfeil *et al*., 2020; Fawzy *et al*., 2020; Sultan *et al*., 2020; Talat *et al*., 2020; Elfeil *et al*., 2022; Mahmoud *et al*., 2022; Sultan *et al*., 2022; Sultan *et al*., 2024).

From the first record of the IBD virus in the 1960s until now, IBD is still considered one of the most important challenges in the poultry production industry (Lasher *et al*., 1997). According to pathotyping IBD viruses are classified into different strains, including classical, variant, and very virulent strains (vvIBDV). Each of these pathotypes showed different pathogenicity and immunogenicity and required an adjusted control strategy for control. The first reported strains are the classical pathotypes followed by variant strains in the United States in the 1980s. The very virulent pathotype which can lead to a severe form of the disease was recorded in Europe and Asia in the 1980s after that spread to different parts of the world, including Asia, the Middle East, and Africa (Sedeik *et al*., 2018; Jackwood *et al*., 2018).

In Egypt, the first report of IBDV occurred in the 1970s and is still considered a threat affecting the poultry production sector which is a vital section of the Egyptian agricultural sector (El-Sergany *et al*., 1976). From IBD's first record till now its epidemiological situation become more complicated due to the emergence of new strains. Despite massive vaccination against IBD using both live attenuated and inactivated vaccines, not only the disease remains endemic in Egypt but also the vvIBDV emerged in Egypt in the 1990s which is considered a significant turning point. The presence of vvIBDV leads to extra severe outbreaks which require more measures to monitor and control the disease (Samy *et al*., 2020).

Recently, the Egyptian epidemiological situation of the IBD has been complicated by the emergence of variant IBDV in addition to the previously circulating classical and very virulent strains. These variant strains have considerable antigenic mutation from classical and very virulent strains that increase their ability to evade immunity produced by traditional vaccines (Shahein *et al*., 2024). These Variant strains cause subclinical infection which leads to bursal atrophy without obvious clinical signs usually seen in vvIBDV infection. Even with the lower mortality rates of these variant strains, it usually incriminated with a major significant immunosuppression effect which predisposes chicken to secondary infections and vaccination program failure (Li *et al*., 2023).

Several studies have documented the co-circulation of classical, variant, and very virulent IBDV strains in the Egyptian fields, The co-circulation of these different strains not only confuses diagnosis and complicates the vaccination process but also increases the chance of reassortment between different viruses, potentially leads to the emergence of novel strains with unpredictable character (Samy *et al*., 2020; Legnardi *et al*., 2023; Morsi *et al*., 2024).

Recent studies in Egypt have identified naturally occurring reassortant strains of infectious bursal disease virus (IBDV), posing new challenges for disease control in poultry (Samy *et al*., 2020). These reassortants often combine genetic material from very virulent IBDV (vvIBDV) strains, potentially increasing pathogenicity. In Poland, a novel reassortant population with vvIBDV segment A and an unidentified "transitional-lineage" segment B was discovered, showing expansion into Finland (Pikuła *et al*., 2020). Contrary to previous assumptions of intermediate pathogenicity in reassortants, a field isolate in Poland (Bpop/03) with vvIBDV segment A and classical attenuated D78-like segment B caused 80% mortality in experimentally infected chickens (Pikuła *et al*., 2018). These findings highlight the complex nature of IBDV virulence and emphasize the need for continued surveillance and updated control strategies.

Molecular tools have employed to sequence and study the VP2 and VP1 genes, which give insights into the genetic among various IBDV strains and aiding in the identification of reassortment processes. Islam *et al*. (2021) developed a genotype classification system of IBDV based on two segments of the viral genome, segment A and segment B which encodes the structural proteins and RNA-dependent RNA polymerase, respectively. This system combines the classical, very virulent, variant, and recombinant strains based on the genetic properties of both segments and provides a more accurate genotyping of IBDV. The classification helps to explain the virus's evolution and to develop vaccines and disease control measures because it reveals the possible consequences of genetic reassortment and recombination for viral behaviour and pathogenicity.

The virulence and pathogenicity of infectious bursal disease virus (IBDV) are primarily determined by specific amino acid residues in the VP2 protein. Studies have identified key mutations in VP2 that affect viral replication, cell tropism, and virulence. Residues 253 and 284 in VP2 play a crucial role in cell tropism and virulence of very virulent IBDV (vvIBDV) (Qi *et al*., 2009). Additionally, residues 249 and 256 in VP2 contribute to viral replication efficiency and virulence (Qi *et al*., 2013). The VP1 protein, encoded by segment B, also influences viral pathogenicity and

Table 1: Samples background information of analysed in this study.

	Samples Name	Province	Collection Year	Host	Age (days)	Vaccination
3	IBDV-KN28-2024	Menoufia	2024	Broiler	33	Transmune 2512
15	IBDV-KN64-2024	Giza	2024	Broiler breeder	23	Vaxitech
16	IBDV-KN83-2024	Menoufia	2024	Broiler	25	Vaxitech
17	IBDV-KN19-2024	Giza	2024	Broiler breeder	27	Transmune
18	IBDV-KN36-2024	Giza	2024	Broiler	17	Vaxitech
19	IBDV-KN42-2024	Giza	2024	Broiler breeder	26	Bursine
20	IBDV-KN39-2024	BEHIRA	2024	Broiler	30	Bursine
22	IBDV-KN63-2024	BEHIRA	2024	Broiler	23	Vaxitech
24	IBDV-KN45-2024	Menoufia	2024	Broiler	18	Transmune 2512
27	IBDV-KN81-2024	Dakahlia	2024	Broiler	28	Vaxitech
30	IBDV-KN68-2024	Dakahlia	2024	Broiler	23	Transmune 2512
1	IBDV-KN15-2023	Giza	2023	Broiler	25	NV
2	IBDV-KN18-2023	Giza	2023	Broiler breeder	26	Transmune 2512
4	IBDV-KN31-2023	Dakahlia	2023	Broiler breeder	28	Transmune 2512
5	IBDV-KN29-2023	Dakahlia	2023	Broiler	31	Transmune 2512
6	IBDV-KN21-2023	BEHIRA	2023	Broiler	21	Vaxitech
7	IBDV-KN25-2023	BEHIRA	2023	Broiler breeder	24	Vaxitech
8	IBDV-KN23-2023	Giza	2023	Broiler	18	Vaxitech
9	IBDV-KN24-2023	Giza	2023	Broiler breeder	18	Bursine
10	IBDV-KN33-2023	Giza	2023	Broiler	23	Vaxitech
11	IBDV-KN73-2023	Menoufia	2023	Broiler breeder	19	Vaxitech
12	IBDV-KN34-2023	Giza	2023	Broiler	26	Transmune
13	IBDV-KN48-2023	Giza	2023	Broiler breeder	28	Vaxitech
14	IBDV-KN49-2023	BEHIRA	2023	Broiler	20	Vaxitech
21	IBDV-KN16-2023	Menoufia	2023	Broiler	21	Vaxitech
23	IBDV-KN32-2023	BEHIRA	2023	Broiler	27	Bursine
25	IBDV-KN22-2023	Menoufia	2023	Broiler	19	Vaxitech
26	IBDV-KN27-2023	Dakahlia	2023	Broiler	25	Transmune 2512
28	IBDV-KN14-2023	BEHIRA	2023	Broiler	20	Bursine
29	IBDV-KN50-2023	Menoufia	2023	Broiler	29	Vaxitech

replication. A single amino acid substitution (V4I) in VP1 can attenuate vvIBDV virulence in chickens while increasing replication in cell culture (Yu *et al*., 2013). These findings provide valuable insights into the molecular determinants of IBDV virulence and pathogenicity, which can be utilized for the development of more effective vaccines against this economically important poultry pathogen. This work aims to examine the issues by analysing the prevalence and genetic variation of IBDV strains collected from different areas of Egypt, with a particular focus on understanding the co-circulation of these strains and their impact on virus reassortment.

MATERIALS AND METHODS

Study Design and Sample Collection

To examine the incidence and genetic diversity of IBDV in four Egyptian governorates: Giza, Menoufia, Dakahlia, and Behira, bursal samples (10 bursa of Fabricius/farm) from 30 chicken broiler farms were collected as showen in Table 1. The flock ages range from 17 to 33 days. Birds in these flocks have a history of various immunization programs, including live, immunocomplex, and recombinant vaccines. The selection of these flocks was based on clinical signs and postmortem examination for IBD and/or immunosuppression. The chicks in these flocks showed ruffled feathers, severe depression, trembling, white watery diarrhoea, severe prostration, vent picking, urate-soiled vent feathers, anorexia, and dehydration. On postmortem examination, chickens show enlargement of the Fabricius bursa and renal nephrosis, extremely dehydrated carcasses, and an enlarged spleen with grey foci. All samples were collected aseptically, deposited directly into sterile containers, and transported to the laboratory in a controlled environment.

IBDV Screening in Field Sample

To make 10% tissue suspensions, the bursa was homogenised with phosphate-buffered saline with Kanamycin (700 µg/ml). To remove cell debris, the tissue suspension was frozen and thawed three times before being centrifuged at 8000 rpm for five minutes. The supernatant was collected, filtered using a 0.45µm Sartorius filter (Germany), and stored in a -80°C freezer (Panasonic, Japan) until use. RNA was extracted directly from Fabricius pooled samples using a QiaAmp® Viral RNA Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. The isolated RNAs were kept at -80°C for future studies. The extracted RNAs were evaluated using the Kylt® IBDV kit (AniCon Lab-GmbH Germany-www.kylt.eu), a closed Real-Time RT-PCR detection system that targets the IBDV VP2 gene, according to the manufacturer's instructions. The reactions were incubated at the kit's temperature parameters in a Stratagene® real-time machine.

Virus Isolation

The tissue suspensions from the positive samples were prepared and then inoculated into 10- to 11-day-old embryonated chicken eggs (ECE) using the chorioallantoic membrane (CAM) route. This process involved gently piercing the eggshell at a designated point, allowing direct access to the CAM, where the suspension was introduced to facilitate viral replication. The inoculated eggs were then incubated at 37°C for several days and monitored for any signs of infection or embryonic death, which would indicate successful viral growth.

Table 2: Primer sequences used for amplification of VP 1-2 gene.

VP2-F673	GTAACAATCACACTGTTCTCAGC
VP2-R1345	TTATGTCTTAGAAGCCAAATGC
VP1-R1150	GAGATCATGAGGTGTGTTGG
VP1-F750	GGGCTTGTCATCCTCACCGG

Genetic Characterization of Positive Samples

The positive RNA extracts for IBDV were sequenced for partial VP1 and VP2 genes. First, both genes were amplified for partial sequence using primers specific to each gene, as shown in Table 2. These primers were used to amplify a 380-bp fragment of VP1 and 620 bp fragment of the VP2 gene, including the hypervariable region HVR, using an Easyscript® one-step RT-PCR kit (cat. No. AE411-02 TransGen Biotech) at an annealing temperature of 55°C for 1 minute. After amplification, 5µl of PCR products were electrophoresed on a 1.5% agarose gel with ethidium bromide (final concentration of 0.5µg/ml) at 95 V for 30 minutes in 1x TBE buffer against the GeneRuler™ 100 bp Plus DNA ladder (Fermentas). The amplified products were purified using the QIAquick Gel Extraction Kit (QIAGEN, USA), following the manufacturer's protocol. The purified amplified products of both the VP1 and VP2 genes were processed using a big dye terminator kit (Applied Biosystems CA, USA), and the prepared sequence reactions were read by an ABI (Applied Biosystems 3500xl genetic analyser, USA).

Sequence and Phylogenetic Analysis of VP2 Gene Hypervariable Region and VP1

As previously stated, these isolates were molecularly identified using RT-PCR, and the PCR products were visualised using gel electrophoresis. The gel containing the expected size was excised and purified using the QIAquick Gel Extraction Kit (QIAGEN, USA) according to the manufacturer's protocol. The purified PCR products were sequenced directly with the Big Dye Terminator V3.1 cycle sequencing kit (Perkinelmer, Foster City, CA, USA). The sequencing reactions were then purified using a spin column Centrisep® kit (Applied Biosystems, USA) to remove any extra free dNTP bases, before being loaded into an ABI sequencer plate (Applied Biosystems 3500xl genetic analysers, USA) to determine the virus nucleotide sequences. The deduced amino acid and nucleotide sequences of IBDV were aligned using the CLUSTAL-W method and subjected to BLAST searches [https://blast.ncbi.nlm.nih.gov/Blast.cgi] to determine their identity with other strains.

The nucleotide sequence data were analysed using BioEdit software. A total of 84 Serotype-I IBDV sequences with the highest homology were compared and identified using the study sequence. The phylogenetic tree for nucleotide sequences was constructed using Egyptian viruses, as well as other international reference and vaccine strains. The bootstrap method was used to estimate the topological accuracy of the phylogenetic tree with 1000 replicates, and the neighbour-joining method was used to determine the inference of phylogenetic relationships in MEGA-6 (Tamura *et al.*, 2013).

RESULTS AND DISCUSSIONS

RNA Extraction, PCR Amplification, and Sequencing

All samples were successfully extracted, yielding high-quality RNA that is appropriate for further investigation. The VP2 and VP1 genes of IBDV were amplified using RT-PCR, resulting in expected amplicons of 620 bp for VP2 and 380 bp for VP1. The gel electrophoresis analysis revealed distinct bands that corresponded to the genes present in each sample.

Phylogenetic Analysis of VP2 Gene

The study of VP2 gene sequences using phylogenetic methods revealed significant genetic diversity across IBDV

strains. As shown in Table 3 and Figure 1, The strains were analysed from in four Egyptian governorates categorised into three distinct groups: classical, variant, and very virulent IBDV strains. More precisely, 15% of the strains were categorised as classical, 80% as variant, and 5% as vvIBDV. Significantly, the high percentage of variant strains, suggests that these strains were more prevalent than very virulent and classical IBD strains.

Table 3: Gene Bank Accession Numbers of VP2 gene and related genotype pf each sample.

Samples Name	Gene Bank Accession No	genotype
IBDV-KN15-2023	PQ232523	very virulent strain A3
IBDV-KN18-2023	PQ232504	Classical A1
IBDV-KN28-2023	PQ232505	Classical A1
IBDV-KN31-2023	PQ232506	Classical A1
IBDV-KN29-2023	PQ232507	nVAR strain A2
IBDV-KN21-2023	PQ232508	nVAR strain A2
IBDV-KN25-2023	PQ232509	nVAR strain A2
IBDV-KN23-2023	PQ232510	nVAR strain A2
IBDV-KN24-2023	PQ232511	nVAR strain A2
IBDV-KN33-2023	PQ232512	nVAR strain A2
IBDV-KN73-2023	PQ232513	nVAR strain A2
IBDV-KN34-2023	PQ232514	nVAR strain A2
IBDV-KN48-2023	PQ232515	nVAR strain A2
IBDV-KN49-2023	PQ232516	nVAR strain A2
IBDV-KN64-2023	PQ232517	nVAR strain A2
IBDV-KN83-2023	PQ232518	nVAR strain A2
IBDV-KN19-2023	PQ232519	nVAR strain A2
IBDV-KN36-2023	PQ232520	nVAR strain A2
IBDV-KN42-2024	PQ232521	nVAR strain A2
IBDV-KN39-2023	PQ232522	nVAR strain A2

As shown in Figure 2, the variant strains showed diverse amino acid substitutions in the hypervariable region of VP2, specifically in Major Hydrophilic Peaks A and B, as well as variations in Manor Hydrophilic Peaks 1 and 2, all of which were associated with antigenicity. These alterations may let the variant strains bypass the immunity afforded by standard immunizations.

Phylogenetic Analysis of VP1 Gene and Detection of Reassortment Events

As shown in Table 4 and Figure 3. We analysed five selected samples one of them related to very virulent strains and four related to variant strains. The analysed strains revealed that the VP1 sequence of the very virulent (vvIBDV) strain clustered closely with the B1 genotype, matching known vvIBDV strains with high pathogenicity. In contrast, the VP1 sequences of the four variant strains were found to be like the B2 genotype. The distinct separation of VP1 sequences into B1 and B2 genotypes for vvIBDV and variant strains, respectively, suggests a well-defined genetic divergence between these groups. The consistency of these findings across the sampled strains demonstrates the stability of the VP1 gene within each strain category, as well as the lack of significant genetic drift within these genotypes in the current study.

Figure 1: Neighbor-joining phylogenetic tree with 1000 bootstrap repeats tests phylogeny method and Tamura 3-parameter model of the nucleotide's sequences of 620bp IBDV-VP2 gene hypervariable region. The IBDV –VP2 isolates in the study marked as red rhombus.

In this study, it compared the phylogenetic trees for both segments to investigate the possibility of reassortment events between segments A (which contains the VP2 gene) and B (which contains the VP1 gene). Our analysis found no evidence of reassortment between segments A and B in the tested strains. The very virulent strain's VP1 gene consistently aligned with the B1 genotype, whereas the VP2 gene corresponded to the typical vvIBDV profile. Similarly, the variant strains' VP1 genes were identical to the B2 genotype, indicating that they didn't undergo reassortment

between segments A and B with other circulating strains. The lack of reassortment observed in this study suggests that the classical, vvIBDV, and variant strains coexisted in Egypt and maintained their genetic integrity in these two genomic segments.

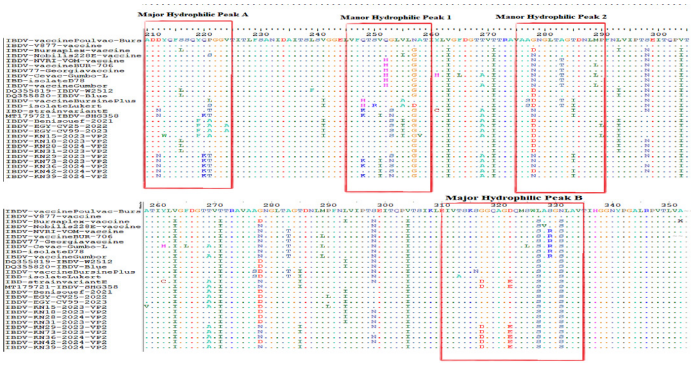

Figure 2: Amino acids alignment report of partial sequence of VP2 gene of IBDV and other Egyptian and representative vaccinal strains.

Table 4: Gene Bank Accession Numbers of VP1 gene and related genotype of each sample.

Samples Name	Gene Bank Accession No	Phenotype
IBDV-KN23-2023	PQ232499	Classical or nVAR strain B1
IBDV-KN34-2023	PQ232500	Classical or nVAR strain B1
IBDV-KN42-2023	PQ232501	Classical or nVAR strain B1
IBDV-KN64-2023	PQ232502	Classical or nVAR strain B1
IBDV-KN15-2023	PQ232503	very virulent strain B2

Together with the development of vaccines, it is essential to have a comprehensive understanding of the genetic diversity, evolutionary dynamics, and epidemiological conditions of IBDV in Egyptian poultry to develop effective disease control strategies. Three different strains of the virus coexist in the Egyptian poultry production sector, and these strains are classical, variant, and very virulent. The significant genetic variability that was found among IBDV strains in this study is in line with the findings that were discovered in the past in regions that include a significant amount of poultry farming. Significantly, we discovered that variant strains of IBDV were prevalent and that these strains accounted for the majority of the isolates that were investigated. Recent research carried out in the Middle East and Asia indicates that variant strains are gradually replacing classical and vvIBDV strains in the field. Furthermore, these strains are becoming more prevalent (Legnardi *et al*., 2023; Shahein *et al*., 2024).

The immune response that is elicited by the established vaccination methods may be the reason why these viruses have a distinct advantage, as the persistent prevalence of variant strains suggests that they have this advantage. The ability of variant strains to evade the immunity induced by the existing traditional vaccines is something that has been observed in several different studies, (Pikuła *et al*., 2021; Rautenschlein and Alkie, 2016). The current findings highlight the importance of updating existing vaccine formulations, which are primarily based on classical strains, to better line up with the antigenic characteristics of variant strains that are currently in circulation. According to the findings of the analysis of the VP1 and VP2 gene sequences, distinct clustering patterns were discovered that differentiated vvIBDV from variant strains without recorded reassortment.

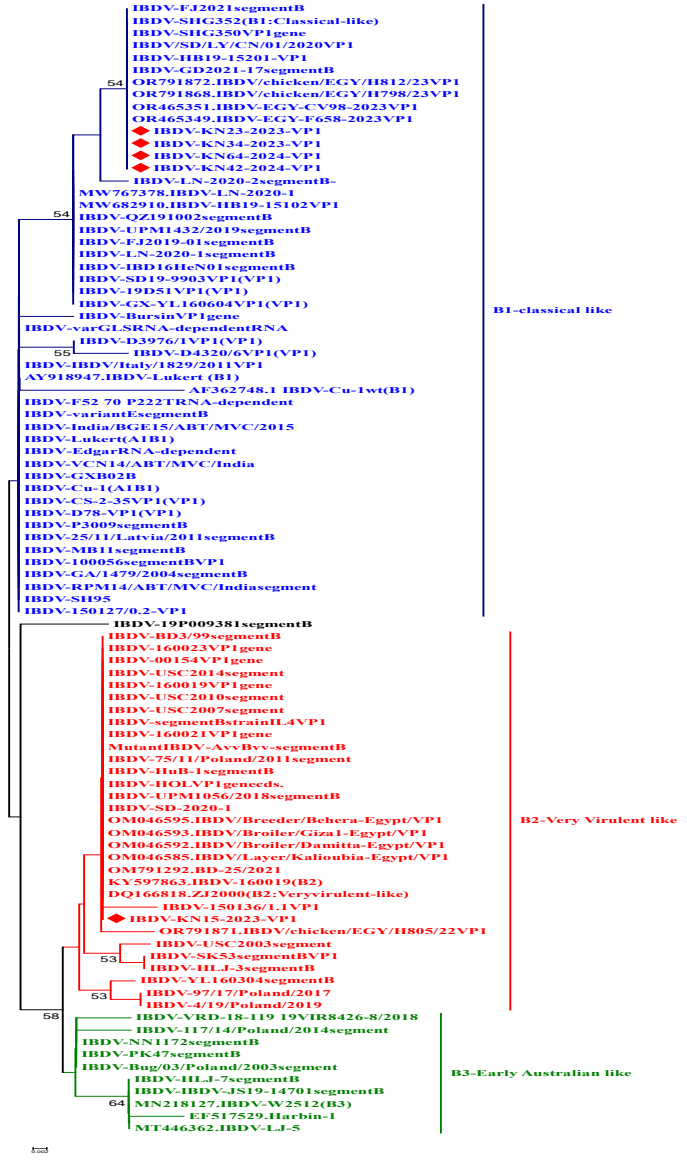

Figure 3: Neighb.or-joining phylogenetic tree with 1000 bootstrap repeats tests phylogeny method and Tamura 3-parameter model of the nucleotide's sequences of 420bp IBDV-VP1 gene hypervariable region. The five IBDV – VP1 isolates in the study marked as red rhombus.

This discovery adds to the evidence that demonstrates the long-term stability of these lineages in Egypt. When compared to findings from other areas where reassortment has played a role in the development of new IBDV strains

with modified pathogenicity, the absence of reassortment between VP1 and VP2 genes in our study is remarkable. This is especially true when compared to the findings from other areas (Reddy *et al*., 2024; Pikuła and Lisowska 2022; Mató *et al*., 2020).

Based on the findings that we have obtained; it appears that the evolutionary patterns of IBDV in Egypt are characterised by consistent lineages rather than frequent genetic recombination. The stability that was observed here may make it less likely that new strains will emerge with a higher level of virulence. Having said that, it also highlights the continued presence of established lineages, particularly the variant strains. Despite this, the fact that this study did not include any reassortment does not declare the possibility of future genetic shifts. Because the introduction of new IBDV strains or changes in farming practices could potentially alter the evolutionary environment, which could lead to the emergence of reassorted or recombinant strains with unique antigenic properties, continuous monitoring and genomic surveillance are extremely important (Pikuła *et al*. 2021).

Comparing our findings to those of earlier research carried out in Egypt and the countries that are geographically neighbouring to it, we discovered that the genetic composition of IBDV presents both similarities and differences. According to the findings of (Legnardi *et al*., 2023; Shahein *et al*., 2024), who also observed a high occurrence of variant IBDV in Egyptian poultry, the prevalence of variant strains in our study is consistent with those findings. On the other hand, the results of our study, oppose those of earlier research, which found that vvIBDV was the predominant strain in certain regions of Egypt during the early stages of the disease (Samy *et al*., 2020). This shift in the main circulating strains may be attributed to changes in the evolutionary dynamics of the virus, as well as the impact of vaccination and biosecurity measures that have been implemented over the past decade.

The strategy of this disease control in addition to the current vaccination programs can be the main reason for this recorded result of the high prevalence of variant IBDV strains in Egypt. It is possible that those that primarily use classical strains, do not provide sufficient immunity against the dominant variant strains. This could ultimately lead to the ineffectiveness of the vaccine and the ongoing spread of the virus (Thai *et al*., 2021). Specifically tailored to the antigenic characteristics of the local variant strains, our findings provide strong support for the necessity of developing new vaccines that are specifically tailored to these characteristics. These vaccines need to be tested to determine whether they can protect against multiple strains to guarantee widespread immunity. In addition, the results of our study emphasise the importance of maintaining a consistent monitoring and genetic analysis of IBDV. These efforts are necessary to identify shifts in the viral population and to determine how vaccination strategies should be adjusted appropriately. The incorporation of molecular tools, such as the sequencing of the next generation, into routine surveillance programs, presents an opportunity to enhance the early detection of new strains and the overall control of IBD in the poultry industry (Nour *et al*., 2023). In the future, research should concentrate on expanding the geographical scope of IBDV surveillance by incorporating a greater number of regions across Egypt. This will allow for a more comprehensive understanding of the situation throughout the whole country. Because of this, we will be able to acquire a more comprehensive conception of the variations in genotype prevalence and reassortment that the virus exhibits across all countries.

CONCLUSIONS AND RECOMMENDATIONS

The purpose of this study is to provide a comprehensive analysis of the genetic distribution of IBDV in four governorates in Egypt. Within this analysis, the prevalence of variant strains and the consistent evolution of established lineages are brought to light. We have an urgent need for updated vaccination strategies that can deal with the immunological variability of IBDV in the Egyptian field, and the findings of our study support this need. In the future, it will be essential to conduct genetic analyses and continuous monitoring to lessen the significant impact that IBD has on the poultry industry and to guarantee Egypt's food security.

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NOVELTY STATEMENT

This study provides a comprehensive genetic assessment of Infectious Bursal Disease Virus (IBDV) strains in Egypt, focusing on the unique co-circulation of classical, variant, and very virulent (vvIBDV) strains. Through detailed VP1 and VP2 gene analysis, the research reveals that variant strains dominate Egyptian poultry fields.

AUTHOR'S CONTRIBUTIONS

The authors contributed to this study as follows: Khalid Nabieh and Wael K. Elfeil conceived and designed the study, carried out sample collection, and drafted the initial

manuscript. Magdy F. Elkady and Wael K. Elfeil supervised the project, provided critical revisions, and contributed to data interpretation. Ahmed Ali assisted in data analysis and interpretation, performed molecular characterization, and reviewed the manuscript. Khalid Nabieh and Ali Zanaty conducted the phylogenetic analysis and contributed to the interpretation of results, while Wael K. Elfeil supported laboratory work and data processing and contributed to manuscript editing and proofreading. All authors read and approved the final manuscript.

Consent For Publication

Not applicable.

Availability of Data and Material

On request, the corresponding author will give the datasets generated and/or analysed during the present work.

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Conflict of Interest

The authors have declared that they have no conflicting interests.

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