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# Characterization of vvIBDV in Vaccinated Broilers and Evaluation of Protection of Different Vaccination Programs

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**Abstract**

INFECTIOUS bursal disease (IBD) represents one of the most economically significant diseases affecting young chickens. The objective of this study was to isolate circulating IBDV strains from diseased commercial broiler flocks (n=35) distributed in 7 Egyptian governorates during 2020-2022 and to evaluate the efficacy of different vaccination programs in the protection of commercial broilers against challenge by a very virulent IBDV (vvIBDV) isolate. The detected IBDV were 16 out of 35 (45.7%), from which we selected two pure isolates for a partial sequence of viral protein 2 (VP2). The phylogenetic analysis classified them as A3 genotype, vvIBDV. Experimentally, 135 one-day-old Cobb 500 broilers were used to evaluate 3 vaccination programs against the vvIBDV challenge. Chickens of group 1 (G1) were vaccinated with HVT-IBD vector vaccine, while those in G2 were vaccinated with live intermediate and intermediate plus vaccines (Lukert strain), and G3 were vaccinated with intermediate and intermediate plus vaccines. At the time of the challenge, 28 days of age, each group was subdivided into two categories: vaccinated-challenged (n=15) and vaccinated unchallenged (n=10). Furthermore, 25 broilers in G7 were maintained as a positive control (unvaccinated-challenged) and 25 broilers in G8 were maintained as a negative control (unvaccinated-unchallenged). All vaccination programs are protected from vvIBDV mortality. The vector HVT-IBD vaccine exhibited the greatest performance, the minimal impact on bursal tissue and viral replication, despite a relatively lower antibody titer when compared with classical IBDV vaccines.

**Key words:** Infectious bursal disease virus (IBDV), vvIBDV, Protection, Live IBD Vaccines, HVT-IBD vector vaccine.

## Introduction

Introduction Infectious bursal disease (IBD) represents a significant economic concern due to its immunosuppressive effects on young chickens (3–6 weeks of age) [1]. The infectious bursal disease virus (IBDV) is the causative agent of the disease. It is a non-enveloped virus belonging to the family Birnaviridae and has a bi-segmented double-stranded RNA (dsRNA) genome. The virus primarily targets B lymphocytes in the bursa of Fabricius, leading to immunosuppression [2,3]. The first report of IBD was in the USA in 1957 [4], followed by the first report in Egypt by El-Sergany et al. [5]. The disease caused significant economic losses due to its ability to impair growth and cause immunosuppression [6,7]. During IBDV infection, the bursal activated T-cells limit the replication of IBDV and stimulate damage to the bursal tissue, as well as subsequent

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recovery, by releasing proinflammatory cytokines [8]. The most common inflammatory cytokines showing an increased expression during IBDV infection are transforming growth factor-beta (TNF-β), tumour necrosis factor-α (TNF-α), interferon- γ (IFN-γ), interleukins (IL)-6, 7, 8, 10, and 1β [9-12]. The cytokine responses and IBDV tissue distribution in the bursa of Fabricius have been investigated following a virulent IBDV challenge [12-14]. Given the economic importance of Gumboro disease and the high prevalence of IBDV, vaccination has remained a crucial control measure. The practice of hyperimmunization of breeders, which allows them to transmit high levels of maternal-derived antibodies (MDA), has been successfully employed until the advent of atypical or serological escape variants in the United States and the emergence of very virulent strains in Europe during the 1985- 1987 period [7]. A variety of live-attenuated vaccines against classical IBDV are commercially available and are classified according to their degree of attenuation as "mild," "intermediate," or "intermediate plus or hot" IBD vaccines [15]. Mild and intermediate vaccines are safer than intermediate plus or hot vaccines, which can cause 4 higher levels of MDA. However, they may harm the bursal follicles, leading to immunosuppression [16]. Accordingly, the timing of IBD vaccine administration should be based on the level and half-life of MDA, the age of the chickens at sampling and vaccination, the type and age of IBDV challenge, the genetic background of the chickens, and the IBD vaccine strain [17]. With the advancement of technology, next-generation vaccines have been developed with the advantage of overcoming MDA and are now commercially available. One such example is the IBD vector vaccine, which uses the turkey herpes virus (HVT) as a vector for the IBDV viral protein 2 (VP2) gene [18,19]. The objective of this study was to isolate recently circulating IBDV genotypes and evaluate the efficacy of different vaccination programs in protecting commercial broilers against challenge with a vvIBDV isolate.

## Material and Methods

*Sample collection*

Total of 35 commercial broiler flocks, were distributed across 7 Egyptian governorates (Alexandria, n=11; Beheira, n=10; Qalyubia, n=5; Matrouh, n=4; Gharbia, n=2; Kafr Elsheikh, n=2 and Menoufia, n=1) were investigated for IBDV infection during 2020-2022, as illustrated in Table 1. All the investigated flocks exhibited the typical signs of IBD, which included general weakness, watery-whitish diarrhea and variable increased mortalities. The post-mortem examination revealed the presence of edematous gelatinous bursitis, along with nephritis and splenitis. A total of 10 pooled bursae were collected aseptically from each examined flock and prepared for virus isolation and confirmation via real-time polymerase chain reaction (rRT-PCR)

*RNA extraction*

Viral RNA was extracted from collected bursal samples (n = 10 pooled bursae from each examined flock) using the QIAamp Viral RNA Mini kit (Qiagen, Germany) according to the manufacturer's instructions.

*Real time-polymerase chain reaction (rRT-PCR)*

The primers and probe utilized for the detection of IBDV [20] and IBV [21] were provided by Metabion (Germany). The preparation of the PCR Master Mix for rRT-PCR was conducted according to the instructions provided by the manufacturer of the QuantiTect kit (Qiagen-Germany). The following cycling conditions were employed: 95°C for 10 minutes, followed by 45 cycles at 95°C for 15 seconds (consisting of denaturation) and 60°C for 30 seconds (annealing/extension). The results were analyzed using a computer 7 system (version 2.2.2) supplied with AB Applied Biosystems (Thermos Fisher Scientific - USA).

*Virus isolation and challenge virus titration in specific pathogen-free-embryonated chicken eggs (SPF-ECE*)

A total of 300 SPF-ECE were used. Bursal homogenate of PCR-confirmed samples was subjected to three successive freeze–thaw cycles, after which the supernatants were centrifuged at 5000 g for 10 minutes. The supernatants were introduced into the chorioallantoic membrane (CAM) of 11-days-old SPF-ECE (Nile SPF eggs, Koom Oshiem, Fayoum, Egypt) and incubated at 37°C. The inoculated embryos were candled daily for a period of five days following inoculation. The embryos were examined for the presence of any abnormalities, including subcutaneous hemorrhages, urate flacks and pathological changes in the liver and kidneys. The embryos and CAM were subsequently homogenized and stored at −80°C until required for use. A vvIBDV isolate no. 34 (accession No. OQ756336) was selected as a challenge isolate and inoculated into SPF eggs aged 11 days via the chorioallantoic membrane (CAM) route for propagation and titration purposes, respectively, and subsequently subjected to rRT-PCR analysis.

*Partial sequence analysis of VP2 gene in IBDV isolates and phylogenetic analysis.*

A set of forward, 5'-GCCCAGAGTCTACACCAT-3', and reverse, 5'-CCCGGATTATGTCTTTG-3', primers were used for the amplification of a 742 bp fragment within the VP2 gene, as described by Jackwood and Sommer [22]. The VP2 gene of 2 IBD isolates no. 32 and 34 was partially sequenced in both directions by Macrogen Inc. (Korea). The sequences were then

aligned with those of IBDV retrieved from the GenBank database. The evolutionary analyses were conducted using the MEGA X software. The evolutionary history was inferred using the Neighbor-Joining method with 8 1,000 bootstrap replicates. The evolutionary distances were calculated using the maximum composite likelihood method. All ambiguous positions were removed for each sequence pair, employing the pairwise deletion method. The nucleotide and amino acid alignment and identity percentage were generated using Generous R Basic software, version 7.1.3 (Copyright © 2005–2014 Biomatters Ltd.). The sequences generated in this study have been submitted to the GenBank database.

*Experimental design*

Chickens A total of 135 one-day-old Cobb 500 broilers, obtained from a local commercial hatchery, were randomly divided into five groups (25 birds per group). Blood samples were collected from the 10 birds that were euthanized to detect maternal derived antibody (MDA) levels. The remaining 125 birds were vaccinated with different vaccination programs and subsequently challenged (n=15 in each group). The vaccinated groups were designated as G1, G2, and G3. Groups 4, 5 and 6 were vaccinated and not challenged (n=10 in each group) and were maintained in close contact with groups 1, 2 and 3, respectively. Furthermore, G7 was maintained as a positive control (unvaccinated-challenged, n=25), while G8 was reared as a negative control (unvaccinated-unchallenged, n=25), as illustrated in Table 2.

*vvIBD challenge virus.*

The isolate no. 34 (with accession number OQ756336) was selected for further propagation and experimental challenge. The IBDV challenge dose was 104.5 EID50/mL. One hundred µL per bird was administered via two routes: 50 µL via the oculonasal route and 50 µL orally.

*Performance assessments*

Body weights (Bwt), body weight gain and feed conversion ratio (FCR) were recorded and calculated (n=10 birds/group). Furthermore, all groups were observed daily for the G2 (n=15) Live Intermediate, Lukert strain (Bursine2®, Zoetis, USA) Live Intermediate plus, Lukert strain (Bursine-Plus®, Zoetis, USA) 12 18 + G3 (n=15) Live Intermediate, LIBDV strain (CEVAC GUMBOL®, Ceva Sante Animale, France) Live Intermediate plus, Winterfield 2512 strain (CEVAC IBD-L®, Ceva Sante Animale, France) 12 18 + G4 (n=10) Vector HVT-IBD (Vaxxitek®, Boehringer Ingelheim) 1 - G5 (n=10) Live Intermediate, Lukert strain (Bursine2®, Zoetis, USA) Live Intermediate plus, Lukert strain (Bursine-Plus®, Zoetis, USA) 12 18 - G6 (n=10) Live Intermediate, LIBDV strain (CEVAC GUMBOL®, Ceva Sante Animale, France) Live Intermediate plus, Winterfield 2512 strain (CEVAC IBD-L®, Ceva Sante Animale, France) 12 18 - G7 (n=25) Non + G8 (n=25) Non - 10 presence of any clinical signs, as well as for any mortalities that may have occurred. In addition, a post-mortem (PM) examination was conducted on each animal.

*Blood samples*

Blood samples were collected from the wing vein (10 samples per group) at 3- and 10-days post challenge (DPC). The sera were then separated and stored at -20°C until testing. Before testing, the sera were inactivated at 56°C for 30 minutes.

*Enzyme linked immunosorbent assay (ELISA).*

The presence of maternal derived antibodies (MDAs) and antibody response prior and following the challenge with IBDV were determined in the collected sera using commercial indirect ELISA kits, namely the classical and BD-plus kits (ID Vet, France). This was done in accordance with the instructions provided by the manufacturer.

*Bursa body weight ratio and viral load in bursa of Fabricius post challenge*

At 3 DPC, bursae from all groups (3 bursae per group) were randomly selected, weighed, and taken during postmortem examination. Prior to slaughter, the birds were weighed individually to calculate the bursa/body weight ratio [23]. The extracted RNAs from the individual bursal tissues (n=3 per each group) were analyzed. The amplification and data acquisition were conducted using an AB Applied Biosystems real-time PCR machine.

*Proinflammatory IL-1β and IL-6 cytokines gene expression*

At 3 DPC, individual bursal homogenates were prepared from eight groups (3 bursae per group) for the detection of IL-1β and IL-6 gene expression using rRT-PCR. The genomic RNA was extracted as previously described. Real-time PCR analysis was conducted using the primers outlined in Table 3, with data analysis performed using the ABI Prism 7900HT Sequence Detection System software (AB Applied Biosystems). Duplicate sets of each reaction sample were subjected to the following thermal profile: one cycle of 48°C for 30 minutes and 95°C for 20 seconds, followed by 40 cycles of 95°C for 3 seconds and 60°C 11 for 30 seconds. The amplification data for the cytokines were normalized against 28S rRNA [24], and the fold change in cytokine gene expression was calculated as previously described [25].

*Histopathological examination*

The histopathological specimens of the tested bursae (3 bursae per group at 3 and 10 DPC, respectively) were prepared by a graded process of

dehydration in alcohol, embedded in paraffin, and sectioned. They were then stained using the H&E stain [28]. The mean severity index (MSI) of bursal lesions was calculated in different groups at 3 and 10 DPC.

*Statistical Analysis*

All data were analyzed using one-way ANOVA, and Tukey's post hoc test was used to determine the significant differences between groups at ($p < 0.05$).

*Ethics statement*

All experimental procedures were approved by the Animal Health Research Institute, Egypt.

## Results

*IBDV detection by real-time RT-PCR*

IBDV detection by real-time RT-PCR was conducted and the result revealed that 12 Out of the 35 samples tested, 16 (45.7%) were positive when tested by rRT-PCR and confirmed by isolation in ECE via CAM. The embryos exhibited curling, dwarfing, greenish enlarged liver and congested kidney with hemorrhagic and edematous CAM containing urates deposition within 3-5 days post inoculation. Ten samples were excluded from further analysis due to the presence of hemagglutinating viruses. An additional three samples were excluded as they were positive for IBV when tested by rRT-PCR. Two pure IBDV samples (No. 32 and 34) were used for sequencing and phylogenetic analysis. Results of sequencing and phylogenetic analysis A phylogenetic tree was constructed based on the most recent classification of IBDV (Fig. 1), which revealed a close relation between the two isolates and their positioning within a group that is closely related to circulating strains in Egypt during the same period. The degree of identity and homology between our isolates and other Egyptian isolates clustered into genetic group 3 with previously isolated vvIBDV strains was 96%–100%. At the amino acid level, the two obtained isolates exhibited 95% amino acid sequence similarity with each other and 98-100% and 93-95% similarity the previously isolated vvIBDV strains [for isolates No. 34 (OQ756336) and 32 (OQ929506), respectively].

*Results of challenge study*

*Clinical disease and mortality*

The challenged birds in positive control G7 exhibited more pronounced signs of weakness, including ruffled feathers, decreased feed intake, huddling, and whitish diarrhea than in the vaccinated bird. Twenty percent mortality (3/15) was recorded in G7 while no mortalities were recorded in all vaccinated groups. The severity of signs and bursal lesions are illustrated in table 4. The vaccinated-challenged groups (1, 2 and 3) and the positive control 14 group (G7) exhibited a firm consistency with swelling and gelatinous exudate in bursa of Fabricius, in addition to swollen kidneys at 3 DPC compared to G8 or the vaccinated, unchallenged groups (4, 5 and 6). However, at 10 DPC, the bursae of all vaccinated, challenged birds exhibited a reduction in size without the presence of exudates.

*Body weight and FCR,*

Group 1, which received the HVT-IBD vector vaccine on day 1, demonstrated a significantly ($p < 0.05$) higher Bwt than the other groups in all records except at 5 DPC in which chickens of G4 exhibited a significantly ($p < 0.05$) higher weight gain (1578 grams). There was no significant difference ($p > 0.05$) between G2 and G3 during the first three weeks. However, in the 4th week, G3 exhibited a significantly ($p < 0.05$) higher weight (1113 grams) compared to G2. At 5 DPC, the challenged birds (G1, 2, 3 and 7) exhibited lower weight records (1366, 1214, 1341 and 1168 grams, respectively) and higher FCR 15 (1.63, 1.5, 1.36 and 1.56) compared to G4-6 and 8. At 10 DPC, the highest significant ($p < 0.05$) Bwt was in chickens of G4 (1703 g) followed by G1 (1629 g) then G8 (1574 g) as indicated in Tables 5 and 6.

*Enzyme linked immunosorbent assay (ELISA)*

The MDAs were measured, and the age of vaccination was calculated. The findings indicated that the optimal age for vaccination was 11 days for the intermediate vaccine and 18 days for the intermediate plus vaccine. At days 18 and 25, G3 exhibited the highest significant ($p < 0.05$) antibody titer (1648.5 and 1979.8), while G2 demonstrated the lowest 16 record (1496.5 and 1902). At 5 DPC, vvIBDV antibody titers were significantly ($p < 0.05$) higher in vaccinated challenged groups than in vaccinated unchallenged groups. Group 2 exhibited a significantly ($p < 0.05$) higher antibody titer (2779.7) than the other two challenged groups, G1 (1978.5) and G3 (2569.5). The positive control G7 exhibited the highest seroconversion of vvIBDV (4222.3), Table 7.

*Bursal body weight ratio*

The bursa body weight ratio was calculated at 3 DPC in all groups. No significant ($p > 0.05$) differences were observed between the vaccinated-unchallenged groups 4, 5 and 6, which recorded 1.314, 1.3 and 1.338, respectively, and G8, which recorded 1.3. The significantly ($p < 0.05$) highest BB ratio was in the positive control G7 as 2.511. Bursa of chickens in G2, vaccinated with Lukert strain vaccines, exhibited a significantly ($p < 0.05$) higher ratio (1.79) than G1 (1.401) and G3 (1.467), which showed no significant ($p > 0.05$) difference between each other (Table 8).

*Results of rRT-PCR to evaluate IBDV viral load in bursal tissue*

Results of rRT-PCR to evaluate IBDV viral load in bursal tissue, At 5 DPC, the findings indicated undetectable IBDV in unchallenged groups, including those that had received vaccination (G4, G5, and G6) and the unvaccinated G8. While the 17 virus was detected in all challenged groups, with significantly different viral loads in chicken groups, G1 exhibited the lowest viral load (1.48 Log10 RNA copies/ml), while the G2 group, recorded the highest viral copy number (5.27 Log10 RNA copies/ml) as indicated in Table 9.

*Proinflammatory IL-1β and IL-6 cytokines gene expression*

To ascertain the role of proinflammatory cytokines in vvIBDV infection, IL-1β and IL-6 were quantified in the bursae of experimental chickens using real-time PCR at 5 DPC. Chickens in G7 exhibited a notable upregulation of both IL-1β and IL-6, with fold changes of 8.11 and 7.2, respectively. The vaccinated-challenged groups (G1, 2, and 3) demonstrated a significant ($p > 0.05$) upregulation in the gene expression levels of both IL1β and IL-6, with fold changes of 2.2, 3.6 and 3.5-fold, respectively, for IL-1β and 1.43, 4.38 and 2.41-fold, respectively, for IL-6. In contrast, the vaccinated-unchallenged groups (4, 5 & 6) exhibited a significant ($p > 0.05$) downregulation (Table 10).

*Histopathology*

Histopathological examination,The architecture of the bursa appears to be normal in the G8 at 3 and 10 DPC, while G4, G5 and G6 also exhibited normal bursal tissue at 3 DPC. In G2 and G7, the examined birds exhibited hyperplasia of lining epithelium, with depletion of lymphocytes and cysts formation, in addition to microcysts and large follicular cysts formation at 3 and 10 DPC. These lesions were more prominent in G2 at 3 DPC. Furthermore, interfollicular oedema, inflammatory cell infiltration, focal subepithelial hemorrhage and mild to moderate connective tissue proliferation were also observed. In G4 and G6 bursa showed degeneration and necrosis of lymphocytes and interfollicular edema with depletion and interfollicular connective tissue formation in G6 at 10 DPC. While Bursa of G5 shows depletion of lymphocytes, with necrosis of lymphocytes, interfollicular oedema and fibroblast infiltration. Histopathological examination of chickens of all groups is indicated in Figures 2 (3 DPC) and 3 (10 DPC) and the MSI in Table 11.

## Discussion

The objective of this study was to investigate the epidemiology and attempt to isolate IBDV in several Egyptian governorates (n=7), as well as to evaluate the efficacy of different vaccination programs for the protection of livestock against an experimental challenge with a recently isolated strain of vvIBDV. IBDV surveillance was conducted on 35 broiler flocks with clinical and PM pictures of IBD. Bursa samples from suspected flocks were collected and tested for the presence of IBDV using rRT-PCR and revealed 16 (45.7%) positive samples and were confirmed by inoculation in ECE. Two pure positive samples were used for virus isolation, sequencing, and phylogenetic analysis. Both isolates with GenBank accession No. of OQ929506 (for isolate 32) and OQ756336 (for isolate 34) were found to be closely related to previously isolated Egyptian vvIBDV isolates of genogroup 3 according to the HVR of VP2 classification scheme that was previously designed by Michel and Jackood [29]. In Egypt, there are numerous IBDV vaccination programs vary in terms of efficacy and protection along with continuous circulation and evolution of IBDV[21]. This situation necessitates the continuous evaluation of vaccination programs against recently isolated vvIBDV. The evaluated vaccination programs in this study revealed absence of mortalities indicating their clinical protection against vvIBDV experimental infection in broilers in accordance with previously reported results by Sultan et al. [30], Manal et al. [32)], Abou El-Fetouh et al. [33] and Gewaily et al. [34]. Despite the moderate clinical disease and bursal lesions in the vaccinated-challenged groups 1, 2 and 3, they were much lower than the positive control G7 which agreed with previous documents by Seddik et al. [35] and Eterradossi and Saif [1]. The results of superior Bwt and lower FCR obtained in this study demonstrated that vector HVT-IBD vaccine had the most optimal performance in broilers. In contrast, the conventional vaccines had adversely impacted the birds' performance, either in vaccinated or vaccinated-challenged groups in comparison to conventional vaccines of which Winterfield 2512 strain-based exhibited a more protective effect than Lukert strain-based vaccination program. These findings agreed with those of Laszlo et al. [36], Wegner [37], Chung et al. [38] and Wegner et al. [39], who observed that broiler chickens vaccinated with the HVT+IBD vector vaccine exhibited higher Bwt at slaughter, a higher European Production Efficiency Factor and a lower FCR and mortality rate compared to birds vaccinated with other classical IBD vaccines. The study of Elbestawy et al. [40], recorded that the genetic similarity in the hypervariable region of VP2 (aa 206-350) between the Egyptian vvIBDV and the vaccinal strains revealed 95.5%, 94.1% and 88.9% to F52/70, W2512 and Lukert strains, respectively, which may explain the superiority of efficacy of the vector HVT-IBD-followed by Winterfield 2512- then Lukert strain-based vaccines. Twenty two Humoral antibodies play an important role in protection against IBDV infection [15]. In our study, the conventional vaccines were evaluated using classical ELISA kits,

while the vector HVT-IBD vaccine was evaluated using the BD-plus ELISA kit as previously recommended by Chang et al. [41] and Lemiere et al. [42]. In this study, chickens of G3, which received the Winterfield 2512 vaccine, exhibited the highest seroconversion, indicating that the vaccinal strain was highly immunogenic. This was in contrast with G2, which received the Lukert strain at 18 and 25 days of age. These results agreed with those reported by Wyeth and Chettle [43], who stated that seroconversion to intermediate plus vaccines in commercial broilers with a high level of MDAbs could be expected at 18 days after vaccination. At 5 DPC, all vaccinated-challenged groups demonstrated higher titers than the vaccinated-unchallenged groups. However, these titers were significantly lower than that observed in the positive control G7. This may be attributed to the protective neutralization of humoral antibodies against vvIBD challenge as previously mentioned by De Wit [44]. The results of the histopathological examination of the bursa of Fabricius indicated that both the positive control group and G2 (vaccinated with Lukert strain intermediate/intermediate plus vaccines) had the most severe bursal damage at 3- and 10- DPC, with the highest lesion score (2.5) in G2. Previously, Uddin et al. [31] documented severe lymphoid depletion in bursal follicles, follicular atrophy, cystic formation of follicles, and mixed cellular infiltration in the bursal follicles, as well as bursal hemorrhage and B-cell depletion during vvIBDV infection. The Lukert vaccinated-challenged G2 demonstrated the most negative impact on the bursa of Fabricius among all vaccinatedchallenged groups, offering the least protection against vvIBDV-induced bursal damage. Such results agreed with those reported by Manal et al. [32], who observed that the bursa of Fabricius of chickens vaccinated with Bursine Plus® exhibited pronounced 23 interfollicular oedema and substantial inflammatory cell infiltration at 3 DPC. Additionally, they noted the presence of lymphocytic necrosis, depletion, follicular atrophy and interfollicular fibroblast proliferation at 10 DPC. The other vaccinated-challenged groups (G1 and G3) exhibited mild bursal lesions. The HVT-IBD vaccinated-challenged G1 demonstrated the lowest bursal lesion score, indicating that HVT-IBDV provides the highest protection of the bursa against bursal damage following challenge with vvIBDV. These results matched with those previously reported by Perozo et al. [45]; Le Gros et al. [46]. Higher BBR were recorded in the chickens of G7 and G2 compared to all others, which may be attributed to an inflammatory process in the affected bursa [47]. The gene expression levels of IL-1β and IL-6 in this study revealed upregulation of both cytokines occurred in all challenged groups, indicating the adverse effect of vvIBDV in infected birds and their stimulation of bursal inflammatory process. These results are agreed with previous reports by Liu et al. [48] and Xu et al. [12], who demonstrated that IBDV activates T cells in the bursa of chickens and upregulates the expression levels of IL-1β and IL-6. Furthermore, our findings align with those of Abel et al. [49] and AbdulCareem et al. [50], who demonstrated that a high viral load in the bursa is associated with significantly elevated expression levels of cytokine genes. Additionally, a previous study indicated a pronounced upregulation of IL-1β in IBDV-infected chicken bursa in comparison to that observed in an uninfected bursa [51]. Furthermore, Long et al. [52] reported that IBDV infection resulted in lymphocyte depletion within the bursa and the promotion of IL-6 expression. However, an alternative study has indicated that IL-1β may be subject to temporary downregulation following infection of chickens with IBDV [53]. To assess the impact of the different vaccination regimens on viral load in bursal tissue following challenge, quantitative real-time RT-PCR (RT-qPCR) was conducted to quantify nucleic acid copy number in bursal tissues at 5 DPC [20]. The highest viral load was 24 recorded in chickens of G2, followed by G3, while the minimal viral load was recorded in G1 compared to chickens in the control positive G7. These results were confirmed by viral isolation in SPF-ECE.

## Conclusion

The obtained results indicated that all the applied vaccination programs afford protection from mortality against the vvIBDV challenge with varying degrees of impact on bird performance. However, the HVT-IBDV vector vaccine provided the highest level of protection against challenge with vvIBDV exhibiting the lowest effect on, and the minimal viral load in bursal tissue and the minimum histopathological lesion score despite a relatively lower antibody titer when compared with classical IBDV vaccines. Also, W2512 based live vaccines had superior protective efficacy than Lukert strain-based one, regarding on bursal damage, bursal viral load, histopathological lesion score and bird performance.

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*Declaration of Conflict of Interest*

The authors declare that there is no conflict of interest.

*Ethical of approval*

This study was ethically approved by the Faculty of Veterinary. Medicine, Damanhour University ethics and animal use committee with a reference number DMU\VetMed-2025/005.

**TABLE 1. History of examined broiler flocks**

| Sample no. | Locality | Age (days) | Vaccination program (day of vaccination) | Real time RT-PCR results | Year |
|---|---|---|---|---|---|
| 1 | Matrouh | 28 | Live Intermediate (14) | Negative | 2020 |
| 2 | Matrouh | 37 | Live Intermediate (13) | Negative | 2020 |
| 3 | Beheira | 24 | Live Intermediate (10)<br>Live Intermediate plus (17) | Negative | 2020 |
| 4 | Kafr Elsheikh | 31 | Live Intermediate (10)<br>Live Intermediate plus (17) | Positive | 2020 |
| 5 | Alexandria | 30 | Live Intermediate (10)<br>Live Intermediate plus (15) | Negative | 2020 |
| 6 | Alexandria | 25 | Live Intermediate (14) | Negative | 2020 |
| 7 | Alexandria | 31 | Live Intermediate (10)<br>Live Intermediate plus (15) | Positive | 2020 |
| 8 | Alexandria | 33 | Live Intermediate (9)<br>Live Intermediate plus (14) | Positive | 2020 |
| 9 | Beheira | 22 | Live Intermediate (11) | Negative | 2020 |
| 10 | Qaliubia | 29 | Vector vaccine (1)<br>Live Intermediate plus (12) | Positive | 2020 |
| 11 | Menoufia | 30 | Vector vaccine (1)<br>Live Intermediate plus (13) | Positive | 2020 |
| 12 | Gharbia | 28 | Live Intermediate plus (13) | Negative | 2020 |
| 13 | Qalyubia | 25 | Live Intermediate plus (14) | Positive | 2020 |
| 14 | Beheira | 31 | Vector vaccine (1)<br>Live Intermediate plus (14) | Positive | 2020 |
| 15 | Beheira | 36 | Live Intermediate plus (12) | Negative | 2020 |
| 16 | Qaliubia | 32 | Live Intermediate plus (14) | Positive | 2021 |
| 17 | Beheira | 28 | Live Intermediate plus (13) | Negative | 2021 |
| 18 | Beheira | 23 | Live Intermediate plus (12) | Negative | 2021 |
| 19 | Matrouh | 30 | Live Intermediate (10 and 15) | Negative | 2021 |
| 20 | Alexandria | 30 | Live Intermediate (8 and 14) | Positive | 2021 |
| 21 | Beheira | 28 | Live Intermediate (9 and 15) | Negative | 2021 |
| 22 | Matrouh | 30 | Live Intermediate (13) | Negative | 2021 |
| 23 | Alexandria | 35 | Immune complex (1)<br>Live Intermediate (12) | Negative | 2021 |
| 24 | Kafr Elsheikh | 25 | Live Intermediate (9)<br>Live Intermediate plus (15) | Negative | 2021 |
| 25 | Alexandria | 24 | NR | Positive | 2021 |
| 26 | Alexandria | 28 | NR | Negative | 2021 |
| 27 | Qaliubia | 27 | NR | Negative | 2022 |
| 28 | Alexandria | 25 | NR | Positive | 2022 |
| 29 | Beheira | 30 | NR | Positive | 2022 |
| 30 | Beheira | 25 | NR | Negative | 2022 |
| 31 | Alexandria | 24 | NR | Positive | 2022 |
| 32 | Qaliubia | 28 | NR | Positive | 2022 |
| 33 | Gharbia | 25 | NR | Positive | 2022 |
| 34 | Alexandria | 26 | NR | Positive | 2022 |
| 35 | Beheira | 30 | NR | Negative | 2022 |

NR: Not Recorded

**TABLE 2. Experimental design for assessment of protection of commercial broilers vaccinated with different vaccination programs against challenge with an vvIBDV isolate**

| Group | Vaccination program | | Challenge at 28 days old |
|---|---|---|---|
| | Type of vaccine | Age of vaccination (days) | |
| G1 (*n*=15) | Vector HVT-IBD (Vaxxitek®, Boehringer Ingelheim) | 1 | + |
| G2 (*n*=15) | Live Intermediate, Lukert strain (Bursine2®, Zoetis, USA)<br>Live Intermediate plus, Lukert strain (Bursine-Plus®, Zoetis, USA) | 12<br>18 | + |
| G3 (*n*=15) | Live Intermediate, LIBDV strain (CEVAC GUMBO-L®, Ceva Sante Animale, France)<br>Live Intermediate plus, Winterfield 2512 strain (CEVAC IBD-L®, Ceva Sante Animale, France) | 12<br>18 | + |
| G4 (*n*=10) | Vector HVT-IBD (Vaxxitek®, Boehringer Ingelheim) | 1 | - |
| G5 (*n*=10) | Live Intermediate, Lukert strain (Bursine2®, Zoetis, USA)<br>Live Intermediate plus, Lukert strain (Bursine-Plus®, Zoetis, USA) | 12<br>18 | - |
| G6 (*n*=10) | Live Intermediate, LIBDV strain (CEVAC GUMBO-L®, Ceva Sante Animale, France)<br>Live Intermediate plus, Winterfield 2512 strain (CEVAC IBD-L®, Ceva Sante Animale, France) | 12<br>18 | - |
| G7 (*n*=25) | Non | | + |
| G8 (*n*=25) | Non | | - |

**TABLE 3. Oligonucleotide primers and probes used for cytokine gene expression analysis.**

| Gene | Primer sequence | Reference |
|---|---|---|
| 28S | F: GGCGAAGCCAGAGGAAACT<br>R: GACGACCGATTTGCACGTC | (26) |
| IL-6 | F: GCTCGCCGGCTTCGA<br>R:GGTAGGTCTGAAAGGCGAACAG | |
| IL1β | F:GCTCTACATGTCGTGTGTGATGAG<br>R: TGTCGATGTCCCGCATGA | (27) |

**TABLE 4. Severity of clinical signs, mortality rate and bursal lesions in all chicken groups**

| Group | Clinical signs severity* | | Mortalities | | Bursal lesions | |
|---|---|---|---|---|---|---|
| | 3 DPC | 10 DPC | 3 DPC | 10 DPC | 3 DPC | 10 DPC |
| G1 | ++ | Non | Non | Non | ++ | + |
| G2 | ++ | Non | Non | Non | +++ | + |
| G3 | ++ | Non | Non | Non | ++ | + |
| G4 | Non | ++ | Non | Non | Non | ++ |
| G5 | Non | ++ | Non | Non | Non | ++ |
| G6 | Non | ++ | Non | Non | Non | ++ |
| G7 | +++ | Non | 3/15 (20%) | Non | +++ | + |
| G8 | Non | Non | Non | Non | Non | Non |

*Clinical signs were general signs of weakness with ruffled feather, decreased feed intake, huddling and whitish diarrhea. The severity of clinical signs and bursal lesions were expressed as +: Mild; ++: Moderate; +++: severe.

**TABLE 5. Average mean ± SD values of weekly record of body weight (in grams) in all chicken groups at the first 4 weeks and at 5 and 10 DPC.**

| Group | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 |
|---|---|---|---|---|---|---|---|---|
| W1 | 205.8±9.1$^{a}$ | 166.1±7.1$^{b}$ | 166.3±7.6$^{b}$ | | | | 163.9±7.7$^{b}$ | 165.7±7.3$^{b}$ |
| W2 | 526.5±16.5$^{a}$ | 402.5±12.7$^{b}$ | 400.5±15.1$^{b}$ | | | | 448±14.7$^{c}$ | 460±16.3$^{c}$ |
| W3 | 858±13.6$^{a}$ | 702.5±15.1$^{b}$ | 693±13.4$^{b}$ | | | | 782.5±15.9$^{c}$ | 787±17$^{c}$ |
| W4 | 1179±37$^{a}$ | 1068.5±39.4$^{b}$ | 1113±28.3$^{c}$ | | | | 1138±19.3c | 1143±15.7$^{ac}$ |
| 5 DPC | 1366±41.2$^{a}$ | 1214±23.2$^{dg}$ | 1341±37.3$^{ad}$ | 1578±25.7$^{c}$ | 1314±18.4$^{dg}$ | 1311±24.7$^{f}$ | 1168±40.2$^{b}$ | 1314±22.7$^{g}$ |
| 10 DPC | 1629±55.3$^{b}$ | 1338.5±33.2$^{b}$ | 1470±19.4$^{c}$ | 1703±47.2$^{a}$ | 1401±40.7$^{d}$ | 1456.5±24$^{e}$ | 1302±31.6$^{f}$ | 1574±28.8$^{c}$ |

Values have different scripts at the same row are significantly different at $P \leq 0.05$ ($n$ = 10).

**TABLE 6. Average mean values of weekly record of FCR in all chicken groups at the first 4 weeks and at 5 and 10 DPC.**

| Group | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 |
|---|---|---|---|---|---|---|---|---|
| W1 | 1.09 | 1.14 | 1.08 | | | | 1.10 | 1.09 |
| W2 | 1.09 | 1.22 | 1.20 | | | | 1.09 | 1.07 |
| W3 | 1.25 | 1.31 | 1.34 | | | | 1.20 | 1.19 |
| W4 | 1.55 | 1.47 | 1.37 | | | | 1.42 | 1.42 |
| 5DPC | 1.63 | 1.50 | 1.36 | 1.63 | 1.73 | 1.51 | 1.56 | 1.61 |
| 10DPC | 1.76 | 1.83 | 1.69 | 1.66 | 1.85 | 1.63 | 1.74 | 1.70 |

Values have different scripts at the same row are significantly different at $P \leq 0.05$ ($n = 10$).

**TABLE 7. Mean ± SD of IBDV antibody titer by ELISA of all chicken groups at 18, 25 days old and 5 DPC.**

| Group | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 |
|---|---|---|---|---|---|---|---|---|
| 18 DO | 1561.2 ±262[a] | 1496.5 ±220[a] | 1648.5 ±16[a] | | | | 285.7 ±54[b] | 394.5 ±43[b] |
| 25 DO | 1793.5 ±22[a] | 1902.9 ±36[b] | 1979.8 ±15[c] | | | | 251.2 ±52[d] | 251.2 ±52[d] |
| 5 DPC | 1978.5±30[a] | 2779.7±292[b] | 2569.5±275[c] | 2222.4±273[d] | 3002.8±150[e] | 3415.9±99[f] | 4222.3±340[g] | 223.1±17[h] |

Values have different scripts at the same row are significantly different at $P \leq 0.05$ ($n = 10$).

**TABLE 8. Average mean ± SD values of bursa body weight ratio of all chicken groups at 3 DPC.**

| Group | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 |
|---|---|---|---|---|---|---|---|---|
| 5 DPC | 1.401±0.03a | 1.79±0.04b | 1.467±0.04a | 1.314±0.05c | 1.3±0.06c | 1.338±0.04ac | 2.511±0.2d | 1.3±0.03c |

Values have different scripts at the same row are significantly different at $p < 0.05$ ($n = 3$).

**TABLE 9. Mean Log10 (RNA copies/ml) ± SD of IBDV load in bursal tissue of all chicken groups at 5 DPC.**

| G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 |
|---|---|---|---|---|---|---|---|
| 1.479798[a] | 5.2736[b] | 2.58558[c] | undetermined | undetermined | undetermined | 3.433196[d] | undetermined |

Values have different scripts at the same row are significantly different at $p < 0.05$ ($n = 3$).

**TABLE 10. Mean ± SD values of comparative IL-1β and IL-6 in all chicken groups at 5 DPC.**

| Group | **IL-1β** | **IL-6** |
|---|---|---|
| G1 | 2.225816±0.002[a] | 1.43±0.008[ad] |
| G2 | 3.606676±0.007[b] | 4.38±1.03[b] |
| G3 | 3.561129±0.004[b] | 2.41±0.17[d] |
| G4 | 0.001918±0.0007[c] | 0.079±0.04[a] |
| G5 | 1.112286±0.038[d] | 0.64±0.37[ad] |
| G6 | 0.057698±0[c] | 0.00008±0.05[a] |
| G7 | 8.117909±1.08[e] | 7.2±2.03[c] |
| G8 | 1 | 1 |

Values have different scripts at the same column are significantly different at $p < 0.05$ ($n = 3$).

**TABLE 11. Histopathological mean severity index (MSI) of bursa in different groups at 3 and 10 DPC.**

| Group | 3 DPC | 10 DPC |
|---|---|---|
| G1 | 1.1 | 1 |
| G2 | 2.5 | 1 |
| G3 | 1.1 | 0.8 |
| G4 | 0 | 1 |
| G5 | 0 | 1 |
| G6 | 0 | 0.8 |
| G7 | 1.8 | 2.5 |
| G8 | 0 | 0 |

0: No lesions. 1: Mild lesions. 2: Moderate lesions. 3: Severe lesions.

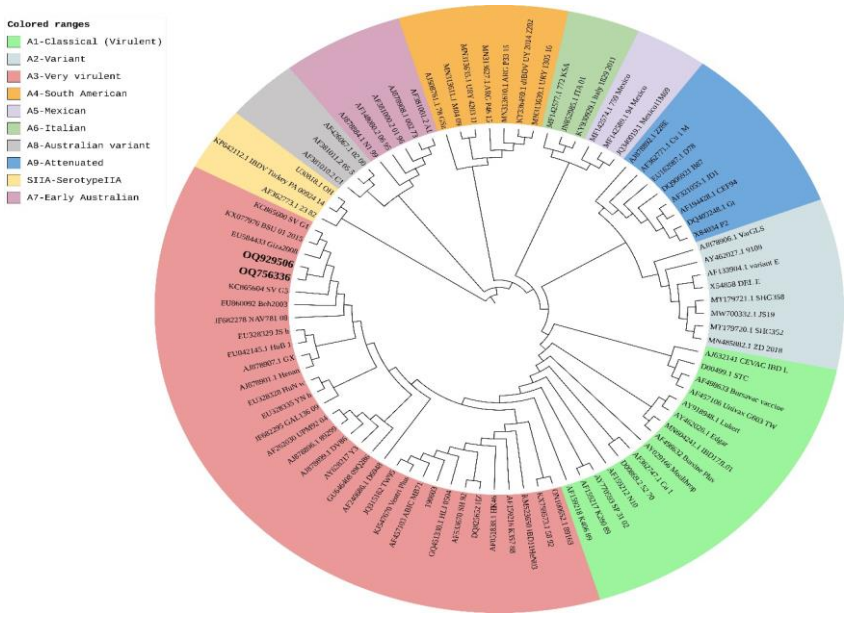


**Fig. 1. Maximum likelihood-based phylogenetic analysis (polar format) of the hypervariable region of the 2 isolates (black bold) compared to other isolates from different genogroups (including global, Middle East and other Egyptian isolates as well as commercially available vaccines method. Interactive Tree of Life (iTOL) v5 program was used to produce the phylogenetic tree. Bootstrapping using UFBoot2 method was applied. Bootstrap=100,000 replicates.**

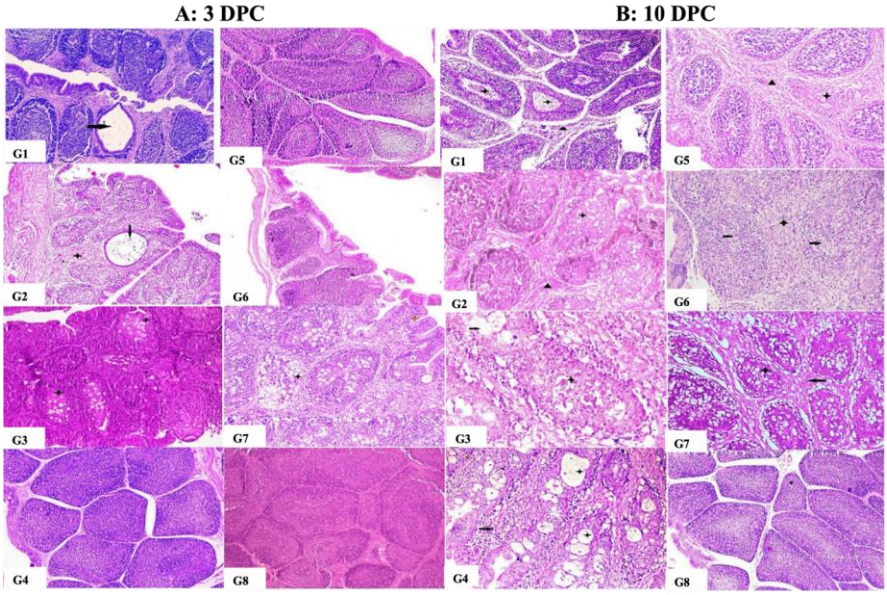


**Fig. 2. A. Bursa of Fabricius collected at 3 DPC. Chickens of G1, bursa showing cyst within one follicle surrounded by normal follicles (H&E X100). G2: showed hyperplasia of lining epithelium, depletion of lymphocytes, interfollicular edema (star) and cyst formation (arrow) (H&E X200). While G3 had follicular microcysts (stars) (H&E X400). In G4, G5 and G6, the bursa showed apparently normal structures (H&E X100). In G7, the bursa had hyperplasia of lining epithelium (red star), with depletion of lymphocytes (black arrow) and cysts formation (H&E X200) compared to the normal bursal structure in G8 (H&E X100). B: Bursa of Fabricius collected at 10 DPC. Chickens of G1 showed depletion of lymphocytes in the follicular medulla (star), and interfollicular edema (triangle) (H&E X200). While in G2, the bursa had depletion of lymphocytes (star) with degeneration and focal necrosis, in addition to interstitial connective tissue proliferation (triangle) (H&E X200). In G3, the bursa showed degeneration and necrosis of lymphocytes (star) and multiple cysts formation (arrow) (H&E X200). In G4, the bursa showed degeneration and necrosis of lymphocytes (star) and interfollicular edema (arrow) (H&E X200). In G5, the bursa had depletion of lymphocytes, with necrosis of lymphocytes (star), and interfollicular edema and fibroblasts infiltration (arrow) (H&E X200). While the bursa of chickens in G6 showed depletion of lymphocytes in the follicular medulla (star), and interfollicular connective tissue formation (triangle) (H&E X200). In G7, the bursa severe depletion and degeneration of follicular lymphocytes with microcysts formation and interfollicular connective tissue proliferation (arrow) (H&E X200) compared to the normal bursal structure in G8 (H&E X100).**

**References**


1. Eterradossi, N., Saif, Y. M. Infectious Bursal Disease. *In:* Swayne, D. E. *Diseases of Poultry*. 14th Ed. Hoboken, NJ: John Wiley & Sons, Inc. pp. 257–283 (2020).
2. Sharma, J. M., Kim, I. J., Rautenschlein, S. and Yeh, H. Y. Infectious bursal disease virus of chickens: Pathogenesis and immunosuppression. *Dev. Comp. Immunol.*, **24**, 223–235 (2000).
3. Kegne, T. and Chanie, M. Review on the incidence and pathology of infectious bursal disease. *Br. Poult. Sci.*, **3**, 68–77 (2014).
4. Cosgrove, A. S. An apparently new disease of chickens: Avian nephrosis. *Avian Dis.*, **6**, 385–389 (1962).
5. El-Sergany, H. A., Moursi, A., Saber, M. S. and Mohamed, M. A. A preliminary investigation on the occurrence of gumboro disease in Egypt. *Egypt. J. Vet. Sci.*, **11**, 185–208 (1974).
6. Phong, S. F., Hair-Bejo, M., Omar, A. R. and Aini, I. Sequence analysis of Malaysian infectious bursal disease virus isolate and the use of reverse transcriptase nested polymerase chain reaction enzyme-linked immunosorbent assay for the detection of VP2 hypervariable region. *Avian Dis.*, **47**,154–162 (2003).
7. Van den Berg, T. P., Morales, D., Eterradossi, N., Rivallan, G., Toquin, D., Raue, R., Zierenberg, K., Zhang, M. F., Zhu, Y. P., Wang, C. Q., Zheng, H. J., Wang, X., Chen, G. C., Lim, B. L. and Müller, H. Assessment of genetic, antigenic and pathotypic criteria for the characterization of IBDV strains. *Avian Pathol.*, **33**,470–476 (2004).
8. Rautenschlein, S., Yeh, H. Y. and Sharma, J. M. The role of T cells in protection by an inactivated infectious bursal disease virus vaccine. *Vet. Immunol. Immunopathol.*, **89**, 159–167 (2002). https://doi.org/10.1016/s0165-2427(02)00202-7
9. Boo, S. Y., Tan, S. W., Alitheen, N. B., Ho, C. L., Omar, A. R. and Yeap, S. K. Transcriptome analysis of chicken intraepithelial lymphocyte natural killer cells infected with very virulent infectious bursal disease virus. *Sci. Rep.*, **10**, 18348 (2020). https://doi.org/10.1038/s41598-020-75340-x
10. Eldaghayes, I., Rothwell, L., Williams, A., Withers, D., Balu, S., Davison, F. and Kaiser, P. Infectious bursal disease virus: strains that differ in virulence differentially modulate the innate immune response to infection in the chicken bursa. *Viral Immunol.*, **19**, 83–91 (2006). https://doi.org/10.1089/vim.2006.19.83
11. Huang, X., Liu, W., Zhang, J., Liu, Z., Wang, M., Wang, L., Zhou, H., Jiang, Y., Cui, W., Qiao, X., Xu, Y., Li, Y. and Tang, L. Very virulent infectious bursal disease virus-induced immune injury is involved in inflammation, apoptosis, and inflammatory cytokine imbalance in the bursa of Fabricius. *Developmental & Comparative Immunology*, **114**, 103839 (2021). https://doi.org/10.1016/j.dci.2020.103839
12. Xu, Z.Y., Yu, Y., Liu, Y., Ou, C.B., Zhang, Y.H., Liu, T.Y., Wang, Q.X. and Ma, J.Y. Differential expression of proinflammatory and anti-inflammatory genes of layer chicken bursa after experimental infection with infectious bursal disease virus. *Poultry Science*, **98**, 5307-5314 (2019). https://doi.org/10.3382/ps/pez312
13. Lee, C.C., Kim, B.S., Wu, C.C. and Lin, T.L. Bursal transcriptome of chickens protected by DNA vaccination versus those challenged with infectious bursal disease virus. *Archives of Virology*, **160**, 69-80 (2015). https://doi.org/10.1007/s00705-014-2232-y
14. Yu, Y., Cheng, L., Xu, Z., Zhang, Y., Ou, C., Wang, Q. and Gao, P. Tissue distribution and developmental changes of interferon regulatory factors in chickens and effects of infectious bursal disease virus infection. *Microbial Pathogenesis*, **104601**, 385-398 (2020).
15. Muller, H., Mundt, E., Eterradossi, N. and Islam, M.R. Current status of vaccines against infectious bursal disease. *Avian Pathology*, **41**, 133-139 (2012). https://doi.org/10.1080/03079457.2012.661403
16. Prandini, F., Simon, B., Jung, A., Poppel, M., Lemiere, S. and Rautenschlein, S. Comparison of infectious bursal disease live vaccines and a HVT-IBD vector vaccine and their effects on the immune system of commercial layer pullets. *Avian Pathology*, **45**, 114-125 (2016). https://doi.org/10.1080/03079457.2015.1127891
17. De Wit, J.J., Heijmans, J.F., Mekkes, D.R. and Van Loon, A.A. Validation of five commercially available ELISAs for the detection of antibodies against infectious bursal disease virus (serotype 1). *Avian Pathology*, **30**, 543-549 (2001). https://doi.org/10.1080/03079450120078743
18. Bublot, M., Pritchard, N., Le Gros, F.X. and Goutebroze, S. Use of a vectored vaccine against infectious bursal disease of chickens in the face of high-titred maternally derived antibody. *Journal of Comparative Pathology*, **137**, S81-S84 (2007).
19. Taghavian, O., Spiegel, H., Hauck, R., Hafez, H.M., Fischer, R. and Schillberg, S. Protective oral vaccination against infectious bursal disease virus using the major viral antigenic protein VP2 produced in *Pichia pastoris*. *PLoS One*, **8**, e83210 (2013). https://doi.org/10.1371/journal.pone.0083210
20. Tomas, G., Hernandez, M., Marandino, A., Panzera, Y., Maya, L., Hernandez, D., Pereda, A., Banda, A., Villegas, P., Aguirre, S. and Perez, R. Development and validation of a TaqMan-MGB real-time RT-PCR assay for simultaneous detection and characterization

of infectious bursal disease virus. *Journal of Virological Methods*, **185**, 101-107 (2012)

21. Meir, R., Maharat, O., Farnushi, Y. and Simanov, L. Development of a real-time TaqMan® RT-PCR assay for the detection of infectious bronchitis virus in chickens, and comparison of RT-PCR and virus isolation. *Journal of Virological Methods*, **163**, 190–194 (2010).

22. Jackwood, D.J. and Sommer, S.E. Restriction fragment length polymorphism in the VP2 gene of infectious bursal disease virus. *Avian Diseases*, **41**, 637 (1997).

23. Sharma, J.M., Dohms, J.E. and Metz, A.L. Comparative pathogenesis of serotype 1 and variant serotype 1 isolates of infectious bursal disease virus and the effect of those viruses on humoral and cellular immune competence of specific pathogen-free chickens. *Avian Diseases*, **33**, 112-124 (1989).

24. Kaiser, P., Rothwell, L., Galyov, E.E., Barrow, P.A., Burnside, J. and Wigley, P. Differential cytokine expression in avian cells in response to invasion by *Salmonella typhimurium*, *Salmonella enteritidis*, and *Salmonella gallinarum*. *Microbiology*, **146**, 3217-3226 (2000). https://doi.org/10.1099/00221287-146-12-3217

25. Livak, K.J. and Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods*, **25**, 402-408 (2001). https://doi.org/10.1006/meth.2001.1262

26. Suzuki, K., Okada, H., Itoh, T., Tada, T., Mase, M., Nakamura, K., Kubo, M. and Tsukamoto, K. Association of increased pathogenicity of Asian H5N1 highly pathogenic avian influenza viruses in chickens with highly efficient viral replication accompanied by early destruction of innate immune responses. *Journal of Virology*, **83**, 7475-7486 (2009). https://doi.org/10.1128/JVI.01434-08

27. Samy, A.A., El-Enbaawy, M.I., El-Sanousi, A.A., Abd El-Wanes, S.A., Ammar, A.M., Hikono, H. and Saito, T. In-vitro assessment of differential cytokine gene expression in response to infections with Egyptian classic and variant strains of highly pathogenic H5N1 avian influenza virus. *International Journal of Veterinary Science and Medicine*, **3**, 1-8 (2015). https://doi.org/10.1016/j.ijvsm.2015.01.001

28. Bancroft, J.D., Stevens, A., Turner, D.R. *Theory and Practice of Histological Techniques*. 4th Edition, New York: Churchill Livingstone (1996).

29. Michel, L.O. and Jackwood, D.J. Classification of infectious bursal disease virus into genogroups. *Archives of Virology*, **162**, 3661-3670 (2017).

30. Sultan, H., Hussein, H.A., Abd El-Razik, A.G., El-Balall, S., Talaat, S.M. and Shehata, A.A. Efficacy of HVT-IBDV vector vaccine against recent Egyptian vvIBDV in commercial broiler chickens. *International Journal of Poultry Science*, **11**, 710 (2012).

31. Uddin, M.M., Islam, M.S., Basu, J. and Khan, M.Z.I. Distribution and quantification of lymphocytes in the major lymphoid organs of naturally Gumboro infected broilers. *International Journal of Morphology*, **30**, 1585-1589 (2012).

32. Manal, A., Sahar, A., Heba, M., Eman, A., Kawkab, A., Abel-Alim, G.A. and Helmy, M.A. Efficacy of some infectious bursal disease (IBD) vaccines against recently isolated IBD virus. *Journal of Animal Science*, **10**, 1-15 (2019).

33. Abou El-Fetouh, M.S., Hafez, M.H., El-Attar, E.-S.R., El-Agamy, M.E. and Ali, A. Comparative bursal cytokine gene expression and apoptosis in vaccinated chickens following virulent infectious bursal disease virus challenge. *Virology*, **558**, 126-133 (2021).

34. Gewaily, M.S., El-Khyat, F., Tahoon, A.E., Al-Rasheed, M., Abdo, S.E., Gado, A. and Ismail, M.M. Cytokines, serological, and histopathological assessment of recombinant vaccination strategies for combatting infectious bursal disease in broiler chickens. *Vaccines*, **12**, 27 (2023).

35. Sedeik, M.E., Awad, A.M., Rashed, H. and Elfeil, K.W. Variation in pathogenicity and molecular characterization of infectious bursal disease virus (IBDV) in Egypt. *American Journal of Animal and Veterinary Sciences*, **13**, 76-86 (2018).

36. Laszlo, K., Janos, P., Laszlo, P. and Imre, S. Results of field experiments with Vaxxitek HVT plus IBD vaccine in Hungary. *Magyar Állatorvosok Lapja*, **130**, 718-726 (2008).

37. Wegner, M. To evaluate the effect of vaccines on the immune response and production performance of broiler chickens of various origins. *UTP University of Science and Technology, Bydgoszcz, Poland*, Doctoral Dissertation (2016).

38. Chung, E.L.T., Alghirani, M.M., Kamalludin, M.H., Nayan, N., Jesse, F.F.A., Wei, O.T.A. and Loh, T.C. Do different vaccination regimes affect the growth performance, immune status, carcass characteristics, and meat quality of broilers? *British Poultry Science*, **62**, 32-37 (2021).

39. Wegner, M., Kokoszyński, D. and Włodarczyk, K. Effect of different vaccination programs on production parameters, carcass, leg bones, and digestive system characteristics of broilers. *Poultry Science*, **102**, 102668 (2023).

40. Elbestawy, A.R., Abd El-Hamid, H.S., Ellakany, H.F., Gado, A.R., El-Rayes, S.H. and Salaheldin, A.H. Genetic sequence and pathogenicity of infectious bursal disease virus in chickens in Egypt during 2017–2021. *Avian Diseases*, **68**, 99-111 (2024).

41. Chang, H.C., Lin, T.L. and Wu, C.C. DNA vaccination with plasmids containing various fragments of large segment genome of infectious bursal disease virus. *Vaccine*, **21**, 507-513 (2003).

42. Lemiere, S., Gauthier, J.C., Kodjo, A., Vinit, L., Delvecchio, A. and Prandini, F. Evaluation of the protection against infectious bursal disease (IBD) challenge in progeny born to parents having received a vaccination program using a herpesvirus of turkey-infectious bursal disease (HVT-IBD) vector vaccine. *World Journal of Vaccines*, **3**, 31505 (2013).

43. Wyeth, P. and Chettle, N. Use of infectious bursal disease vaccines in chicks with maternally derived antibodies. *Veterinary Record*, **126**, 577-578 (1990).

44. De Wit, J.J. Gumboro disease: estimation of optimal time of vaccination by the Deventer formula. *Polish Journal of Veterinary Sciences*, **3**, 19–22 (1998).

45. Perozo, F., Villegas, A.P., Fernandez, R., Cruz, J. and Pritchard, N. Efficacy of single-dose recombinant herpesvirus of turkey infectious bursal disease virus (IBDV) vaccination against a variant IBDV strain. *Avian Diseases*, **53**, 624-628 (2009). DOI: https://www.doi.org/10.1637/8687-31009RESNOTE.1

46. Le Gros, F.X., Dancer, A., Giacomini, C., Pizzoni, L., Bublot, M. and Graziani, M. Field efficacy trial of a novel HVT-IBD vector vaccine for 1-day-old broilers. *Vaccine*, **27**, 592-596 (2009).

47. Corley, M.M. and Giambrone, J.J. Immunosuppression in specific-pathogen-free broilers administered infectious bursal disease virus vaccines by in ovo route. *Avian Diseases*, **46**, 810-815 (2002).

48. Liu, H., Zhang, M., Han, H., Yuan, J. and Li, Z. Comparison of the expression of cytokine genes in the bursal tissues of the chickens following challenge with infectious bursal disease viruses of varying virulence. *Virology Journal*, **7**, 364 (2010). DOI: https://doi.org/10.1186/1743-422X-7-364

49. Abel, K., Rocke, D.M., Chohan, B., Fritts, L. and Miller, C.J. Temporal and anatomic relationship between virus replication and cytokine gene expression after vaginal simian immunodeficiency virus infection. *Journal of Virology*, **79**, 12164-12172 (2005).

50. Abdul-Careem, M.F., Hunter, D.B., Lambourne, M.D., Read, L.R., Parvizi, P. and Sharif, S. Expression of cytokine genes following pre- and post-hatch immunization of chickens with herpesvirus of turkeys. *Vaccine*, **26**, 2369-2377 (2008).

51. Khatri, M., Palmquist, J.M., Cha, R.M. and Sharma, J.M. Infection and activation of bursal macrophages by virulent infectious bursal disease virus. *Virus Research*, **113**, 44-50 (2005).

52. Long, F.Y., Guo, Y.M., Wang, Z., Liu, D., Zhang, B.K. and Yang, X. Conjugated linoleic acids alleviate infectious bursal disease virus-induced immunosuppression in broiler chickens. *Poultry Science*, **90**, 1926-1933 (2011). DOI: https://doi.org/10.3382/ps.2011-01447

53. Jones, S.A. Directing transition from innate to acquired immunity: defining a role for IL-6. *Immunology*, **175**, 3463-3468 (2005).

# تصنيف فيروس التهاب غدة فابريشيوس شديد الضراوة في دجاج التسمين المحصن وتقييم الحماية من خلال برامج التحصين المختلفة

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**الملخص**

يمثل مرض التهاب غدة فابريشيوس المعدي (IBD) أحد أكثر الأمراض الاقتصادية أهمية التي تصيب دجاج التسمين. الهدف من هذه الدراسة هو عزل سلالات IBDV المنتشرة من قطعان دجاج التسمين التجارية المصابة (35 مزرعة) والموزعة في 7 محافظات مصرية خلال الفترة بين 2020-2022، وتقييم كفاءة برامج التحصين المختلفة في حماية دجاج التسمين التجاري ضد التحدي بسلالة شديدة الضراوة من فيروس الجمبورو (vvIBDV) . تم اكتشاف الفيروس في 16 من أصل 35 (45.7%)مزرعة ، وتم اختيار معزولتين نقيتين لتحديد التسلسل الجزيئي لبروتين الفيروس 2VP أ حيث أظهر التحليل الوراثي أن هذه المعزولات تنتمي إلى النمط الجيني 3A، وهو فيروس الجمبورو شديد الضراوة vvIBDV. تجريبيًا، تم استخدام 135 كتكوتًا عمر يوم واحد من سلالة Cobb 500 لتقييم 3 برامج تحصين مختلفة ضد التحدي بـفيروس الجمبورو شديد الضراوة vvIBDV . تم تحصين الكتاكيت في المجموعة الأولى (1G) باستخدام لقاح HVT-IBD المحمل، بينما تم تحصين الكتاكيت في المجموعة الثانية 2G باستخدام لقاحات حية متوسطة ومتوسطة معززة (lukert strain)، وتم تحصين المجموعة الثالثة 3G باستخدام لقاحات حية متوسطة(سلالة LIBDV) ومتوسطة معززة (سلالة winterfield 2512()). عند التحدي في عمر 28 يومًا، تم تقسيم كل مجموعة إلى فئتين: مجموعة محصنة متحداة (العدد15) تحكم إيجابية (غير محصنة – متحداة)، و25 دجاجة في المجموعة الثامنة 8G كمجموعة تحكم سلبية (غير محصنة - غير متحداة). وقد اظهرت النتائج ان جميع برامج التحصين وفرت حماية من النفوق الناتج عن الاصابة بفيروس الجمبورو شديد الضراوة vvIBDV. وأظهر لقاح HVT-IBD المحمل أفضل أداء من حيث التأثير على أنسجة غدة فابريشيوس وتكاثر الفيروس، على الرغم من أنه أدى إلى مستوى أقل نسبيًا من الأجسام المضادة مقارنة باللقاحات الكلاسيكية لفيروس IBDV.

**الكلمات المفتاحية:**فيروس التهاب غدة فابريشيوس المعدي ((**IBDV**، **vvIBDV**، الحماية، اللقاحات الحية لمرض **IBD**، لقاح **HVT-IBD** المحمل.