LETTERS IN BIOMATHEMATICS
An International Journal

RESEARCH ARTICLE

OPEN ACCESS

Molecular and Immunohistochemical Characterization of Infectious Bursal Disease Virus in Broilers Using PCR, VP2 Analysis, and CD19/CD57 Markers

Ahmed N. Flayyih[1], Fanar A. Isihak[2]

[1]MSc student, Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Mosul, Iraq.
[2] Department of Microbiology, College of Veterinary Medicine, University of Mosul, Iraq.

ABSTRACT

The infectious bursal disease virus (IBDV) remains one of the most important immunosuppression diseases of broiler chickens because of its targeting the bursa of Fabricius and its adverse effect on flock health and performance. This study aimed to characterize field IBDV infection in broiler chickens by combining molecular and immunohistochemical approaches. An investigation based on a cross-section of 70 broiler chickens aged 25–35 days from 10 commercial farms in Nineveh Governorate, Iraq, showing clinical suspicion of infectious bursal disease. Representative bursal samples were examined by conventional RT-PCR, RT-qPCR, sequencing of the VP2 gene, and immunohistochemistry using CD19 and CD57 markers. Conventional RT-PCR successfully amplified the expected VP2 fragment in 20 out of 28 bursal samples tested, with positive results found in 9 out of 10 farms. All samples were also confirmed positive for IBDV RNA by RT-qPCR, which corresponds to an overall positivity rate of 83.3% (n = 24). However, there were significant variations in RNA load within positive samples with Ct values ranging from 21 to 39. Melt curve analysis revealed a single sharp peak for all positive samples, which showed single peak melt curves with a specific melting temperature at 82.89°C. Phylogenetic analysis of four study isolates demonstrated separation into two distinct clusters, with two isolates grouping with Mosul-related reference sequences and two clustering with Egyptian reference strains, indicating genetic heterogeneity among circulating field viruses. Immunohistochemically, bursal tissues showed graded expression of both CD19 and CD57, ranging from negative to high immunoreactivity, reflecting variable immune-cell involvement and bursal immunopathological damage. In conclusion, the combination of RT-qPCR, conventional RT-PCR, VP2-based sequence analysis, and CD19/CD57 immunohistochemistry provided a comprehensive framework for confirming IBDV infection and characterizing the associated molecular and tissue-level changes in field cases.

ARTICLE HISTORY
Received September 19, 2026
Accepted October 29, 2026

KEYWORDS
Molecular,
Immunohistochemical,
Characterization,
Infectious Bursal Disease Virus,
Broilers,
PCR,
VP2 Analysis,
CD19,
CD57,
Markers.

1. Introduction

Infectious bursal disease is a highly contagious viral infection of young chickens and remains one of the most economically damaging infections in poultry production, not only because it can cause acute illness and mortality but also it affects the immune system of poultry, which is characterized by destruction of the lymphoid organs (Wang *et al*., 2025). The etiology of the disease is a non-enveloped, icosahedral birnavirus with a bi-segmented double-stranded RNA genome (segments A and B), whose replication is determined by an RNA-dependent RNA polymerase that contributes to constant genetic diversification (Messaï *et al*., 2024). The field strains of IBDV are classified into three main phenotypic groups: classical, variant and very virulent strains (Legnardi *et al*., 2023). Classical IBDV (cIBDV) are the original field strains previously described in the early

CONTACT Ahmed N. Flayyih:

outbreaks of IBD and represent the classical strains previously described in the region. Variant IBDV (vIBDV) presents marked antigenic variation and most of the changes are found in the VP2 protein. These strains are responsible for subclinical infection with bursal atrophy and varying degrees of immunosuppression (Messaï *et al*., 2024; Eterradossi & Saif, 2013). Very virulent IBDV (vvIBDV) presents partial antigenic similarity with classical viruses but has been associated with severe outbreaks and high mortality in backyard and commercial broilers (Legnardi *et al.,* 2023; Myint *et al*., 2021; Dey *et al*., 2019).

Polymerase chain reaction (PCR) is a powerful molecular tool for the detection and characterization of IBDV. Amplification of the VP2 gene, especially its hypervariable region, has been widely used for identification of field isolates and for subsequent sequencing and phylogenetic analysis. Several recent filed studies have employed VP2-specific primers to confirm IBDV infection and to investigate the genetic relationships among circulating strains (Bayraktar *et al*., 2025; Abd El-Fatah *et al*., 2024).

In parallel, Real-time PCR (RT-PCR) is still the most commonly used technique for the molecular identification of IBDV genomic RNA directly from clinical samples, including bursa of Fabricius tissues from young birds with overlapping clinical features with other immunosuppressive diseases. The technique is used to amplify diagnostic segments of the IBDV genome, notably the hypervariable segment of the VP2 (hvVP2) of genome segment A, which contains multiple antigenic determinants and significant viral markers for pathogenesis and molecular typing (Abd El-Fatah *et al*., 2024; Enyetornye *et al.*, 2024; Mosad *et al*., 2024).

Immunohistochemistry (IHC) offers a powerful tool to view IBDV infection at the tissue level and can complement molecular approaches. Host cellular immune response within the bursa of Fabricius can be viewed in considerable detail and IHC can highlight localized changes in immune cells. The distribution of IHC-stained antigens within the follicles could be related to areas of follicular damage as well as subsequent tissue repair. Viewing the pathogenesis of IBDV infection at a spatial level, in addition to detection of viral RNA, and an assessment of bursal follicle damage, and affected immune cell populations (Williams & Davison, 2005; Rautenschlein *et al*., 2003). CD19 is a type I transmembrane cell surface glycoprotein expressed predominantly on B lymphocytes across much of their life span, from early developmental stages through maturity, making it a widely used marker of the B-cell lineage (Younis & Isihak, 2025). In chickens, CD57 most detected using the HNK-1 monoclonal antibody, is a carbohydrate epitope whose expression is associated with immune-cell differentiation/activation rather than serving as a strict lineage-defining marker. Accordingly, its immunostaining pattern is interpreted in relation to the functional state of lymphoid cells within tissues (Mast & Goddeeris, 1997). So, this study was designed to confirm IBDV infection in broiler chickens using conventional RT-PCR and RT-qPCR and to estimate viral burden based on Ct values. Furthermore, characterize bursal immunopathological changes by immunohistochemistry using CD19 (B-cell compartment) and CD57 (activation-associated lymphoid phenotype).

2. Materials and Methods

Ethical Approval

The research received approval from the Institutional Animal Care and Use Committee at the University of Mosul/College of Veterinary Medicine. The article was licensed according to ethical number UM.VET.2025.064.

Study design and sample size

This study was designed as a field-based cross-sectional investigation targeting broiler flocks with suspected infectious bursal disease (IBD). The study included 70 samples of broiler chickens of 25-35 days of age from 10 commercial farms, where suspected clinical symptoms of infectious bursal disease were observed. 6-8 specimens (putative cases of IBD) from each flock, as well as data of general clinical symptoms, were subjected to gross examination of the bursa of Fabricius for finding typical changes. 2-3 fragments of bursal tissues from 3 specimens with most pronounced lesions of the bursa of Fabricius from each of 10 farms were used for detecting IBDV by conventional PCR and for real-time RT-PCR for determining the virus load. Fragments of bursa of Fabricius from affected birds fixed in formalin and embedded in paraffin wax were subjected to immunohistochemical study.

Study area and sampling period

The study was conducted in the Nineveh Governorate (Iraq). which is the most intensive broiler production area within the country. The survey was conducted over a five-month period, starting in September and ending in January. The survey included broiler farms where flocks showed clinical symptoms comparable to those observed with IBD. These farms were visited at the occurrence of clinical cases, the best time to identify representative lesions with a measurable amount of viral RNA. Samples were submitted to the laboratory over a period of five months and stored at appropriate refrigerated temperatures. RNA extraction and IHC examination were carried out at the laboratory.

Sample collection and handling

A post-mortem examination was carried out on the broiler chickens and gross lesions were observed. All

samples were coded and identified on receipt of samples. Samples of Bursa of Fabricius were removed aseptically and kept in separate containers for conventional PCR and RT-PCR for long term storage at −20°C and for immunohistochemistry after processing, embedding in paraffin wax and sectioning.

RNA extraction and cDNA synthesis

Total RNA was extracted from 50–100 mg of frozen bursa of Fabricius tissues using a commercial spin-column RNA kit (Addbio, Korea) according to the manufacturer's instructions. Tissue samples were lysed and homogenized, clarified by centrifugation, and the RNA was purified through sequential binding and washing steps. An on-column DNase I treatment was applied to remove residual genomic DNA, and RNA was finally eluted in 50 µL of elution buffer and stored at −20 °C until analysis. For reverse transcription, RNA templates were converted to cDNA using the Addbio One-Step RT-qPCR SYBR Master Mix, in which cDNA synthesis is performed during the initial reverse-transcription step prior to real-time amplification.

Conventional RT-PCR assay for detection of IBDV

Conventional RT-PCR was used to detect infectious bursal disease virus (IBDV) through amplification of the VP2 gene using specific primers obtained from Macrogen Co. (Korea). The primer sequences were as follows: forward, 5′- TCACCGTCCTCAGCTTACCCACATC-3′; and reverse, 5′-GGATTTGGGATCAGCTCGAAGTTG-3′; (Ramzy *et al*., 2015). After extraction of viral RNA from bursal tissues and synthesis of cDNA, the PCR reaction was prepared in a total volume of 30 µL using AddBio Master Mix (2X) (AddBio, Korea), containing 15 µL of master mix, 11 µL of PCR-grade water, 1 µL of each primer (10 µM), and 2 µL of cDNA template. The reactions were run in a T100 thermal cycler (Bio-Rad, USA) with an initial denaturation at 95°C for 10 min, followed by 35 cycles at 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 5 min. The PCR products were resolved on a 1.5% agarose gel stained with GelRed dye and electrophoresed in 1X TBE buffer at 75 V for 1 hour. A 100 bp DNA ladder was included as a size marker, and the amplified bands were visualized under UV light using a Gel Doc EZ imaging system (Bio-Rad, USA).

Real-time (RT-qPCR) detection of IBDV

IBDV was screened in bursal samples using a VP2-targeted one-step RT-qPCR (SYBR Green) assay with primers designed by authors from the reference sequences, which were as follows: forward, 5′-AGCATCGGGGGAGAACTCG-3′; and reverse, 5′ CCGTCAGCCCATTGTCTGC-3′. For real-time PCR, 20 µL RT-qPCR reactions were assembled containing RT-qPCR master mix, specific forward and reverse primers, RNA and nuclease-free water. Real-Time PCR amplifications were performed on a Step One Plus Real-Time PCR system following the manufacturer's protocol for RNA template reactions. Each reaction started with a reverse transcription step at 48°C for 10 minutes, followed by 40 cycles of amplification and data detection at 95°C for denaturation for 15 seconds and 60°C for annealing and primer extension for 1 minute. Samples were interpreted as positive based on a clear amplification signal within the defined cycle range and supported by melt-curve specificity where applicable.

Sequencing and bioinformatic analysis

VP2 sequences from individual viruses (purified from PCR-positive samples) were subcloned for sequencing to confirm the identity of the viruses and for strain level comparison. Samples were then set up for Sanger sequencing. The raw data from the Sanger sequencing gel lanes were viewed for quality and poor regions were removed. Forward and reverse reads assembled into a consensus sequence. These sequences were then searched against the NCBI GenBank database using BLAST for confirmation of IBDV identity and for the determination of the most closely related reference strain for preliminary assignment of a genogroup. The sequences were then aligned with the appropriate reference strains to construct a phylogenetic tree to deduce evolutionary relationships between the field viruses sequenced in this study. The final sequences were submitted to GenBank and assigned the following accession numbers: PX585089, PX585090, PX585091 and PX585092.

Immunohistochemical staining

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded sections of the bursa of Fabricius according to samples collected. The sections were deparaffinized in xylene and rehydrated through descending grades of ethanol. Antigen retrieval was then carried out prior to antibody incubation, and the slides were warmed in an oven for 10 min. Immunostaining was completed using a Poly-HRP detection system, followed by visualization with DAB chromogen and counterstaining with hematoxylin. Stained sections were examined microscopically, and digital images were captured using a digital camera and analyzed with ImageJ software. The degree of immunostaining was evaluated according to the intensity and distribution of brown staining in immunoreactive cells and was classified semiquantitatively as negative (-), low (+), moderate (++), or high (+++).

CD19 immunostaining

For CD19 detection, bursal sections were incubated with a rabbit polyclonal antibody obtained from Elabscience (USA). According to the manufacturer's datasheet, this antibody is suitable for immunohistochemistry and has a recommended working dilution of 1:100–1:500. After primary antibody incubation, the sections were treated with the corresponding Poly-HRP anti-rabbit secondary antibody, and the reaction was visualized using DAB chromogen followed by hematoxylin counterstaining. The stained sections were then examined microscopically, and digital images were captured using a digital camera and analyzed with ImageJ software to assess the degree of CD19 immunostaining in the bursa of Fabricius.

CD57 immunostaining

For CD57 detection, the sections were incubated with a mouse monoclonal antibody obtained from Elabscience (USA). According to the manufacturer's instructions, this antibody is suitable for IHC-P applications, with a recommended dilution of 1:50–1:200 for the concentrated form and antigen retrieval in EDTA buffer (pH 9.0) before antibody incubation. The antibody was incubated for 60 min at room temperature or overnight at 4°C. After primary antibody incubation, the sections were treated with the appropriate anti-mouse secondary antibody, and the reaction was visualized with DAB followed by hematoxylin counterstaining. Positive staining was identified as tan-to-brown staining in the expected cells without background signal, whereas the absence of such staining was interpreted as negative.

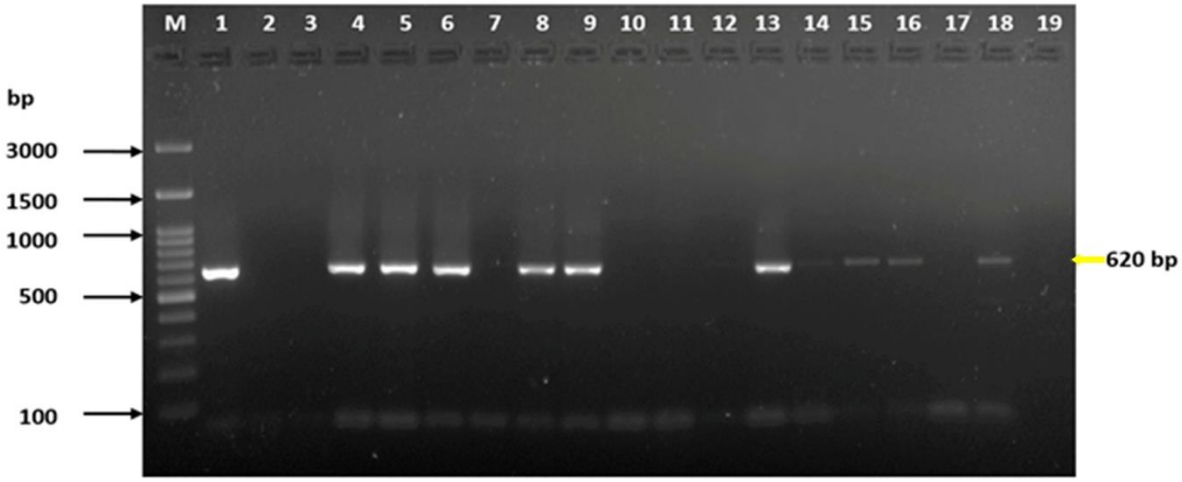
3. Results

Conventional RT-PCR targeting the VP2 gene of IBDV successfully amplified the expected viral fragment in 20 of the 28 examined bursal samples, giving a positivity rate of 71.4%. At the farm level, positive amplification was detected in 9 of the 10 farms investigated, indicating a wide distribution of IBDV between the examined broiler flocks. Complete positivity was observed in Farms 1-4 and Farm 9, whereas Farms 5, 6, and 7 showed mixed results, with both positive and negative samples identified within the same farm. Farm 8 and 10 showed no detectable amplification (Table 1). As shown in Figures 1 and 2, which together represent the gel electrophoresis profile of the analyzed samples, positive cases produced clear bands relating to the expected VP2 amplicon size of 620 bp. In contrast, no amplification was observed in the negative control lanes, supporting the specificity of the reaction.

Table 1. Farm-level detection pattern of IBDV by conventional RT-PCR in examined bursal samples

Farm No.	No. of tested samples	Positive samples	Negative samples	Detection pattern by conventional RT-PCR
1	3	3	0	Complete positivity
2	3	3	0	Complete positivity
3	3	3	0	Complete positivity
4	3	3	0	Complete positivity
5	3	2	1	Mixed pattern
6	2	1	1	Mixed pattern
7	3	2	1	Mixed pattern
8	2	0	2	Negative
9	3	3	0	Complete positivity
10	3	0	3	Negative

Figure 1. Gel electrophoresis for VP2 gene of IBDV. Lane 1 represents the 100 bp DNA marker. Lanes 1,



4, 5, 6, 8, 9, 13, 14, 16, and 18 are positive samples; lane 19 is the negative control.

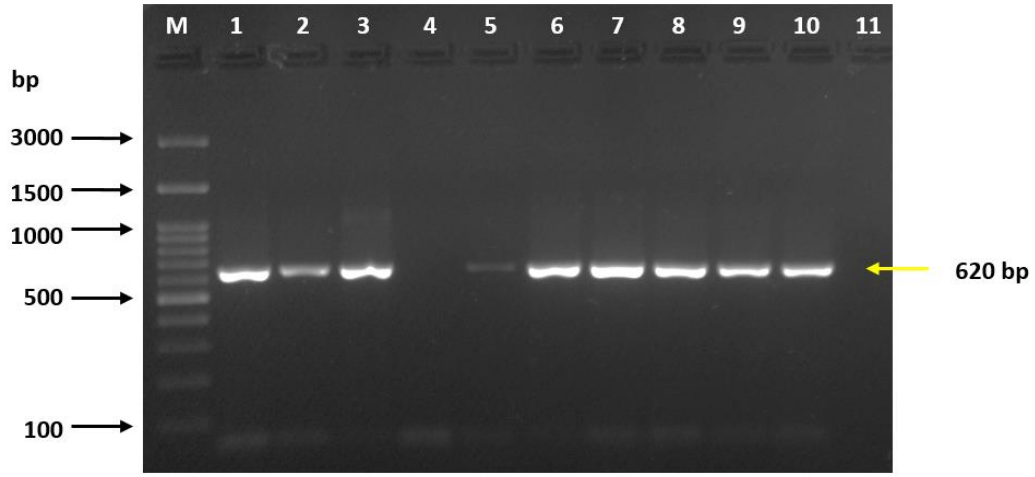


Figure 2. Gel electrophoresis for VP2 gene of IBDV. Lane 1 represents the 100 bp DNA marker. Lanes 1-3 and 5-10 are positive samples, and lane 11 is a negative control.

Quantitative real-time PCR analysis detected IBDV RNA in 20 out of 24 examined bursal samples, corresponding to an overall infection rate of 83.3% at the sample level. At the farm level, 9 out of 10 farms (90%) had at least one qPCR-positive sample, while one farm showed no detectable IBDV RNA. Complete positivity across all tested samples was observed in 5 farms (50%), whereas 3 farms (30%) exhibited mixed results, with both positive and negative samples detected within the same farm. One frame (10%) showed exclusively negative results. A significant proportion of the isolated samples were IBDV-positive. Virus RNA was detected in most of the investigated farms in the broiler production chain. Similar patterns of Ct values from qPCR were observed when comparing Ct values from different time points. All categories of Ct values (low, moderate, high, and negative) were present in all months of sampling. Low Ct values were found on day 33 (Farm 9: Ct 21). Moderate Ct values were found across a wide age range (25–35 days), especially in the farm groups 1-4. Higher Ct values or negative results were found in the age groups 27–30 days (Farms 5–8) and 35 days (Farm 10). In addition, Ct values were uniformly positive, mixed, or negative on the same day on different farms (Table 2).

Table 2: Relationship between farms, samples, and qPCR results

Farm No.	Sample No(s).	Age (days)	Ct values	qPCR interpretation
1	1, 2, 3	25	34, 32, 34	All positive (moderate viral load)
2	4, 5, 17	33	30, 31, 33	All positive (moderate–high viral

Farm No.	Sample No(s).	Age (days)	Ct values	qPCR interpretation
				load)
3	6, 7, 19	31	32, 33, 35	All positive (moderate to low viral load)
4	8, 9, 20	33	34, 31, 29	All positive (variable viral load)
5	14, 10	28	Negative, 37	Mixed (one negative, one low positive)
6	13, 18	27	34, Negative	Mixed infection status
7	15, 21	27	33, Negative	Mixed infection status
8	24	30	Negative	Negative
9	11, 12, 16	33	32, 28, 21	All positive (one very high viral load)
10	22, 23	35	36, 39	Low viral load positives

There was considerable variation within and between farms. Farms 1–4 consistently showed positive results for IBDV, with Ct values varying from 29 to 35. Farm 9 had some samples with low Ct values as well as samples with moderate Ct values. Farms 5, 6, and 7 had mixed results, with one sample in each case having high Ct values and the other samples having negative results. Farm 8 had no positive results for IBDV in the sample examined. All samples from farm 10 were positive, with Ct values ranging from 36 to 39.

Melt curves of the VP2 SYBR Green qPCR assays showed single sharp melt peaks at 82.89 °C for all positive samples with uniform melt profiles and no additional peaks detected throughout the temperature range analyzed. All negative samples had no specific melt peaks corresponding to the VP2 amplicon.

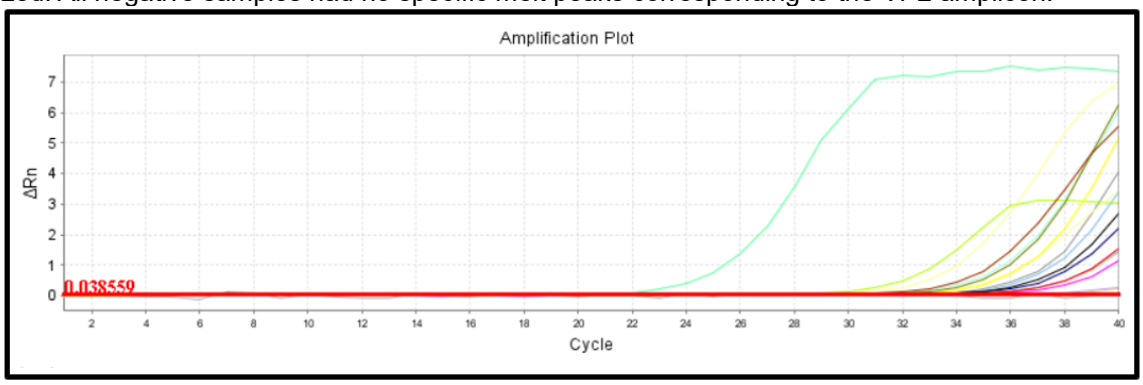


Figure 3. Quantitative real-time PCR (qPCR) amplification profiles of individual broiler chicken samples using VP2 specific primers and probes for the detection of Infectious Bursal Disease Virus (IBDV). Each curve shows an individual sample. The amplification plots show the ΔRn fluorescence values for each sample plotted against the cycle number. The horizontal red line refers to the threshold level and is used to determine the cycle threshold (Ct) of the samples that have positively amplified the IBDV VP2 gene.

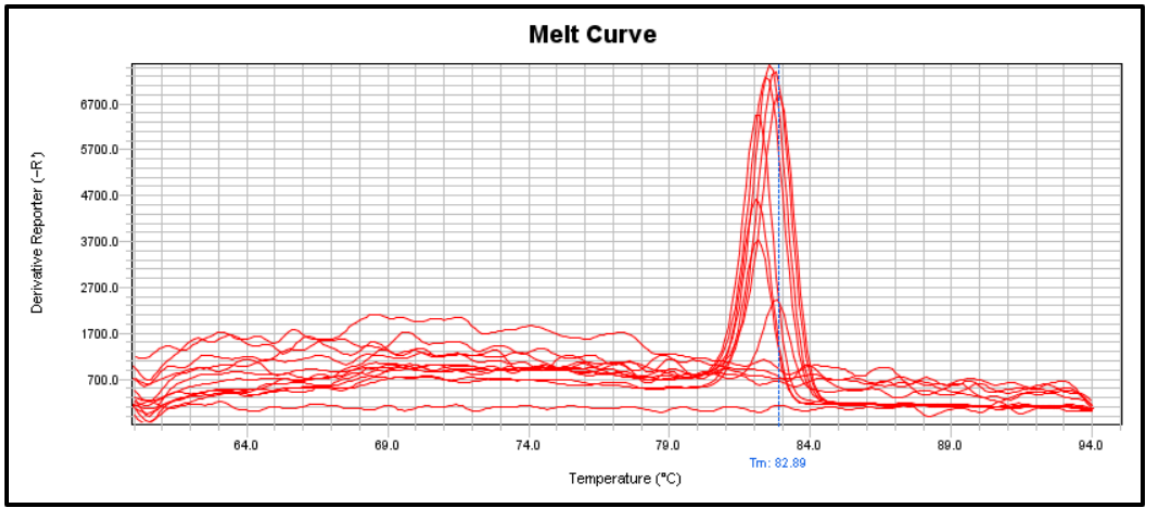


Figure 4. Melt curve profiles for VP2-based qPCR products of IBDV showed single sharp melt peaks at 82.89 °C, indicating specific amplification without any amplification of non-specific products or primer-dimer.

A phylogenetic analysis of Infectious Bursal Disease Virus (IBDV) based on VP2 gene was carried out to know the genetic relationship among the four study isolates along with reference isolates from different parts of the world. The phylogenetic tree revealed that all the four isolates of IBDV under study formed two distinct clusters. The isolates PX585089 and PX585090 were placed in a closely related cluster with Mosul, Iraq reference sequences PX488385, PX488379, PX488383 and some other reference sequences from Iran and Italy with very short branch lengths. The two other isolates PX585091 and PX585092 formed another cluster that included reference sequences from Egypt (PX488389, PX488383, PX488378 and PX488374) and some other foreign isolates. Phylogenetic analysis revealed a tree-like topology with branch lengths, which places the isolates closer to Egyptian strains than to other isolates of the same collection (Mosul).

These sequence references were from Iraq (Diyala), Israel, Iran, China, Nigeria, Hungary and the United States. Most of them were placed in a distant position from the studied IBDV isolates and formed distinct, separate lineages. However, the four IBDV isolates under this study did not cluster within a specific genetic group. They were divided into two distinct clusters based on their VP2 gene sequences, with two isolates clustering with the Mosul reference sequences and two with Egyptian reference sequences.

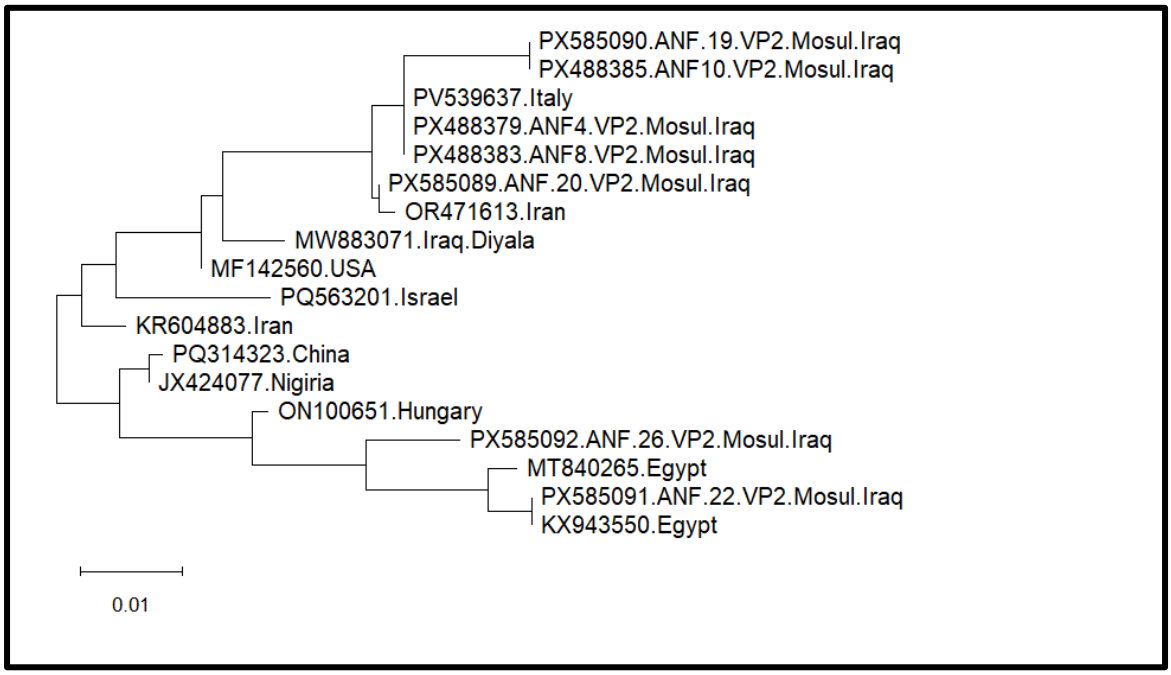


Figure 5. Phylogenetic relationships of Infectious Bursal Disease Virus (IBDV) isolates from Mosul, Iraq, based on the VP2 gene

Immunohistochemistry was performed on bursal tissues. Sections were stained with specific antibodies

against CD19 and CD57, and the intensity of the staining was scored from negative to strong. Negative: No brown staining within the bursal section. +: Low positive; faint staining of a few scattered infiltrating cells. ++: Moderate positive; clear staining of the majority of infiltrating cells. +++: High positive; strong, intense brown staining of the majority of infiltrating B cells. These samples were classified as strongly positive for the respective marker.

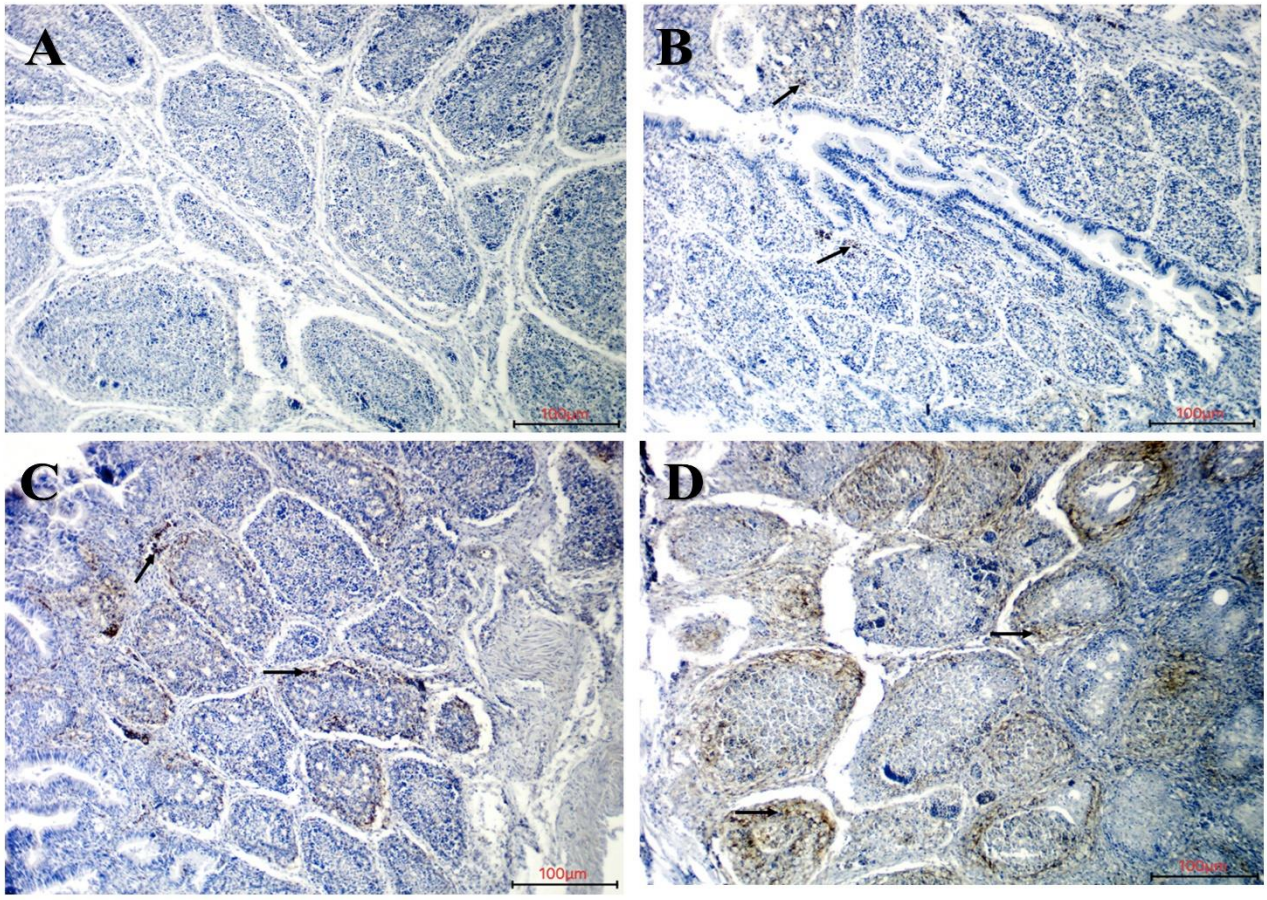


Figure 6. (A) Immunohistochemical detection of CD19 in avian bursa of Fabricius. Showing a negative expression (-) of CD19 in the bursa of Fabricius (100µm IHC). **(B)** Immunohistochemical detection of CD19 in avian bursa of Fabricius infected with IBDV. Showing a low positive immunoreactivity (+) of CD19, which appears as a brown stain in the infiltrating B cells (black arrows). (100µm IHC). **(C)** Immunohistochemical detection of CD19 in avian bursa of Fabricius infected with IBDV. Showing moderate positive immunoreactivity (++) of CD19, which appears as a brown stain in the infiltrating B cells (black arrows). (100µm IHC). **(D)** Immunohistochemical detection of CD19 in avian bursa of Fabricius infected with IBDV. Showing a high positive immunoreactivity (+++) of CD19, which appears as a brown stain in the infiltrating B cells (black arrows). (100µm IHC).

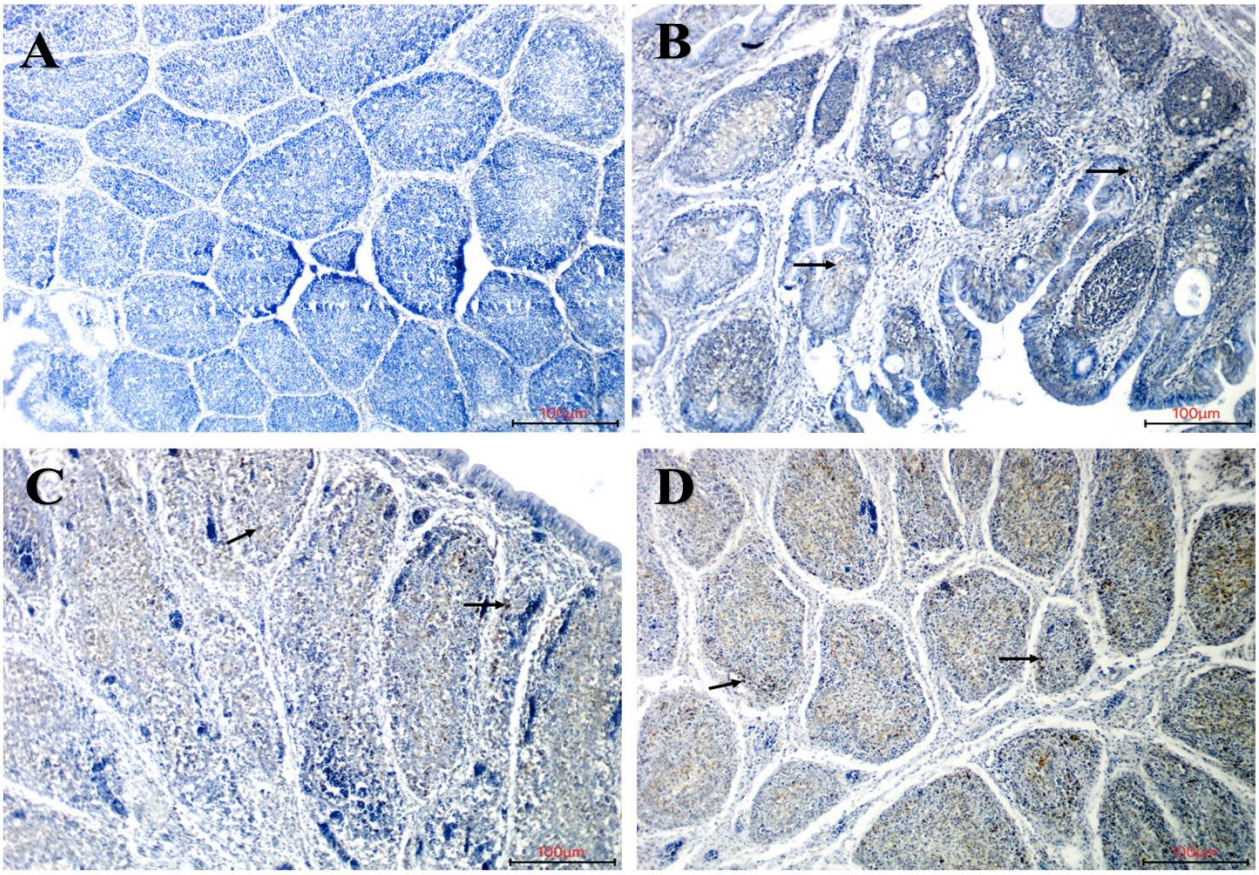


Figure 7. (A) Immunohistochemical detection of CD57 in avian bursa of Fabricius. Showing a negative expression (-) of CD57 in the bursa of Fabricius (100µm IHC). **(B)** Immunohistochemical detection of CD57 in avian bursa of Fabricius infected with IBDV. Showing a low positive immunoreactivity (+) of CD57, which appears as a brown stain in the infiltrating B cells (black arrows). (100µm IHC). **(C)** Immunohistochemical detection of CD57 in avian bursa of Fabricius infected with IBDV. Showing a moderate positive immunoreactivity (++) of CD57, which appears as a brown stain in the infiltrating B cells (black arrows). (100µm IHC). **(D)** Immunohistochemical detection of CD57 in avian bursa of Fabricius infected with IBDV. Showing a high positive immunoreactivity (+++) of CD57, which appears as a brown stain in the infiltrating B cells (black arrows). (100µm IHC).

4. Discussion

Infectious bursal disease remains one of the most important viral diseases of chickens because of its tropism for the bursa of Fabricius and its well-recognized immunosuppressive effect on the developing immune system. The disease is caused by infectious bursal disease virus (IBDV), a bi-segmented double-stranded RNA virus whose ongoing genetic evolution has contributed to substantial diversity among circulating field strains. This epidemiological complexity, together with the persistence of Classical, variant, very virulent, and reassortant viruses in poultry populations, continues to challenge disease control and reinforces the importance of accurate laboratory diagnosis (Zhang *et al*., 2024; Michel & Jackwood, 2017).

Diagnosis of IBDV is not just based on gross lesions and should include an integrated approach using both molecular and tissue-based diagnosis. While conventional RT-PCR can be used to amplify specific regions of the IBDV genome for diagnosis, the amplified products can be further characterized. In contrast, RT-qPCR is more sensitive and provides rapid detection and estimation of viral load by determining the Ct value. Immunohistochemistry on tissues provides vital information on the localization of antigens and markers of immune response within affected bursal tissues. This study therefore demonstrates the use of a combination of methods for IBDV diagnosis in field conditions (Adel *et al*., 2024; Aliyu *et al*., 2021; Singh *et al*., 2015).

A conventional RT-PCR assay based on the amplification of the VP2 gene of IBDV was used for detection of the virus. In this study, the virus was detected in 20 (71.4%) out of 28 tested bursal samples from 10 farms. Positive amplification was obtained from samples of 9 out of the 10 investigated farms. The conventional RT-

PCR using bursal tissue is useful for the diagnosis of IBD since IBDV primarily replicates in the bursa of Fabricius. The observed positivity rate appears consistent with previous reports in several previous field studies (Aliyu *et al*., 2021; Jiang *et al*., 2021). However, the percentages of detected positivity varied widely among studies. Jiang *et al*. (2021) reported the positive rate of 45.1% for bursal samples and 61.9% for flocks in China. Bayraktar *et al*. (2025) demonstrated IBDV in 91.4% of broiler samples in a Turkish flock, focusing on the fact that the frequency of detection may differ according to outbreak stage, vaccination background, circulating genotype, and sample selection. Also, IBDV was confirmed in bursal homogenates from various Malaysian field isolates using VP2-targeted RT-PCR by Aliyu *et al*. (2021).

The farm level pattern in results is also meaningful. Complete positivity in several farms, together with mixed positive and negative results in others, possibly reflects heterogeneity in viral circulation within flocks, differences in the timing of sampling relative to infection, and variation in viral load between individual birds. This reading is steady with the known field behavior of IBDV, where multiple genotypes may circulate at the same time and produce variable molecular detection reports within and between flocks (Jiang *et al*., 2021; Bayraktar *et al*., 2025). Furthermore, the clear 620-bp VP2 bands observed on the agarose gel, together with the lack of amplification in the negative control lanes. Although real-time RT-PCR is generally more sensitive than endpoint conventional RT-PCR, conventional RT-PCR remains respected as a practical confirmatory tool and as a reliable method for generating VP2 amplicons suitable for sequencing and phylogenetic analysis (Adel *et al*., 2024; Aliyu *et al*., 2021).

Molecular evidence of ongoing circulation of IBDV in the tested broiler farms was found by the RT-qPCR test. The test detected specific IBDV viral RNA signals from 20 out of 24 (83.3%) tested bursal samples. The distribution of active infections was not limited to a few isolated cases; specific positive samples were found in 90% (9/10) of tested farms. Detection of viral RNA in 20 of 24 bursal samples (83.3%), with 9 of 10 farms (90%) yielding at least one positive sample, indicates that the virus was not confined to isolated cases but was widely distributed among the investigated flocks. The high detection rate is likely related both to the epidemiological status of the selected farms and to the analytical sensitivity of RT-qPCR, which remains one of the most reliable methods for detecting IBDV RNA directly in bursal tissue. This agrees with recent studies showing that real-time RT-PCR provides rapid, sensitive, and specific detection of IBDV, including its emerging variants, and is particularly valuable in field situations where clinical and gross lesions may relate to other immunosuppressive conditions (Adel *et al*., 2024; Tomás *et al*., 2017).

The RT-qPCR positivity rate recorded in the present study was relatively high, indicating substantial viral circulation in the broiler flocks investigated. At the sample level, this detection rate was clearly higher than that reported by Legnardi *et al*. (2023), who found only 7 of 24 samples (29.2%) positive during molecular surveillance in Egypt. Likewise, Abd El-Fatah *et al*. (2024) reported positive in 20 of 52 field samples (38.4%), which is also markedly lower than the proportion observed in the present work. In contrast, the current sample-level positivity was lower than that described by Bayraktar *et al*. (2025), who reported IBDV detection in 91.4% of broiler samples in Turkish flocks, but it remained broadly comparable to the high flock positivity observed by Cheggag *et al*. (2018), whose real-time RT-PCR survey in Morocco identified positive flocks in 84 of 102 suspected cases (82.3%) over a four-year period. At the farm level, the present finding of 90% positive farms was also notably higher than the 36.4% farm positivity reported by Ekiri *et al*. (2021) in Nigeria and the 61.9% flock positivity described by Jiang *et al*. (2021) in China. From the comparisons, it could be inferred that the molecular load of IBDV on these farms was high. The high detection rate in this study was partly due to the study design where cases that were clinically and pathologically compatible were targeted. The use of bursal tissue as the main sample for diagnostic purposes and the high analytical sensitivity of the study's method, RT-qPCR, which could detect IBDV RNA from different loads from field samples, also contributed to the high detection rate. Variation was seen in the individual Ct values. Within Farm 9, the Ct values ranged from a very low value of 21 in one sample to higher values of 36–39 in samples from Farm 10. Within Farms 5–7, the Ct values showed mixed positive and negative results and indicated varying levels of viral RNA in the different flocks within a farm as well as between farms. This variation may be attributed to a lower viral load or uneven distribution of the virus within the flocks (Jayasundara *et al*., 2017). Farms 1–4 (including Farm 9) tested positive; however, other farms in this survey had mixed results. Sampling multiple birds per flock is key to accurately determine IBDV status. RT-qPCR allowed for the determination of largely heterogeneous detection within individual field-exposed flocks, possibly due to level of exposure, genetic diversity, and/or ongoing vaccination programs. (Adel *et al*., 2024; Wang *et al*., 2023).

A VP2-based phylogenetic tree was constructed to clarify the molecular characteristics of the four IBDV isolates studied in this manuscript. The VP2 sequences of the four field isolates did not represent a single lineage and were distributed into two distinct groups. Two isolates grouped closely with earlier reported Mosul-related sequences, whereas the other two clustered with Egyptian reference strains, suggesting that more than one genetically related IBDV lineage may be circulating in the local epidemiological setting. The VP2 gene, especially the hypervariable region, is a main marker for IBDV genogrouping and molecular differentiation

among different strains (Bayraktar *et al*., 2025; Abd El-Fatah *et al*., 2024; Michel & Jackwood, 2017). Although this assignment is based on limited sequence data from the VP2 gene, full-length genome sequences would be required to confirm the possibility of reassortment and to better elucidate long-term evolutionary relationships among these strains (Bayraktar *et al*., 2025; Islam *et al*., 2021).

The immunohistochemical findings in this study showed a clear graded pattern of CD19 and CD57 expression in the bursa of Fabricius, ranging from negative to high immunoreactivity, which suggests marked variation in the degree of immune-cell involvement among the examined IBDV-affected tissues. Particularly, the variable CD19 staining observed here is broadly consistent with the report of Shahsavandi *et al*. (2023), who showed that CD19 expression is altered in association with IBDV replication, supporting the view that changes in CD19 immunoreactivity reflect disruption of the B-cell compartment during infection. Furthermore, the presence of low, moderate, and high CD19/CD57 staining across different sections agrees with the general IHC observations of Singh *et al*. (2015), who demonstrated that immunohistochemistry could reveal variable antigen and lesion-associated patterns in IBD-affected lymphoid tissues. To our knowledge, there is no comparative study on the expression of CD57 in IBDV-infected bursal tissues. The graded expression of CD57 in this immunohistochemical study likely correlates with the different numbers and populations of activated or redistributed lymphoid cells in the pathological follicles. This study is in line with the current understanding of the effects of IBDV on immune cells and its interference with adaptive immune responses (Zhang *et al*., 2024; Trapp & Rautenschlein, 2022). Immunohistochemistry is therefore a useful complementary tool in studying the immunopathology of IBDV infection.

5. Conclusions

This survey conducted on 10 broiler farms revealed a high prevalence of IBDV. A strategy using molecular detection in conjunction with immunohistochemistry was found to be useful for a meaningful field diagnosis of IBDV. RT-qPCR prepared sensitive detection and useful assessment of viral burden, while conventional RT-PCR and VP2 sequencing supported molecular confirmation and revealed genetic heterogeneity between the detected isolates. Together, CD19 and CD57 immunohistochemistry added tissue-level comprehension to the variable immunopathological changes occurring in the bursa of Fabricius. Overall, the combination of RT-qPCR, conventional RT-PCR, VP2 analysis, and IHC offers a consistent and biologically informative approach for characterizing field IBDV infection in broiler chickens.

Acknowledgements

The researchers would like to express their serious appreciation to the College of Veterinary Medicine, University of Mosul, for supporting this work. Additionally, we would like to thank the laboratory staff and colleagues for their scientific assistance during the sample collection, administration, and analysis processes. Special thanks are extended to the broiler farm owners and workers for their assistance during the field investigation.

6. References:

1. Abd El-Fatah, A.H., Ayman, D., Samir, M., Eid, S., Elgamal, M., El-Sanousi, A.A., Ibrahim, M., AlKhazindar, M., Ali, M.M. & Afify, A. (2024). Molecular characterization of circulating infectious bursal disease viruses in chickens from different Egyptian governorates during 2023. Virology Journal, 21(1), 312.
2. Adel, A., Zanaty, A., Mosaad, Z., Selim, K., Hagag, N.M., Badr, M., Ellakany, H., Shahien, M. & Samy, A. (2024). Advancing IBDV diagnostics: A one-step multiplex real-time qRT-PCR for discriminating between vvIBDV and non-vvIBDV viruses, including the newly emerged IBDV variant. *Frontiers in Veterinary Science*, *11*, 1421153.
3. Aliyu, H. B., Hair-Bejo, M., Omar, A. R., & Ideris, A. (2021). Genetic diversity of recent infectious bursal disease viruses isolated from vaccinated poultry flocks in Malaysia. *Frontiers in veterinary science*, *8*, 643976.
4. Bayraktar, E., Aydin, O., Tali, H.E., Ozkan, I.E., Umar, S., Tali, H.B., Yilmaz, A., Turan, N., Kolukisa, A., Konuk, C. & Erdem, A. (2025). Predominance of reassortant infectious bursal disease viruses in Turkish poultry flocks. *Poultry science*, 105974.
5. Cheggag, M., Zro, K., Sebbar, G., Rahmatallah, N., Mouahid, M., EL Houadfi, M., & Kichou, F. (2018). Diagnosis of clinical cases of infectious bursal disease using a modified rapid taq man-MGB real-time RT-PCR assay. *Journal of Agricultural Science and Technology A*, *8*, 230-238.
6. Dey, S., Pathak, D. C., Ramamurthy, N., Maity, H. K., & Chellappa, M. M. (2019). Infectious bursal

disease virus in chickens: prevalence, impact, and management strategies. *Veterinary Medicine: Research and Reports*, 85-97.
7. Ekiri, A.B., Armson, B., Adebowale, K., Endacott, I., Galipo, E., Alafiatayo, R., Horton, D.L., Ogwuche, A., Bankole, O.N., Galal, H.M. and Maikai, B.V., & Cook, A. J. (2021). Evaluating disease threats to sustainable poultry production in Africa: Newcastle disease, infectious bursal disease, and avian infectious bronchitis in commercial poultry flocks in Kano and Oyo States, Nigeria. *Frontiers in veterinary science*, *8*, 730159.
8. Enyetornye, B., Abugri, H. A., Kusi-Appiah, A. K., Maboni, G., Odoom, T., Gottdenker, N. L., & Velayudhan, B. T. (2024). Pathology and VP2-Based Characterization of Infectious Bursal Disease Virus Associated with an Outbreak in Layer Chickens in Ghana. *Pathogens*, *13*(12), 1115.
9. Eterradossi, N., & Saif, Y. M. (2013). Infectious bursal disease. *Diseases of poultry*, 219-246.
10. Islam, M.R., Nooruzzaman, M., Rahman, T., Mumu, T.T., Rahman, M.M., Chowdhury, E.H., Eterradossi, N. & Müller, H. (2021). A unified genotypic classification of infectious bursal disease virus based on both genome segments. *Avian Pathology*, *50*(2), 190-206.
11. Jiang, N., Wang, Y., Zhang, W., Niu, X., Huang, M., Gao, Y., Liu, A., Gao, L., Li, K., Pan, Q. & Qi, X. (2021). Genotyping and molecular characterization of infectious bursal disease virus identified in important poultry-raising areas of China during 2019 and 2020. *Frontiers in Veterinary Science*, *8*, 759861.
12. Jayasundara, J. M. K. G. K., Walkden-Brown, S. W., Katz, M. E., Islam, A. F. M. F., Renz, K. G., McNally, J., & Hunt, P. W. (2017). Pathogenicity, tissue distribution, shedding and environmental detection of two strains of IBDV following infection of chickens at 0 and 14 days of age. *Avian Pathology*, *46*(3), 242-255.
13. Legnardi, M., Franzo, G., Tucciarone, C. M., Koutoulis, K., & Cecchinato, M. (2023). Infectious bursal disease virus in Western Europe: the rise of reassortant strains as the dominant field threat. *Avian Pathology*, *52*(1), 25-35.
14. Legnardi, M., Poletto, F., Talaat, S., Selim, K., Moawad, M.K., Franzo, G., Tucciarone, C.M., Cecchinato, M. & Sultan, H. (2023). First detection and molecular characterization of novel variant infectious bursal disease virus (genotype A2dB1b) in Egypt. *Viruses*, *15*(12), 2388.
15. Mast, J., & Goddeeris, B. M. (1997). CD57, a marker for B-cell activation and splenic ellipsoid-associated reticular cells of the chicken. *Cell and tissue research*, *291*(1), 107-115.
16. Messaï, C.R., Chenouf, N.S., Khalouta, O., Chorfa, A., Salhi, O., Tucciarone, C.M., Poletto, F., Franzo, G., Aberkane, C., Cecchinato, M. & Legnardi, M. (2024). Infectious Bursal Disease Virus in Algeria: Persistent Circulation of Very Virulent Strains in Spite of Control Efforts. *Animals*, *14*(23), 3543.
17. Michel, L. O., & Jackwood, D. J. (2017). Classification of infectious bursal disease virus into genogroups. *Archives of virology*, *162*(12), 3661-3670.
18. Mosad, S. M., Elsayed, M. M., Hammad, E. M., Hendam, B. M., Ali, H. S., Eladl, A. H., & Saif, M. A. (2024). Genotype classification and pathogenicity of infectious bursal disease virus circulating in vaccinated broiler chicken farms. *Veterinary Research Communications*, *48*(5), 3089-3104.
19. Myint, O., Suwanruengsri, M., Araki, K., Izzati, U.Z., Pornthummawat, A., Nueangphuet, P., Fuke, N., Hirai, T., Jackwood, D.J. & Yamaguchi, R. (2021). Bursa atrophy at 28 days old caused by variant infectious bursal disease virus has a negative economic impact on broiler farms in Japan. *Avian Pathology*, *50*(1), 6-17.
20. Ramzy, N., & Abdel-fattah, S. H. A. H. E. R. A. (2015). Prevalence and molecular characterization of Gumboro virus in chicken farms in Ismailia. *Assiut Veterinary Medical Journal*, *61*(145), 152-159.
21. Rautenschlein, S., Yeh, H. Y., & Sharma, J. M. (2003). Comparative immunopathogenesis of mild, intermediate, and virulent strains of classic infectious bursal disease virus. *Avian diseases*, *47*(1), 66-78.
22. Shahsavandi, S., Torabi, S., Ebrahimi, M. M., & Ghadiri, M. B. (2023). Down-regulating CD19 surface markers expression correlates with infectious bursal disease virus replication. *Veterinary Immunology and Immunopathology*, *264*, 110658.
23. Singh, J., Banga, H. S., Brar, R. S., Singh, N. D., Sodhi, S., & Leishangthem, G. D. (2015). Histopathological and immunohistochemical diagnosis of infectious bursal disease in poultry birds. *Veterinary world*, *8*(11), 1331.
24. Tomás, G., Hernández, M., Marandino, A., Techera, C., Grecco, S., Hernández, D., Banda, A., Panzera, Y. & Pérez, R. (2017). Development of an RT-qPCR assay for the specific detection of a distinct genetic lineage of the infectious bursal disease virus. *Avian Pathology*, *46*(2), 150-156.
25. Trapp, J., & Rautenschlein, S. (2022). Infectious bursal disease virus' interferences with host immune cells: what do we know?. *Avian pathology*, *51*(4), 303-316.
26. Wang, C., Hou, B., Shao, G., & Wan, C. (2023). Development of a one-step real-time TaqMan reverse

transcription polymerase chain reaction (RT-PCR) assay for the detection of the novel variant infectious bursal disease virus (nVarIBDV) circulating in China. *Viruses*, *15*(7), 1453.
27. Wang, W., Wu, J., Jiang, N., Liang, Q., Liu, R., Fu, Q., Fu, G., Wei, T., Wan, C., Cheng, L. & Chen, H. (2025). Advances in Infectious Bursal Disease Virus Vaccines—A Review. *Microorganisms*, *13*(12), 2801.
28. Williams, A. E., & Davison, T. F. (2005). Enhanced immunopathology induced by very virulent infectious bursal disease virus. *Avian pathology*, *34*(1), 4-14.
29. Younis, Z. T., & Isihak, F. A. (2025). Immunological assessment and homogeneity of commercial inactivated bivalent Newcastle and avian influenza H9N2 vaccines in broilers. *Iraqi Journal of Veterinary Sciences*, *39*(3), 501-510.
30. Zhang, T., Wang, S., Liu, Y., Qi, X., & Gao, Y. (2024). Advances on adaptive immune responses affected by infectious bursal disease virus in chicken. *Frontiers in immunology*, *14*, 1330576.