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Original Article

Molecular characterization and pathogenicity evaluation of recent infectious bursal disease virus strains: implications for Newcastle disease vaccine efficacy

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Infectious bursal disease continues to cause significant economic losses in the Egyptian poultry industry despite intensive vaccination programs. This study was aimed to molecularly characterize the circulating infectious bursal disease virus strains in Egypt and to compare the pathogenic and immunosuppressive effects of very virulent and variant strains. Ten pooled bursal samples from suspected broiler flocks were subjected to reverse transcription polymerase chain reaction for detection and identification. Nine samples tested positive. Sequencing and phylogenetic analysis identified seven samples (D1, D3, D4, D5, D6, D7, and D8) as very virulent-like strains, showing 98.8-99.2% amino acid sequence identity among them, but only 87.8-91% identity with vaccine strains used in Egypt. Two samples (D9 and D10) were identified as variants with 96-96.4% identity with other Egyptian variants. Two isolates (D8 and D10) were selected to study their pathogenicity and immunosuppressive effects in specific pathogen free chickens, which were orally infected with 10^5 egg infective dose 50% of each isolate and vaccinated against Newcastle disease, 5 days before infection. The variant strain caused earlier and more severe bursal damage without clinical signs or mortality, while the very virulent strain led to typical disease symptoms and 60% mortality. The mean hemagglutination inhibition titers were lower in variant-infected chickens, while protection against Newcastle disease virus was 60% and 40% in very virulent and variant-infected chickens, respectively, compared to 90% in uninfected chickens. These findings indicate that variant strains are more pathogenic and immunosuppressive than very virulent strains, highlighting the need for effective control measures.

Keywords: infectious bursal disease virus; RT-PCR; pathogenicity; Newcastle disease virus; hemagglutination inhibition test.

Introduction

Newcastle disease (ND) and infectious bursal disease (IBD) represent a significant threat to the Egyptian poultry industry, leading to considerable economic losses due to reduced productivity, immunosuppression, and increased mortality. Despite ongoing control efforts, sporadic outbreaks continue to affect poultry flocks. The infectious bursal disease virus (IBDV), responsible for IBD, is classified into two serotypes, I and II, within the family *Birnaviridae* and genus *Avibirnavirus*. Serotype I

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strains are pathogenic, targeting immunoglobulin M+ (IgM+) B cells in the bursa of Fabricius, and this leads to immunosuppression, increased susceptibility to secondary infections, and vaccine failures.[1] In contrast, serotype II strains are non-pathogenic.

The viral genome consists of two segments of double-stranded RNA (segments A and B), which are prone to frequent genetic mutations, reassortments, and recombinations. These genetic changes can affect virulence and antigenicity, thereby influencing vaccine efficacy.[2] Segment A encodes structural proteins (VP2 and VP3), a viral protease (VP4), and a nonstructural protein (VP5), while segment B encodes VP1, essential for viral replication.[3] The VP2 protein, organized into base (B), shell (S), and projection (P) domains, features a hyper-variable region (HVR) spanning amino acid residues 206-350. This region contains hydrophilic sites at residues 210-225 (peak A), 247-254 (minor peak 1), 281-292 (minor peak 2), and 312-324 (peak B), which are critical for pathogenicity and vary among strains.[4]

Serotype I strains are classified into four pathotypes: classical virulent (cvIBDV), antigenic variant (VarIBDV), very virulent (vvIBDV), and attenuated (atIBDV), with recent identification of novel variants.[5] Recent reports from Egypt highlight an increase in the virulence and severity of both vvIBDV and VarIBDV strains. These strains pose significant challenges to conventional vaccines, penetrate maternal immunity, and contribute to high mortality rates in young chickens.[6]

As IBDV targets the bursa of Fabricius and causes immunosuppression, it makes chickens more susceptible to other diseases and reduces the effectiveness of vaccines, including those for Newcastle disease virus (NDV). NDV is a highly contagious virus that cause severe respiratory and neurological disease in chickens. Despite vaccination efforts, NDV outbreaks persist, particularly in farms where IBDV is prevalent.[7] The emergence of novel VarIBDV strains exacerbates this issue, as these new variants may penetrate maternal immunity, complicate the immune response, and thereby potentially reduce the effectiveness of NDV vaccines.[8]

Understanding how these novel IBDV variants impact NDV vaccine efficacy is crucial for improving poultry health management. This research aimed to molecularly characterize the currently circulating IBDV strains, assess the pathogenicity of vvIBDV and VarIBDV field isolates, investigate the effect of mutations on virulence, and explore their implications for immune responses to ND vaccination. By providing these insights, the study seeks to optimize vaccination strategies and improve control measures against both IBD and ND in poultry.

Materials and Methods

Specific pathogen free embryonated chicken eggs (SPF-ECE)

SPF-ECE were obtained from the specific pathogen free (SPF) production farm, Koum Oshiem, El-Fayoum, Egypt and were used for isolation and titration of IBDV field strains according to World Organization for Animal Health (WOAH).[9]

Experimental SPF free chickens

One hundred and fifty 18-day-old SPF chickens, obtained from a farm in Koum Oshiem, El Fayoum, Egypt, were raised on the floor under strict hygienic conditions and fed a balanced commercial diet. They were required for pathogenicity and immunosuppression assessment.

Reference virus

The Bursa-Vac® IBD vaccine, supplied by Receiving sample office of Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), was used as a positive control for virus detection by conventional RT-PCR.

Challenge ND virus

The virulent ND virus, with an infectivity titer of 10^6 egg infective dose 50% (EID_{50})/mL, was kindly supplied by the Newcastle Disease Research Department at the Veterinary Serum and Vaccine Research Institute in Abbasia, Cairo.

Sample collection and preparation

Between 2022 and 2023, a total of 10 pooled hemorrhagic bursal tissues (seven bursas from each farm) were collected from freshly dead chickens in El-

Mansoura, El-Menia, and El-Sharkia governorates. All farms had a vaccination history against IBDV, using commercial live vaccines administered one or two times via drinking water. The samples were prepared according to WOAH[9] and coded as D1 to D10.

Molecular identification of IBDV by conventional one-step RT-PCR assay

Viral RNAs were extracted from bursal homogenates using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's guidelines. The RT-PCR was carried out using the forward primer AUS GU (5'-TCA CCG TCC TCA GCT TAC CCA CAT C-3') and reverse primer AUS GL (5'-GGA TTT GGG ATC AGC TCG AAG TTG C-3'), amplifying a 620 bp fragment within the HVR of the VP2 gene. The thermal profile employed was: 20 min at 50°C (RT reaction), followed by 95°C for 15 min (initial PCR activation); then 40 three-step PCR cycles of 94°C for 30 s (denaturation), 59°C for 1 min (annealing), and 72°C for 1 min (extension), with a final extension cycle at 72°C for 10 min on thermocycler (Biometra, Germany) according to Metwalley et al., 2009.[10] The PCR products were analyzed by electrophoresis in a 1.5% agarose gel containing ethidium bromide dye (final concentration 0.5 µg/mL) to show amplification at 620 bp.

Sequencing and phylogenetic analysis

Positive RT–PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. Sequencing of the purified PCR products was carried out using the Bigdye TM Terminator V3.1 cycle sequencing kit (Perkin-Elmer, Foster City, CA, USA) according to the manufacturer's instructions in the ABI PRISM 3130 genetic analyzer (Applied Biosystems) with 80 cm capillaries.

BLAST® analysis was initially conducted to establish the identity of the obtained sequences with other sequences in GenBank. The VP2 gene sequences were then submitted to GenBank using the BankIt tool, the accession numbers are provided in Table 1. The nucleotide and amino acid sequences of the samples were aligned with sequences of IBDV strains, corresponding to different groups (G1-G7), obtained from the National Center for Biotechnology Information. The alignment was performed using the CLUSTAL-W program and the Meg Align module of DNASTAR software (Laser gene version 7.2; DNASTAR, Madison, WI, USA), then exported to MEGA 6 software for construction of a phylogenetic tree using the maximum likelihood methodology with 1,000 bootstrap replicates to assess the robustness of the tree topology. The pairwise nucleotide and amino acid identity percent was calculated using DNA star software (DNA Star, Madison, WI).

Virus isolation and titration

After genetic analysis, two PCR-positive samples were inoculated in SPF-ECE via chorioallantoic membranes (CAMs) route for isolation and titration following standard protocols outlined by WOAH.[9] Subsequently, the isolated IBDV strains were diluted to 10^5 EID_{50}/mL for subsequent experimental infections.

Experimental design for pathogenicity and immunosuppression assessment of IBDV isolates

A total of 150 SPF chickens, 18-day old, were divided into three equal groups (50 chickens/each) for experimental work as follow: the first group (G1) was infected orally with 10^5 EID_{50} of a vvIBDV strain, the second group (G2) was infected orally with VarIBDV strain with the same dose, and the third group (G3) was the uninfected control group. The three groups were housed separately in strictly isolated and disinfected rooms and kept under daily observation for clinical symptoms and mortality. All chickens were vaccinated with inactivated ND vaccine 5 days before experimental infection via subcutaneous route. Three chickens from each group were euthanized at 1st, 2nd, 3rd, 4th and 5th day post-infection (dpi) for postmortem (PM) examination. The bursas were collected and stored in 10% neutral formalin following the methods described by Bancroft and Gamble[11] for pathological examination. Bursa weight ratios (bursa-to-body weight ratio, BBR) were calculated using the formula: bursa weight (g)/live body weight of individual bird (g ×1,000. Additionally, the bursa: body weight index (BBIX) for each day was calculated, including the

Table 1. Accession number for IBDV strains in the current study.

Samples Name	Sample code	Governorate	Age at sampling	Phenotype	GenBank accession number
IBDV-1/Mansoura/Egypt	D1	El-Mansoura	21d	Very virulent	PP508369
IBDV-2/Mansoura/Egypt	D3	El-Mansoura	30d	Very virulent	PP508370
IBDV-3/Mansoura/Egypt	D4	El-Mansoura	25d	Very virulent	PP508371
IBDV-4/Mansoura/Egypt	D5	El-Mansoura	21d	Very virulent	PP508372
IBDV-5/Mansoura/Egypt	D6	El-Mansoura	26d	Very virulent	PP508373
IBDV/Menia/Egypt (A)	D7	El-Menia	28d	Very virulent	PP508374
IBDV/Menia /Egypt (B)	D8	El-Menia	24d	Very virulent	PP508375
IBDV/sharkia/Egypt/ 2023 variant (C)	D9	El-Sharkia	18d	Variant	OR687651
IBD/Sharkia/Egypt/2023 variant (D)	D10	El-Sharkia	19d	Variant	OR687652

standard deviation, using the formula [BBIX= (Bursa: body weight ratios)/(Bursa: body weight ratios in the negative group)]. A BBIX value below 0.7 was regarded as an indicator of atrophy following the criteria established by Lucio and Hitchner.[12]

Individual blood samples were collected from 10 chickens of each group at 7, 14, and 21 days post-vaccination. The antibodies against NDV were measured in each collected serum sample by hemagglutination inhibition (HI) test according to WOAH.[13] On 3rd week post-vaccination, 10 chickens from each group were challenged intramuscularly with NDV at a dose of 10^6 EID_{50}/bird. The chickens were observed daily for clinical symptoms and number of deaths in each group during 14 days post-challenge.

Protection (%) = Number of survivals/Total number of challenged bird X 100.

Ethical approval

All animal experiments were conducted in accordance with ethical standards and protocols approved by the Institutional Animal Care and Use Committee of Zagazig University (ZU-IACUC committee) under approval number ZU-IACUC/2/F/147/2023.

Statistical analysis

The statistical analysis used ANOVA and t-test procedures via the InStat GraphPad program. Differences between mean values were considered statistically significant at $P < 0.05$.

Results

Molecular detection of IBDV

Out of the total 10 of pooled bursal samples, only nine were found positive for IBDV by VP2 gene-based RT-PCR.

Sequencing and phylogenetic analysis

Phylogenetic analysis of VP2 gene revealed that seven samples D1, D3, D4, D5, D6, D7, and D8 were clustered with vvIBDV strains. Conversely, the remaining two isolates (D9 and D10) were grouped with VarIBDV strains. The vvIBDV strains showed 96.8% to 98% amino acid identity with other Egyptian vvIBDV strains, but only 87.8-91% identity with vaccine strains used in Egypt. The VarIBDV strains (D9 and D10) showed 96% to 96.4% identity with other Egyptian variants as shown in Figure 1.

None of the examined samples were of attenuated or vaccinal origin due to absence of 253-Histidine and 284-Threonine substitutions typically found in attenuated vaccine strains.

Virus isolation and titration

Two PCR-positive samples (D8 and D10) were selected for isolation and titration in SPF-ECE. The inoculated

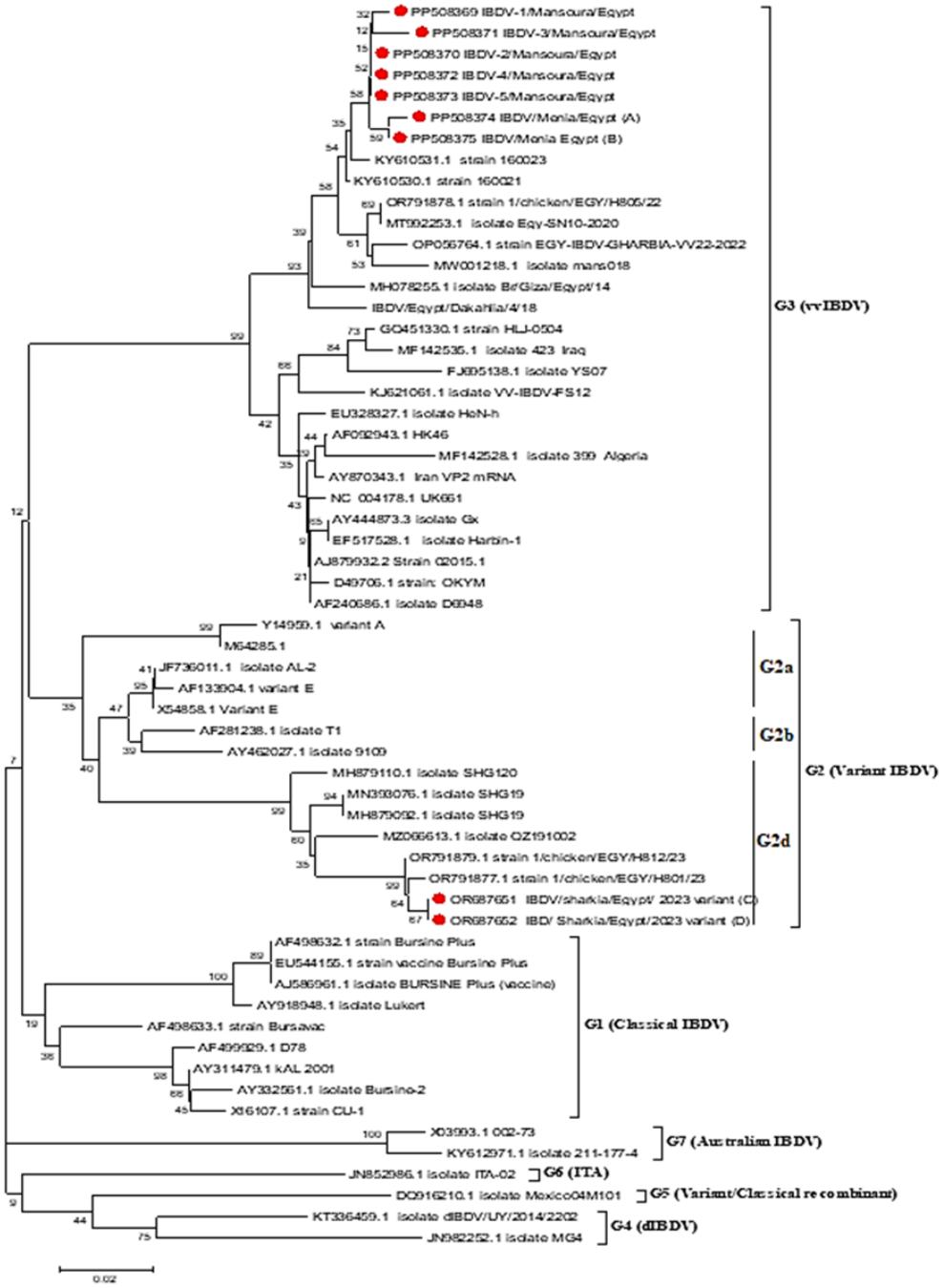

Fig. 1. Phylogenetic analysis of IBDV based on partial nucleotide sequences of the VP2 gene. The isolates from this study are indicated by red circle. G2a and G2b are the American variants; G2d are Chinese variants.

SPF-ECEs showed characteristic embryo lesions during the second passage, including stunted growth, liver necrosis with a mottled appearance, greenish coloration of the liver, and hemorrhage. Additionally, the harvested CAMs exhibited thickening and petechial hemorrhages.

The infectivity titration after the third passage revealed virus titers of $10^{6.5}$ and $10^{5.6}$ $\log_{10}$ EID_{50}/mL for (D8 and D10) isolates, respectively.

Pathogenicity evaluation of the two selected IBDV field isolates

Clinical signs and mortality

Throughout the observation period, the control group (G3) exhibited no mortality or clinical symptoms. In group G1, birds displayed severe clinical manifestations, including depression, anorexia, ruffled feathers, huddling, tremors, prostration and whitish watery diarrhea, resulting in a 60% mortality rate. In contrast, the variant-infected group G2 exhibited mild diarrhea and depression, without mortality. Notably, clinical signs in group G1 were more severe, manifesting as early as 48-hour post-infection.

PM examination

No gross lesions were observed in the control group. In contrast, both infected groups exhibited the characteristic PM lesions of natural IBDV infection. Additionally, hemorrhagic thymic lobes were observed in group G1. As quantified by the BBR, the severity of bursal atrophy was more pronounced in group G2. The VarIBDV strain induced bursal atrophy as early as 72-hour post-infection, while the vvIBDV strain showed bursal atrophy from 96- hour post-infection.

Changes in body weight and relative bursal weight

The infected birds exhibited a significant ($P \leq 0.05$) decrease in both bursal weight and body weight compared to the control group. Additionally, chickens in group G2 demonstrated significantly lower bursal and body weights than those in group G1 throughout the experiment. The BBR decreased from 1.75±0.1 to 0.71±0.12 in group G1 and from 1.93±0.2 to 0.34±0.09 in group G2, in contrast to non-infected control birds in group G3, where the BBR slightly increased from 2.2±0.1 to 2.52±0.1 throughout the experiment. Furthermore, the BBIX decreased significantly ($p < 0.05$) in infected birds compared to negative control group non-infected. The BBIX in group G2 was below 0.7 at 3 dpi and continued to decrease to 0.1 until 5 dpi while in group G1, the BBIX was below 0.7 at 4th dpi and then continued to decrease to 0.28 until 5 dpi. These results suggest that the VarIBDV strain causes earlier and more severe bursal atrophy than the vvIBDV strain, while the control group did not exhibit bursal atrophy, as demonstrated in Table 2.

Immunosuppression effect

The absence of clinical signs, coupled with marked bursal atrophy in chicks infected with the VarIBDV

Table 2. Mean BBR and BBIX in different groups.

Dpi	Groups	Body weight (g)	Bursa weight (g)	BBR	BBIX
1st	G1	723± 0.00	1.3±0.1	1.75±0.1	0.795
	G2	723±0.00	1.4 ±0.17	1.93±0.2	0.877
	G3	739.3±0.57	1.7±0.08	2.2±0.1	-
2nd	G1	748.5±0.00	1.26±0.07	1.66±0.05	0.790
	G2	748.6±1.15	1.3±0.1	1.7±0.1	0.809
	G3	854.6±4.5	1.8±0.1	2.1±0.1	-
3rd	G1	984.6±4.2	1.8±0.1	1.8±0.1	0.782
	G2	985±4.3	1.3±0.1	1.3±0.1	0.565
	G3	1203±5.1	2.8±0.1	2.3±0.1	-
4th	G1	1206.3±5.5	1.56±0.3	1.29±0.25	0.586
	G2	1200.6±0.57	0.73±0.2	0.58±0.16	0.263
	G3	1605±4.58	3.6±0.26	2.2±0.15	-
5th	G1	1204.6±4.1	0.89±0.15	0.71±0.12	0.28
	G2	1202±2.6	0.43±0.1	0.34±0.09	0.134
	G3	1803.3±4.9	4.56±0.2	2.52±0.1	-

Dpi: day post infection. BBR: bursa-to-body weight ratio. BBIX: bursa body weight index. G1: group 1 infected with vvIBDV strain. G2: group 2 infected with VarIBDV strain. G3: uninfected control group. -: BBIX equal to zero.

strain, prompted a focused evaluation of the immunosuppressive properties of this virus compared to a vvIBDV strain.

HI test

The mean HI antibody titers were significantly reduced in the IBDV-infected groups. Group G2 exhibited significantly lower mean HI antibody titers than group G1 at 1st, 2nd, and 3rd weeks post-vaccination. In contrast, control group demonstrated a substantial increase in antibody titers, surpassing those of both groups G1 and G2, as shown in Table 3.

Protection against NDV challenge

Cumulative mortalities in groups G1 and G2 were statistically significantly different from control group ($P < 0.05$). Protection was 60%, 40%, and 90% in groups G1, G2, and G3, respectively.

Table 2. Mean HI antibody titer against NDV vaccine. Data are expressed as mean ±SD.

Chicken group	Mean HI antibody titer (log2)		
	WPV		
	1st	2nd	3rd
G1	2.6 ± 1.07^{d}	4.7 ± 1.16^{d}	5.9 ± 0.94^{a}
G2	1.8 ± 0.63^{d}	3.8 ± 1.03^{d}	5.1 ± 0.94^{a}
G3	4.0 ± 0.94^{d}	6.1 ± 1.10^{d}	7.1 ± 0.99^{c}

Different subscript letters within the same column indicate significant difference at $P<0.05$, a = non-significant ($P>0.05$), b = significant ($P<0.05$), c = highly significant ($P<0.01$) and d = very highly significant ($P<0.001$). HI: hemagglutination inhibition. WPV: weeks post-vaccination. G1: group 1 infected with vvIBDV strain. G2: group 2 infected with VarIBDV strain. G3: uninfected control group. Different superscript letters within the same column indicate significant difference at $P<0.05$.

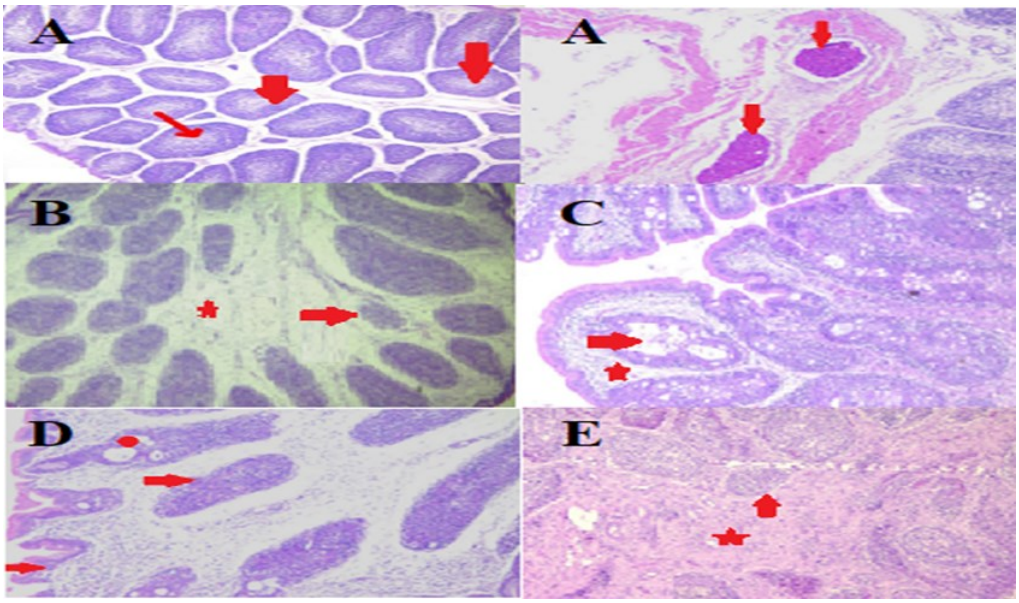

Fig. 2. Histopathology of bursa of Fabricius in group G1. (A) Bursa at 1st dpi showing mild interfollicular edema with mild lymphocyte depletion. The right (A) picture shows congestion and edema in tunica muscularis of bursa. (B) Bursa at 2nd dpi showing lymphocyte depletion, compressed follicles (indicated by arrow), and interstitial edema with infiltration of inflammatory cells (star) H&E X50. (C) Bursa at 3rd dpi showing lymphocyte depletion, follicular cysts (arrow), and interstitial edema with inflammatory cells infiltration (star) H&E X100. (D) Bursa at 4th dpi showing hyperplasia of the epithelium (indicated by arrow) with cysts (circled), lymphocyte depletion, compressed follicles, and interstitial edema with inflammatory cells infiltration H&E X100. (E) Bursa at 5th dpi showing depleted follicles (arrow) and proliferation of interstitial connective tissue (star).

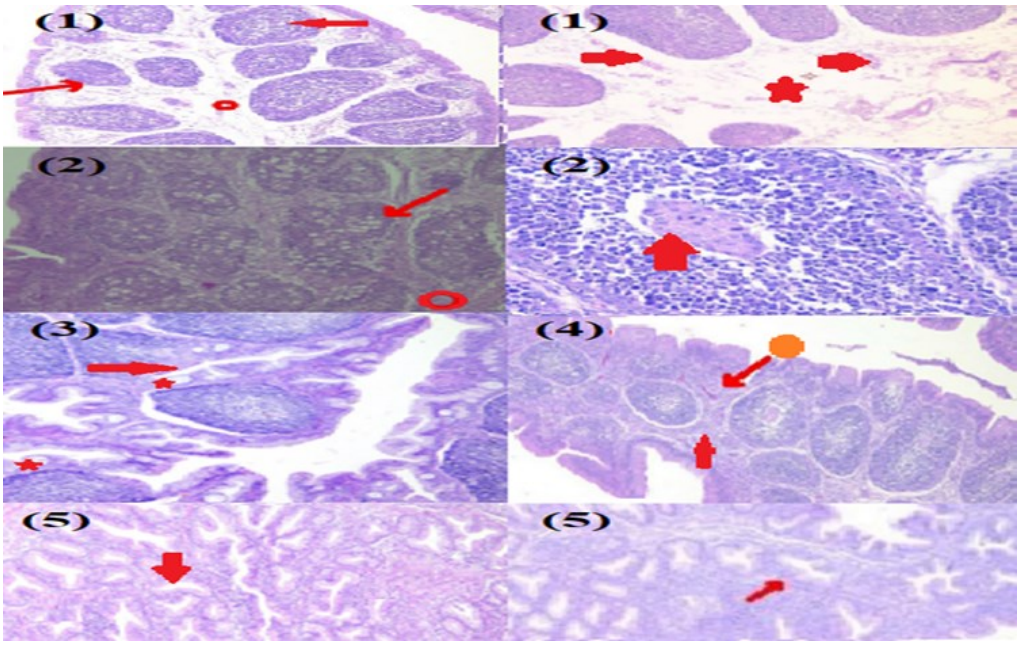

Fig. 3. Histopathology of bursa of Fabricius in group G2. (1) Interfollicular edema with mild lymphocyte depletion (arrow) H&E X100. (2) Bursa at 2nd dpi showing cystic follicles (arrow) with interfollicular connective tissue proliferation (circle) H&E X100, lymphocyte necrosis in medulla (arrow), (3) severe hyperplasia (arrow) and cystic formation of lining epithelium (star), with lymphocyte depletion H&E X100. (4) Atrophy of the plicae with lymphocyte necrosis (circle) in medulla and interfollicular connective tissue proliferation (arrow) H&E X100. (5) Atrophy with severe follicular epithelization & interstitial lymphocytes aggregation.

Histopathological findings

In the assessment of bursal tissues, the control group displayed normal histological architectures, while both infected groups demonstrated mild interfollicular edema with lymphocyte depletion, congestion, and inflammatory cell infiltration.

Significant differences emerged by the 2nd dpi, with group G2 displaying cystic follicles and necrosis, while group G1 exhibited compressed follicles and moderate lymphocyte depletion. By the 3rd dpi, group G2 showed severe hyperplasia of follicular epithelium alongside cystic follicles, severe lymphocyte depletion, and interstitial edema with inflammatory cell infiltration.

Notably, at the 4th and 5th dpi, severe bursal atrophy was more pronounced in group G2 compared to group G1. By the 5th dpi, group G2 exhibited a complete absence of intact lymphoid follicles, substituted by fibrous tissue, with a highly corrugated lining epithelium. These findings collectively indicate a progressive histopathological deterioration, for the VarIBDV strain compared to the vvIBDV strain, as elucidated in Figures 2 and 3.

Discussion

IBD is a highly contagious disease affecting chickens, leading to significant economic losses worldwide through high mortality, immunosuppression, and secondary infections. In Egypt, numerous IBDV outbreaks have been reported within a short period following the emergence of novel VarIBDV strains in 2023.[6,14] Then, updating the current situation is crucial to address this issue effectively and to develop a new strategy for disease control. In this study, IBDV was diagnosed from 10 infected farms in Egypt during 2022-2023. We isolated and molecularly characterized IBDV strains from infected samples and compared their pathogenic features by assessing clinical signs, mortality rates, BBR, and histopathological changes in the bursa. Additionally, we evaluated the impact of these strains on the effectiveness of the inactivated ND vaccine.

Initially, bursal samples were examined using VP2 based RT-PCR, nine out of 10 samples tested were positive. Phylogenetic analysis of the VP2 protein's hypervariable region classified the current isolates into two main pathogenic subgroups. Seven samples (IBDV-1 through

IBDV-5), along with IBDV/Menia/Egypt (A) and (B), were classified as vvIBDV, whereas two (IBDV/sharkia/Egypt/2023 variant (C) and (D) were identified as VarIBDV strains distinct from vaccine strains. The vvIBDV isolates exhibited 98.8% to 99.2% identity between them while they showed a variability of 2-3.2 % with other Egyptian vvIBDV strains at both nucleotide and amino acid sequence. Comparative analysis with classical vaccine strains showed identities ranging from 87.8% to 91%, with the closest similarity observed with Bursa-Vac®. In contrast, VarIBDV strains exhibited higher identities (96% to 96.4%) with other Egyptian variants and approximately 93% with Chinese variants, but lower identities with American strains (89%) and vaccine strains (83.5% to 85.1%). These findings indicate the endemic circulation and rapid evolution of the virus in Egypt over years.[15]

Significant mutations were noticed in the VP2 region's hydrophilic peak A (210-225), crucial for antibody binding, which likely influence virus antigenicity and virulence.[16] VarIBDV strains displayed specific mutations like F220Y, differing from F220S in vvIBDV, potentially impacting immune evasion in vaccinated flocks.[17]

Moreover, specific residues (222A, 256I, 279D, 294I, 299S) were consistently found in all vvIBDV isolates except IBDV-3, which exhibited a different substitution (N instead of D at position 279) compared to other vvIBDV strains.[17] Mutation at position 222 (P222A) may facilitate immune escape, potentially explaining virus persistence in vaccinated flocks.[2] The presence of amino acids 252I and 299S in VarIBDV strains, also found in Chinese variants, underscores their evolutionary relationships.[8]

Amino acids at positions 253 and 284 (Q253H; A284T) are critical for cell tropism and adaptation in cell culture.[18] All IBDV isolates in this study had glutamine at position 253 and alanine at position 284, potentially affecting their growth in cell culture and pathogenicity. Strains with glutamine at 253 are linked to higher pathogenicity compared to those with histidine. This variation is significant given the extensive use of live attenuated viruses in vaccination programs, indicating a potential for these viruses to evolve and change their pathogenicity over time.[19]

Chickens infected with vvIBDV exhibited typical signs and high mortality (60%), consistent with previous reports.[20] In contrast, infection with VarIBDV strains led to severe bursal atrophy without clinical signs, causing immunosuppression and susceptibility to secondary infections.[8,21]

PM examinations revealed that chickens infected with vvIBDV strains exhibited severe bursal atrophy and hemorrhages, including in the thymus which aligns with other findings,[21] suggesting direct viral injury or virus-induced inflammatory responses. VarIBDV infections led to rapid bursal depletion. Both groups had lower body weights compared to controls, with continuous bursal atrophy until the end of the experiment, as reported by others.[8] The BBIX indicated significant atrophy in both groups, with group 2 showing earlier and more severe decline. These findings suggest VarIBDV strains cause more severe bursal atrophy and immunosuppression compared to vvIBDV strains.[8,21]

Histopathological analysis showed that chickens infected with the VarIBDV strain of IBDV experienced more severe and rapid bursal damage compared to those infected with the vvIBDV strain. This aligns with prior studies highlighting the aggressive nature of VarIBDV strains.[8] By the 4th and 5th dpi, the VarIBDV strain group exhibited significantly more pronounced bursal atrophy and complete absence of intact lymphoid follicles by the 5th dpi, with tissue replaced by fibrous tissue and highly corrugated epithelium, indicating severe damage.

Furthermore, the immunosuppressive effect was evident in the VarIBDV strain group, as indicated by significantly lower HI antibody titers against NDV compared to the vvIBDV strain group. This compromised protection against virulent NDV, suggesting a greater impact on NDV vaccine efficacy. This finding is consistent with previous reports that highlight the association between high systemic antibody levels and protection against NDV.[8,9] In contrast, group G3, comprising vaccinated chickens without infection, showed a substantial increase in antibody titers, surpassing those of both infected groups, indicating effective vaccine-induced immunity against NDV despite exposure to VarIBDV. The results of the HI and challenge tests against the NDV vaccine suggest

that both IBD strains induce immunosuppressive effects, with the VarIBDV strain exhibiting a more pronounced effect than the vvIBDV strain.

Our study highlights the coexistence of both vvIBDV and VarIBDV strains in Egypt, underscoring their progressive evolution and persistence. VarIBDV strains exhibit greater pathogenicity and induce more pronounced immunosuppressive effects compared to vvIBDV strains. These findings emphasize the critical need for continuous monitoring and the development of effective vaccines to mitigate the evolving threats to poultry health.

Conflict of interest

The authors declare that there is no conflict of interest.

Author's contributions

Ola Y. Abido: study conception, molecular characterization of collected field samples, design of the work, writing-review and editing.

Karim M. Selim: isolation of collected field samples.

Sara Abdel-Mawgod: molecular characterization of collected field samples.

Mohamed S. Sobh: histopathology for bursa.

Arwa El Naggar: performed the analytic calculation and statistics.

Mohamed M. Shawki: sample collection and study the pathogenicity of variant and very virulent IBDV strains by experimental infection.

Mohamed A. Elhady: supervision, project administration, sample collection.

All authors read and approved the final manuscript.

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Caracterización molecular y evaluación de la patogenicidad de cepas recientes del virus de la bursitis infecciosa: implicaciones para la eficacia de la vacuna contra la enfermedad de Newcastle

Resumen

La bursitis infecciosa continúa causando importantes pérdidas económicas en la industria avícola egipcia a pesar de los intensos programas de vacunación. El objetivo de este estudio fue caracterizar molecularmente cepas del virus de la bursitis infecciosa circulantes en Egipto y comparar los efectos patógenos e inmunosupresores de cepas muy virulentas y variantes. Diez muestras procedentes de pollos de engorde sospechosos de bursitis infecciosa fueron sometidas a la reacción en cadena de la polimerasa de transcripción inversa para su detección e identificación. Nueve muestras resultaron positivas. Mediante la secuenciación y el análisis filogenético se identificaron siete muestras (D1, D3, D4, D5, D6, D7 y D8) como cepas muy virulentas, que mostraban una identidad de secuencia de aminoácidos del 98,8-99,2% entre ellas, pero sólo del 87,8-91% con las cepas vacunales utilizadas en Egipto. Dos muestras (D9 y D10) se identificaron como cepas variantes con un 96-96,4% de identidad con otras variantes egipcias. Se seleccionaron dos aislados (D8 y D10) para estudiar su patogenicidad y efectos inmunosupresores en pollos libres de patógenos específicos, que fueron infectados por vía oral con 10^5 dosis infectivas en huevos 50% de cada aislado y vacunados contra la enfermedad de Newcastle, 5 días antes de la infección. La cepa variante causó lesiones bursales más tempranas y graves sin signos clínicos ni mortalidad, mientras que la cepa muy virulenta provocó síntomas típicos de la enfermedad y un 60% de mortalidad. El título medio de inhibición de la hemaglutinación fue inferior en los pollos infectados por la cepa variante, mientras que la protección contra el virus de la enfermedad de Newcastle fue del 60% y el 40% en los pollos infectados con la muy virulenta y la variante, respectivamente, en comparación con el 90% en los pollos no infectados. Estos resultados indican que las cepas variantes son más patógenas e inmunosupresoras que las cepas muy virulentas, lo que pone de relieve la necesidad de adoptar medidas de control eficaces.

Palabras clave: virus de la enfermedad infecciosa de la bolsa; reacción en cadena de la polimerasa de transcriptasa inversa; patogenicidad; virus de la enfermedad de Newcastle; pruebas de inhibición de hemaglutinación.

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